XXXIII INTERNATIONAL CONGRESS ON MICROBIAL ECOLOGY AND DISEASE

Abstract Proceedings

Organized by the

SOCIETY OF MICROBIAL ECOLOGY AND DISEASE (SOMED)

Greece, 6-10 September 2010

PRESIDENT: Professor Eugenia BEZIRTZOGLOU, MD, PhD

Printing of Abstract Proceedings was sponsored by Journal ANAEROBE (USA) and the Faculty of Agricultural Development, Democritus University of Thrace (Greece)

INTERNATIONAL ADVISORY BOARD

Nazmiye ALTINTAS (Turkey)
Francesca CLEMENTI (Italy)
George CREATSAS (Greece)
Mehmet DOGANAY (Turkey)
Gianfranco DONELLI (Italy)
Christine EDWARDS (UK)
Sydney FINEGOLD (USA)
A.Kadir HALKMAN (Turkey)
Sofia KATHARIOU (USA)
Alexandros KORTSARIS (Greece)
Veronica LAZAR (Romania)
Paola MASTRANTONIO (Italy)
Birce MERCANOGLU-TABAN (Turkey)
Tore MIDTVEDT (Sweden)
Marika MIKELSAAR (Estonia)
Elisabeth NAGY (Hungary)
Carl Erik NORD (Sweden)
Elisabeth NORIN (Sweden)
Andrew ONDERDONK (USA)
Arthur OUWEHAND (Finland)
Marie-Benedicte ROMOND (France)
Sevastianos ROUSSOS (France)
Boris SHENDEROV (Russia)
Shigeru KAMIYA (Japan)
Constantinos SIMOPOULOS (Greece)
Gjalt WELLING (The Netherlands)
Michael WILSON (UK)

LOCAL ORGANIZING COMMITTEE

Athanasios ALEXOPOULOS
Nicholaos ANDRIKOPOULOS
Ioannis ARVANITOGIANNIS
Christos BEZIRTZOGLOU
Dimitris BOSKOU
George BOSKOU
Theodoros CONSTANDINIDIS
Elefterios DROSINOS
George KALANTZOPOULOS
Maria KANELLAKI
Amalia KARAGOUNI
Michalis KONTOMINAS
Katerina KOTZAMPASSI
Ioannis KOURKOUTAS
Evangelia KOUSKOUNI
Athanasios KOUTINAS
Maria KYRIAKOU
Evanthia LITOPOULOU-TZANETAKI
Gerasimos MANTZARIS
Athina MAVRIDOU
Mela NACOU
George- John NYCHAS
Olga PAGONOPOULOU
Christos PAPALOUCAS
Maria PAPAPETROPOULOU
Stavros PLESSAS
Ioannis SKOUFOS
Stefanos SPARTALIS
Petros TAOUKIS
Effie TSAKALIDOU
Athanassios TSAKRIS
Arsenios TSIOTSIAS
Miltiadis TYPAS
Nικolaos TZANETAKIS
Athina TZIORA
Apostolos VANTARAKIS
Despina VASSILIADOU
Chrissoula VOIDAROU

SCIENTIFIC ASSISTANT ORGANIZING COMMITTEE

Maria FOURNOMITI
Vaia GEROKOMOU
Anastasia KARGIOTIDOU
Ioanna MANTZOURANI
Alexandra NERANTZAKI
Angelos NICAS
Elisabeth STAVROPOULOU
Maria SKOUFOU
Christos STEFANIS
Stavros VAVIAS

*Dedicated to the memory of our colleagues **Christopher AUGUR** and **Miltos TSOGAS***

Relevant Perspectives in Clinical Microbial Ecology

Invited Lectures

State of Art: MIcrobiology in Health and Disease

S.M. Finegold, M.D., MACP

To illustrate the theme of this lecture, I will discuss the bowel bacteria in relation to autism. There are probably many other diseases (e.g., Parkinson's disease, multiple sclerosis, rheumatoid arthritis, etc.) in which intestinal bacteria play a role.

There is evidence of genetic predisposition to autism, but the percent of autistic subjects with this background is unknown. It is also clear that other factors, particularly environmental toxins, play a role by damaging the child's immune system. Our study was of a type of autism known as regressive autism with gastrointestinal manifestations and included 33 autistic children of varying severity, seven sibling controls with no evidence of autism, and eight non-sibling healthy controls. We used pyrosequencing with the titanium modification, with the collaboration of Dr. Scot Dowd. This powerful technique detected approximately 1,000 operational taxonomic units in the stools of the children. At the phylum level, the healthy controls showed dominance of *Firmicutes* (64% of the total flora) with *Bacteroidetes* next most common (30%). The autistic children's stools showed a distinctly different pattern, with *Bacteroidetes* predominating (51%) and *Firmicutes* next most common (38%). This is the first study of the entire fecal population showing a statistically significant (p 0.001) abnormality in the bowel flora of autistic children. I will discuss the organisms potential ly involved in contributing to the pathology and others that might be protective.

Previous studies involving study of the upper gastrointestinal (UGI) flora (stomach and duodenum) of a small number of autistic and control children , with the collaboration of Dr. A. Kaul, demonstrated significant overgrowth of UGI flora in two children who had hypochlorhydria with no known cause other than autism.

Microbial ecology of the cervix, HPV infections and vaccination

G. Creatsas, MD, FACS, FACOG, FRCOG
Division of Pediatric-Adolescent Gynecology & Reconstructive Surgery, 2nd Department Obstetrics/Gynecology, University of Athens, Aretaieion Hospital, Athens, Greece.

Cervical infections known as cervicitis might be caused by several aerobic or anaerobic pathogens including Chlamydia trachomatis, Neisseria gonorrhoeae, herpes simplex virus (HSV), or human papillomavirus (HPV). Another cause of cervicitis along with vaginitis is commonly cause by Trichomonas vaginalis [the most frequent curable sexually transmitted infection (STI) in young, sexually active women], while local trauma, radiation, or malignancy can result in noninfectious cervicitis. The infectious etiologies are significantly more common than the noninfectious causes, and all possible infectious causes are STIs.
According to CDC (Centers for Disease Control and Prevention) more than 100 types of HPV exist while above 30 types of them can infect the genital area. The majority of HPV infections are asymptomatic, unrecognized, or subclinical. Especially genital HPV infection is very common, usually self-limited, and occurs more frequently than cervical cell changes among women or visible genital warts among both men and women. High-risk HPV types (e.g. 16, 18, 31, 33, and 35) infect the anogenital region and are strongly associated with cervical neoplasia while HPV types 6 & 11 can cause genital warts. Persistent infection with high-risk types of HPV is the most important risk factor for cervical neoplasia. Papanicolaou test remains the gold standard screening method of cervical cells for HPV infection and squamous intraepithelial lesions (SILs). Although HPV testing may detect more precancerous cells than conventional cytology according to recent publications, must be reserved for women with atypical squamous cells of undetermined significance (ASC-US) or in screening women aged ≥30 years in conjunction with the Papanicolaou test. Counseling of women with HPV infection should include information for the wide spread of this STI and should emphasize that this infection usually goes away on its own. If any Papanicolaou test or biopsy abnormalities have been observed, further evaluation is recommended. Screening women or men with the HPV test, outside of the above recommendations for use of the test with cervical cancer screening, is not recommended according to CDC. Today no therapy has been identified that can eradicate HPV infection. In the presence of coexistent SIL, management should be based on histopathologic findings. On the contrary in the absence of genital warts or cervical SIL, treatment is not recommended for subclinical genital HPV infection.
The U.S. Food and Drug Administration recently approved the HPV vaccine for females aged 9–26 years. The American College of Obstetricians and Gynecologists (ACOG) recommends the vaccination of females in this age group. Current cervical cytology screening recommendations remain unchanged and should be followed regardless of vaccination status. Sexually active women can receive the HPV vaccine and even women with previous abnormal cervical cytology or genital warts can also receive the HPV vaccine although should be counseled that the vaccine may be less effective. Data available regarding vaccination of women older than 26 years and males is currently insufficient to make recommendations.

The Role of Bacteria in Extremely Low Gestational Age Neonates: Studies of Preterm Birth and Fetal Inflammation

A.B. Onderdonk

Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA

The ELGAN study (extremely low gestational age neonates) evaluated the chorionic parenchyma of babies born before the 28th week of gestation using placenta histology and quantitative microbiologic methods. In addition, blood samples were obtained from neonates starting one day post delivery for evaluation of cytokines and chemokines. Specimens were obtained from over 1,500 neonates delivered for a variety of reasons, including preterm rupture of membranes, preterm labor and preeclampsia. All data, including fetal ultrasound and behavioral assessments for the first two years of life were housed in a central data repository. Investigators providing primary data (microbiology, histology etc) were blinded with respect to other parameters and subject demographic information until all data had been deposited.

Evaluation of the microbiologic data indicates that colonization of the chorionic parenchyma is common during the second trimester and that colonization rates decrease with increasing fetal age. When stratified by method of delivery, both those fetuses delivered vaginally (V) and those delivered by caesarian section (CS) showed the same colonization trends. When the data was stratified by reason for delivery, it was noted that fetuses delivered because of preterm labor or preterm rupture of membranes had higher colonization rates than fetuses delivered for preeclampsia, a purely maternal indication. These data strongly suggest that the isolation of bacteria from the chorionic parenchyma is not due to contamination of the placenta at the time of delivery. Organisms isolated from chorionic parenchyma included species commonly found as part of the vaginal and skin microflora as well as organisms associated with bacterial vaginosis. Histological evidence of fetal inflammation was associated with colonization of the chorionic parenchyma with specific microorganisms. The pro-inflammatory cytokine response on the first postnatal day also indicates that there are organism specific neonatal inflammatory response profiles. Evidence of fetal inflammation and colonization of the chorionic parenchyma correlate with white matter damage as demonstrated by ultrasound and by follow up postnatal assessments. The data support the hypothesis that the placenta is not a sterile organ during the second trimester and that bacteria may provoke a fetal inflammatory response including echo lucent lesions in the brain. The importance of these findings will be discussed.

Role of anaerobes in transplant recipients

P. Mastrantonio

Department of Infectious, Parasitic and Immune-mediated Diseases, Istituto Superiore di Sanita, Rome, Italy, e-mailaddress: paola.mastrantonio@iss.it
tel.: +39 0649902335

Infection remains a common complication following transplants. Documented overall rate of infectious complications in adults ranges between 5% and 60% with a significant infection related mortality. Bacterial infections remain a potent source of morbidity and mortality during the immediate post operative period and in addition to nosocomial infections also surgical site and opportunistic infections must be considered. Bloodstream infections (BSIs) in particular are common in solid-organ transplant recipients but also pneumonia and urinary tract infections are frequently reported.The causing bacteria more frequently detected are aerobic gram positive cocci, gram negative rods and non fermentative bacilli,as expected, but also anaerobes are isolated and probably under detected. The few reports often underline the detection of unusual anaerobic bacteria that can be common saprophytes, and represent a challenge for microbiologists since the difficulties to be recognised by conventional techniques.As an example, in our experience, a case of massive pleural effusion caused by Eubacterium plautii in a kidney transplant recipient was reported. The difficulties in isolating anaerobic bacteria can influence the prompt initiation of adequate antibiotic treatments leading to a higher rate of mortality. Last but not least, Clostridium difficile-associated diarrhea has been described in all types of solid organ and stem cell transplantation. Rapid detection of this pathogen is essential in order to initiate timely treatment, since diarrhea during the early post-transplant period can cause severe secondary complications such as toxic megacolon.

Selective decontamination by antimicrobials during long term treatment / Perspectives for saving host indigenous microbiota

M.B. Romond

University of Lille Nord de France, 59000 Lille, France

Short bowel syndrome (SBS) occurs in patients fed by total parenteral nutrition (TPN) following massive intestinal resection. TPN weaning is usually associated with occlusion or sepsis although antibiotherapy is used for intestinal decontamination. In the present study modification to the intestinal biotope was investigated in infants and children (n=14) with recurrent symptoms of sepsis or occlusion during enteral feeds introduction. They were all treated by aminosides for a long-term period. Cause of massive resection was: necrotizing enterocolitis (n=3), Hirschprung disease (n=2), laparoschisis (n=1), atresia (n=1), chronic subocclusion (n=3), visceral myopathy (n=1), necrosis with acute intestinal invagination (n=1), cystic fibrosis (n=2). Effluents from stomies and/or rectal samples were collected before and during enteral feeds introduction. Bifidobacteria, *Bacteroides*, clostridia, enterobacteria, lactobacilli and enterococci were enumerated. Bifidobacteria were commonly isolated. Introduction of enteral feeds induced usually transient reduction to bifidobacteria. A few patients developed occlusion (3) or sepsis (3) during the survey. A drastic drop of bifidobacteria was observed prior occlusion. It was associated with detection of clostridial spores. Sepsis was preceded by detection of *C.perfringens* vegetative forms and followed by a drop in bifidobacteria. Attempt to enhance bifidobacteria with human milk or live bifidobacteria failed to prevent sepsis.

In conclusion, clostridia likely participate in the development of symptoms during enteral feeds introduction. Supplementation with bifidobacterial compounds that were shown to reduce clostridia in human volunteers could be of interest in children with recurrent symptoms.

Antibiotic resistance problem in intensive care units

M. Doganay

Department of Infectious Diseases, Faculty of Medicine, Erciyes University, Kayseri, Turkey,
e-mail: mdoganay@erciyes.edu.tr, tel.: +905333682042

Hospital-acquired infections (HAIs) have become worldwide problem. An infection develops in the 5-15% of the patients during hospital stay. Critically ill patients in intensive care units(ICUs) are 5-10 times more likely to hospital-acquired infection than those in non-critical ward. HAIs are increasing in prevalence due to ageing populations, more immunocompromised patients and greater use of invasive interventions. Patients in the ICU are commonly exposed to broad-spectrum antimicrobial agents and the ICU presents ample opportunities for the cross transmission of resistant bacteria from patient to patient. Antibiotic resistance has reached a significant rate in many ICUs in many hospitals around the world. Many studies showed high rates resistant pathogens, including methicillin-resistant Staphylococcus aureus (MRSA), methicillin-resistant coagulase negative staphylococci, vancomycin-resistant enterococci (VRE), ceftazidime-resistant Pseudomonas aeruginosa and extended-spectrum beta-lactamase producing Enterobacteriacea. Currently, multidrug resistant (MDR) Acinetobacter baumannii and Pseudomonas aeruginosa are concern of importance in ICUs of tertiary care hospitals. The patients in some ICUs are dying from lack of availability of any antibiotic active against certain strains of Acinetobacter baumannii and Pseudomonas aeruginosa. Infections due to resistant microorganisms lead to extended length of stay, higher costs and greater morbidity and mortality in hospital setting. To control the spread of MDR bacterial infections in ICU, an infection control program consisting a good surveillance system, and early warning systems is essential. In the near future, new agents for the treatment of MDR infections in development is not being expected, for this reason, antibiotic use in ICUs must be more rationalized.

Climate changes, environment and infection

E. Charvalos and C. Bezirtzoglou

Technological Institute of Athens, Department of Microbiology, Athens, Greece and European Committee, Brussels, Belgium. e-mail : gasson@aegean.gr

Climate change is a current global concern and, despite continuing controversy about the extent and importance of causes and of its effects, it seems likely that it will affect the incidence and prevalence of both residual and imported infections in Europe. Climate affects mainly the range of infectious diseases, whereas weather affects the timing and intensity of outbreaks. Climate change scenarios include a change distribution of infectious diseases with warming and changes in outbreaks associated with weather extremes. The largest health impact from climate change for Europe doesn't come from vector borne infectious diseases. This does not mean that these types of health impacts will not arise in Europe. The ranges of several vector-borne diseases or their vectors are already changing in altitude due to warming. In addition, more intense weather events create conditions conductive to outbreaks of infectious diseases: Heavy rains leave insect breeding sites, drive rodents from burrows, and contaminate clean water systems. The incidence of mosquito-borne parasitic and viral diseases, are among those diseases most sensitive to climate. Climate change affect disease transmission by shifting the vector's geographic range and by shortening the pathogen incubation period. Climate-related increases in temperature in sea surface and level would lead to higher incidence of water-borne infectious and toxin-related illnesses, such as cholera and seafood intoxication. Climate changes all around the world with impact in Europe are demonstrated by the fact that recent cases of cholera have been imported to Europe from Kenya, where spreading epidemic has been linked to the El Niño phenomenon, originated from the Pacific Ocean. Human migration and damage to health infrastructures from aberrant climate changes could indirectly contribute to disease transmission. Human susceptibility to infections might be further compounded by alterations in the human immune system caused by increased exposure to ultraviolet radiation and malnutrition due to alterations in agricultural products. Different kind of incidents in Europe with extreme weather events demonstrated effects on public health. The recent outbreak of the insect-borne Chikungunya virus in Italy in 2007 is an example of the kind of new health threat that the EU must be vigilant to confront. In addition, health effects of flooding, have been related to an excess cases of leptospirosis and campylobacter enteritis. Such examples have been demonstrated reported after flooding in the Czech Republic. Similarly, an increase of cryptosporidiosis in the United Kingdom has been related to flooding. Changing vector distributions associated with tickborne encephalitis and malaria have also been dempostrated in EU. A recently reported case of malaria in Italy in June 2008, suspected to be indigenously acquired, has shown how easily malaria could be reintroduced into several countries in the region. Another case of malaria in Greece in May 2010 affecting a young man living in a forestry region was claimed at KEELPNO-the Greek Center for disease control. Would this latest case be considered closely related to the one from Italy? If yes, then Public Health Services should elaborate plans to affront possible tick-borne diseases. Heatwaves

are important causes of mortality on mortality are important. The deaths seen in France in 2003 from a heat wave are projected to be repeated, as heat waves become more severe. However, heatwaves impacts on the trnasmission and severity of infectious diseases have not been elucidated. Finally scientific challenges include the elucudation of climate changes and extreme weather condition impact on infection transmission and outcome, human immune system changes and infection response, outbreak scenarios, animal and plant health and public health preparedness. European action plans to affront climate changes related health and infection problems are developed by the EU Commission at different levels and jointly by different DGs. In a few words within the EU the following points on human, animal and plant health are considered a priority: • Strengthening cooperation between the services of these three branches of health (human, animals, plants); • Developing action plans in the event of extreme weather conditions, in order to be better prepared and to react in the best way; • Gathering more reliable information on the risks of climate change whilst maintaining international cooperation, in particular with the WHO, as cooperation beyond that between Member States will be required to be more effective; • Providing additional effort to identify the most effective measures; • Improving the surveillance and the control of the animal diseases. The European Commission has decided to consider climate change, and the consequences it has on health, with greater importance whilst being aware that it is at the root of numerous diseases.

Microbial Ecology Perspectives in Molecular Biology

Invited Lectures

Catching the intestinal microbiota by FISH

G.W. Welling

Department of Medical Microbiology, University Medical Center Groningen, Groningen, The Netherlands

e-mail address: g.w.welling@med.umcg.nl

The first microscopic view of bacteria in 1677 undoubtedly was an exciting event. Morphological differences between bacteria were visible. It took more than 300 years before it was possible to identify bacteria in a microscopic view based on their genetic make-up. Fluorescent oligonucleotide probes targeted at 16S ribosomal RNA of a bacterial family, group, genus, or species were developed. With these probes quantitative determination of the bacterial composition could be achieved. Our first probe in 1995 was targeted at the genus Bifidobacterium.

It soon became clear that the methodology, fluorescent in situ hybridization (FISH) had a large potential to get an accurate insight in the bacterial composition of faecal samples in health in disease. Furthermore it could be applied to determine the effect of modulating substances i.e. pre- pro- and antibiotics. More 16S rRNA probes were developed and the FISH-technique was automated in 1999.

Other methods to study the composition of the intestinal microbiota became available e.g. qPCR, DGGE, HIT-chip analysis and deep-sequencing. These methods do have their advantages e.g. lower detection limit compared with FISH. However none of them is as quantitative as an in situ method with which the bacteria can be seen microscopically and counted.

FISH was and is being applied in several studies involving newborn infants, elderly, HIV-patients, patients with severe pancreatitis, intensive care patients selectively decontaminated with antibiotics and diabetes type 1 patients. The results show that it is relatively simple to catch the intestinal microbiota by FISH.

Metagenomics of the human intestine microbiota

M. Kleerebezem

TI Food and Nutrition, Wageningen University and NIZO food research; P.O. Box 20 6710BA, Ede, The Netherlands. Phone:+31-318659629, fax: +31-318650400,

e-mail: michiel.kleerebezem@nizo.nl

The human gastrointestinal tract harbors a complex community of microbes, which plays a prominent role in human health. Studies of the human intestine microbiota mostly focus on the fecal community. Recent metagenomics efforts have generated the first genetic catalogue of genes of the fecal microbiota, which is complemented with an increasing number of complete genome sequences of representative intestinal isolates. These efforts start to create an inventory of the function-repertoire of the human intestine microbiota that can be used for microbiota function-modeling as a function of health and disease, which can support the rational design of dietary interventions aimed to improve consumer's health via modulation of the intestine microbiota.

Despite these advances, we should realize that the microbiota composition varies between different locations in the GI tract and most studies have been targeting the large intestine (fecal) microbiota. Our knowledge is especially limited when it comes to the small intestine microbiota, which is a consequence of its limited accessibility. At the same time, it is clear that the small intestine is the site where initial interactions between food and intestinal microbes take place, while also important host immune functions are coordinated by small intestinal interactions with the residing microbiota. Therefore, our studies have aimed to deduce an ecological model of the microbiota composition and function in the small intestine using ileostoma patients (individuals where the colon has been removed by surgery) as well as healthy individuals. Phylogenetic analysis of microbial composition was combined with metagenomic determination of the community's function-composition and its stability, while metatranscriptome analyses generated an expression profile of the residing microbes. The combinatorial interpretation of these data, allowed the reconstruction of the human small intestinal microbial community and its functional repertoire and activity, which was shown to display fluctuations over time that correlated with the short chain fatty acid profiles determined in parallel.

This presentation will present recent advances in (functional) metagenomics of the human intestinal tract microbiota, with a special focus on the microbial communities residing in the small intestine.

Molecular languages of synbiotic (probiotic) microorganisms

B.A. Shenderov

Gabrichevsky Moscow Research Institute of Epidemiology and Microbiology, Russia e-mail: shenderof@yandex.ru**,** tel.: +(495) 474 9857 - home; +(495) 7080231-work

Symbiotic gut microorganisms release of various soluble low molecular weight (LMW) molecules of different chemical nature (surface and exogenous proteins, nucleases, serpins, sirtuines, other enzymes, lectins, peptides, amines, bacteriocines, fatty and amino acids, lactones, furanons, miRNA, NO, etc). These LMW molecules are able to sense environment, interact with corresponding cell surface, membrane, cytoplasm and nucleic acid receptors, to reply quickly and coordinately by induction of special sets of genes, to support stability of host genome and microbiome, to modulate epigenomic regulation of gene phenotypic expression, to ensure the information exchange in numerous bacterial and bacteria-host systems playing an important role in the control for many genetic and physiological functions, biochemical and behaviour reactions, in supporting host health in general. Various symbiotic (probiotic) strains produce different spectrum of such LMW molecules. There is chemical and functional similarity between LMW molecules synthesized by host eukaryotic cells, indigenous and probiotic microorganisms and some micronutrients. It means many LMW compounds of different origin may be the universal regulators contributing to the transmission of information, quorum – sensing effects, metagenome stability and epigenomic control for cell growth and development as well as phenotypic expression of different genes. Knowledge accumulated concerning molecular languages of symbiotic microorganisms allows us to better understand the mode of action of known probiotics and to design in principle novel probiotics (metabiotics) with increased health effectiveness. Now we are only at the beginning of a new era of molecular personal biotherapy and nutrition. Soon we can successfully manipulate both the host and its microbiota through interfering in their cross-talk, stability and epigenomic regulation of expression of genes using various types of eukaryotic, prokaryotic and nutrition origin LMW molecules are capable to modulate genetic, metabolic and physiological activities.

Clinical Microbial Ecology
Oral Presentations

Identification and Analysis of the *Shewanella oneidensis* Major hemN Gene Encoding the Oxygen-Independent Coproporphyrinogen III oxidase

S. Al-Sheboul

Faculty of Medical Sciences/ Department of Laboratory Sciences Jordan University of Science and Technology, e-mail: sashboul@just.edu.jo

Shewanella oneidenesis is a facultative anaerobe that can use a large number of electron acceptors including metal oxides. During anaerobic respiration, S. oneidensis synthesizes a large number of c cytochromes, which give the organism its characteristic orange color. Using a modified mariner transposon a number of S. oneidensis mutants deficient in anaerobic respiration were generated. One mutant, BG163, exhibited reduced pigmentation and was deficient in c cytochromes normally synthesized under anaerobic condition. The deficiencies in BG163 were due to insertional inactivation of HemN1, which exhibits a high degree of similarity to genes encoding anaerobic coproporphyrinogen III oxidases which are involved in heme biosynthesis. The ability of BG163 to synthesize c cytochromes under anaerobic conditions and to grow anaerobically with different electron acceptors was restored by the introduction of hemN1 on a plasmid. Complementation of the mutant was also achieved by the addition of hemin to the growth medium. The genome sequence of S. oneidensis contains three putative anaerobic coproporphyrinogen III oxidase genes. The protein encoded by HemN1 appears to be the major enzyme that is involved in anaerobic heme synthesis of S. oneidensis. The other two putative anaerobic coproporphyrinogen III oxidase genes may play a minor role in this process.

Lactose and Human milk oligosaccharides as modulators of intestinal microbiota and bacterial translocation

C.Mielcarek[1] and M.B. Romond[2]

[1] EBI, Cergy-Pontoise, France

[2] Université Lille Nord de France, Lille, France

Massive resection of the small intestine in infants requires feeding by parenteral nutrition. Introduction of enteral feeds-even human milk- frequently leads to chronic bowel obstruction and/or sepsis. Clostridia and more specifically *C. perfringens*, are suspected to participate in the physiopathology. To investigate the effect of lactose and human milk neutral oligosaccharides (HMNOs) on clostridia, germfree mice were inoculated with enterotoxigenic *C.perfringens* strain isolated from a patient with NEC, or with *C.clostridioforme* group containing human flora (HF). To take into account the poor lactase activity in adult mice, different doses of lactose were administrated during 2w. At concentration similar to human milk, lactose (70g/L) and HMNOs (7g/L) induced mortality within one week in *C.perfringens* monoassociated mice although they transiently reduced the intestinal bacterial carriage. At 7 g lactose /L, no toxic effect was observed. In HF mice, no mortality was observed. But increase in clostridia was observed in median ileum of 7gLactose/L drinking mice ($p=0.017$). And prevalence in clostridia increased in caecum of 70gLactose/L($p <0,05$) and HMNOs ($p<0.025$) drinking mice. Bifidobacteria were as well increased from distal ileum to colon in 70gLactose/L drinking mice whereas they decreased in the caecum of mice drinking lower lactose concentrations. Bacteraemia was more frequent in 70gLactose/L mice ($p<0.02$). When lower doses of lactose were administrated bifidobacterial translocation was observed.

In conclusion, oligosaccharides from human milk can favour clostridial population and stimulate enterotoxin translation in *C.perfringens* when they reached the lower intestine at sufficient a concentration. And in infant with massive intestinal resection, the shortness of the intestine does not allow for complete breakdown of lactose. Thus formula deprived in lactose would be more suitable for introduction of enteral feeds in infants fed by parenteral nutrition.

Metagenomics of the small intestinal microbiota

M.M. Leimena[1,2] E.G. Zoetendal[1,2], E.J. Smid[1,3] and M. Kleerebezem[1,2,3]

[1]Top Institute Food Nutrition (TIFN), P.O. Box 557, 6700 AN, Wageningen; [2]Laboratory of Microbiology, Wageningen University and Research Centre, Dreijenplein 10, 6703 HB, Wageningen, Netherlands;
[3]NIZO Food Research B.V., P.O. Box 20, 6710 BA, Ede milkha.leimena@wur.nl**,** tel.: +31317480062

The ecology of microbes in the human small intestine is of particular interest since this is the first site in which (digested) food components, hosts and microbes interact. However, knowledge of those genes functions in the small intestinal track is limited. To gain insights into the genetic potential of a community, a metagenomics approach can be used as it provides a catalogue of potential functions of the microbiota. In this study, metagenomics-metatranscriptomics approach was used to identify the in situ expressed pathways of the small intestine microbiota. A fosmid library containing random microbial DNA fragments of approximately 30kb and a random cDNA library were constructed from ileostomy effluent of one individual. Screening for in situ expressed genes in the library was done using polymerase chain reaction (PCR) strategy and a computational approach by aligning sequences of random cDNA library that encode functional properties to the end reads sequences of ileal metagenome fosmid library. Sequence analysis of 73 inserts containing in situ expressed genes revealed the abundance of genes with highest similarity to Streptococcus species. Based on Cluster of Orthologous Genes (COGs) classification of these genes, 36% belonged to metabolism cluster, with the largest part to carbohydrate transport and metabolism. Two fosmid inserts screened by PCR strategy contained three and two different in situ expressed genes, respectively. These inserts contained pathways belonging to the ascorbate-aldarate and galactose metabolism. Details of the screening strategies and of the results will be presented in the meeting.

A *B. lactis* fermented milk product selectively alters the gut microbiota and ameliorates colitis

P.Veiga[1,2,], C. A. Gallini[1], C. Béal[2], M.L. Delaney[6], I. Chambaud[1], A. DuBois[6], J. Knol[2,4], J.Van Hycklama Vlieg[2,4], A. Khlebnikov[2,3], J. N. Glickman[5], A. B. Onderdonk[5,6], and L. H. Glimcher [4,5] and W. S. Garrett[1,5]

[1]Harvard School of Public Health
[2]Danone Research, R.D. 128, 91767 Palaisceau Cedex, France
[3]The Dannon Company Inc., White Plains, 10603 NY, USA
[4]Danone Research, Centre for Specialized Nutrition, 6700 CA Wageningen, The Netherlands
[5]Harvard Medical School, Boston, MA, USA
[6]Brigham and Women's Hospital,e-mail: wgarrett@hsph.harvard.edu, tel.: 617-512-2041

The gut microbiota is essential for the development of ulcerative colitis (UC) which spontaneously occurs in T-bet-/- Rag2-/- deficient (TRUC) mice. In this model of gut inflammation, microbial modulation in response to antibiotic therapy leads to improvement of UC scores. This raises the possibility that other means of gut microbiota manipulation, in particular food interventions, may also impact the inflammation in this model. The aim of this study was to determine the microbial structure of the gut microbiota of TRUC mice and to test the impact of foods containing living bacteria on chronic intestinal inflammation. The effects of the nutritional intervention were assessed by i) colon histology and ii) gut microbiota quantitative analysis. Mapping the gut microbiota by real time qPCR analysis of stool samples from TRUC and Rag2-/- (control) mice revealed significant differences in bacterial populations, including a dramatic reduction of Bifidobacteria in TRUC. To test whether a fermented milk containing live Bifidobacteria, would improve colitis in this model, TRUC mice consumed either a fermented-milk containing Bifidobacterium animalis subsp lactis DN -173010 or a control product (non-fermented acidified milk) over a 4 week period. Interestingly, the consumption of the test product showed a significant decrease in gut inflammation as demonstrated by histology and indeed coincided with alterations in the gut microbiota. The results show that consumption of fermented milk containing B. lactis DN -173010 cures UC, potentially by direct immune effects or by creating unfavorable conditions for putative pathogenic bacteria that may be implicated in disease progression in this animal model.

Diversity and metabolic impact of intestinal *Lactobacillus sp*. in healthy adults and the elderly

J. Štšepetova[1], E. Sepp[1], H. Kolk[2], K. Lõivukene[3], E. Songisepp[4], M. Mikelsaar[1]

[1]Department of Microbiology, Medical Faculty, University of Tartu, Ravila 19, Tartu 50411, Estonia
[2]Departments of Traumatology and Orthopedics, University of Tartu, Tartu, Estonia
[3]Laboratory of Microbiology, Tartu University Hospital 4 Bio-Competence Center of Healthy Dairy Production Ltd., Tartu, Estonia
e-mail: jelena.stsepetova@ut.ee, tel.: +3727374174

The aim of the study was to assess the counts and species distribution of intestinal lactobacilli and exploring if the data of intestinal lactobacilli are associated with body mass index and blood glucose level in healthy adults and elderly persons. The BMI ($p<0.01$) and the level of fasting blood glucose ($p<0.001$) was significantly higher in the elderly. In the elderly the total counts of lactobacilli were significantly higher than in adults ($p<0.01$ by bacteriology; $p<0.001$ by qRT-PCR). The number of species in adults was lower than in elderly ($p<0.05$). Adults were more often colonized with L. acidophilus ($p=0.031$) and L. helveticus ($p<0.001$). In contrast, L. plantarum ($p=0.035$), L. paracasei ($p<0.001$) and L. reuteri ($p=0.031$) were more prevalent in the elderly. L. rhamnosus was detected in adults ($p<0.001$), but not in any elderly person. A positive correlation between BMI and fasting blood glucose ($r=0.463$; $p<0.0001$) adjusted for gender was found. The BMI was associated with counts of lactobacilli adjusted for age and gender ($r2=0.187$; Adj $r2=0.144$; $p=0.008$). The higher BMI in both groups of persons was directly predicted by presence of obligately homofermentative lactobacilli and L. sakei, both adjusted for age and gender. The plasma glucose values were in negative correlation with colonization with L. paracasei ($r= - 0.460$; $p=0.0238$) in adults and on borderline with L. fermentum ($r= - 0.321$; $p=0.052$) in the elderly. Thus, the species-specific PCR analysis of Lactobacillus sp. combined with viable plating data are indicating substantial age-related structural differences in the lactobacilli community, reflected in host glucose metabolism and body weight.

Intestinal Lactoflora in Patients with Antibiotic Associated Diarrhea (AAD)

P. Naaber [1,2] , J. Stsepetova [1], I. Smidt [1], E. Shkut [1], M. Rätsep[1], S.Kõljalg[1], K. Lõivukene[1], R. Mändar [1], I.H. Löhr [2], O.B.Natås[2], E. Sepp[1]

[1] University of Tartu
[2] Stavanger University Hospitals, e-mail:paul.naaber@kliinikum.ee

To evaluate the qualitative and quantitative composition of intestinal lactobacilli in Clostridium difficile (CD) positive and negative AAD patients from Estonia and Norway. Faecal samples of AAD patients were collected from Norway (n=42) and Estonia (n=37). Quantitative cultures and real-time PCR for CD and lactobacilli (LB) were performed. LB were identified with 16S RNA sequencing. Results: The higher counts of LB in Norwegians as compared to Estonians were found (median 4.14 log10 CFU/g vs. 0, p=0.018). However, the prevalence of LB was not significantly different (64% vs. 46%). Comparing CD positive and negative samples no differences were found in total LB prevalence or counts. Totally 9 different LB species were detected. The species composition of lactobacilli differed in the two groups of AAD patients: L. gasseri was more common in Estonians (19% vs. 2%, p=0.023) and L. plantarum in Norwegians (21% vs. 5%; p=0.05). Comparing CD positive and negative AAD patients L. plantarum was found more frequently in CD negative patients than positive ones (33% vs 9%, p=0.03). Similar trend was in case of L. gasseri (20% vs. 8%) but not significant (p=0.17). Significant differences in the quantitative and species composition of lactoflora were found between Estonian and Norwegian AAD patients. Whereas total count of lactobacilli seems not to predict the protection against CD infection, some particular species may have differential role in colonization resistance against CD both in Estonian and Norwegian AAD patients.

Safety and immunomodulatory properties of Enterococcus faecium IS-27526 in Indonesian young children

I. S. Surono

SEAMEO TROPMED RCCN University of Indonesia Salemba Raya 6, Jakarta 10430 Indonesia, tel.: +628129247889, e-mail: gridsw@yahoo.com

Enterococcus faecium IS-27526 is one of novel probiotics isolated from dadih, a yogurt-like product of Indonesian traditional fermented milk from West Sumatera Indonesia. In vitro study on adhesion properties and antibiotic resistance, in vivo and three human studies had been conducted especially to apparently healthy Indonesian children younger than five, randomised double blind placebo controlled trials, community based trials in five Public Health Centers. The results confirmed safety of novel indigenous probiotic Enterococcus faecium IS-27526. There was no vancomycin resistance, and safety was confirmed even to immuno-compromised undernourished young children. Significant immunomodulatory properties especially humoral immune response of fecal sIgA in undernourished young children supplemented with Enterococcus faecium IS-27526 at 108 cfu/day for 90 days in the form of freeze dried powder or probiotic cream also had been validated. Significant effect on increasing bodyweight of undernourished children was also observed after 90 days supplementation as compared to control group. Key words : novel probiotics, Enterococcus faecium Is-27526, dadih, safety, immune response

Effect of banana consumption on faecal microbiota: a double-blind, randomized, controlled trial

E.K. Mitsou[1], E. Kougia [2], Tz. Nomikos[1], M. Yannakoulia [1], K. Mountzouris [3], A. Kyriacou [1]

[1]Department of Dietetics and Nutritional Science, 70 El. Venizelou str., 17671 Kallithea, Harokopio University, Kallithea, Greece
[2]Inter-university Postgraduate Study Programme (PSP) in 'Food Science and Nutrition', University of Ioannina and TEI of Athens, Greece
[3] Department of Nutritional Physiology and Feeding, Agricultural University of Athens, Athens, Greece *Corresponding author: Dr. Adamantini Kyriacou Dept. of Dietetics and Nutritional Science, Harokopio University, 70, El Venizelou str., 17671, Kallithea, Greece Tel. +30210 9549301, FAX : +30210 9577050 e-mail: mkyriacou@hua.gr

Banana is a widely consumed fruit, which contains considerable amounts of potential prebiotic dietary fibers and resistant starch. In our double-blind, randomized, controlled trial we aimed to evaluate the in vivo prebiotic effect of banana consumption on faecal microbiota. Thirty-four healthy women participated in the study, having BMI ≥24 kg/m2, age 20-45 years, without history of gastrointestinal disease and no antibiotic and other medication use two months prior the initiation and during the study. All women were asked to maintain their usual dietary habits for 60 days and they were randomly assigned to consume twice a day a pre-meal snack, either one medium banana, or one cup of banana-flavoured drink or one cup of water (control group). Stool samples were collected at baseline, on days 30 and 60 of intervention for enumeration of total anaerobes, bifidobacteria and lactobacilli by plate count techniques, as well as for SCFA measurement. Gastrointestinal symptoms were also recorded. Mean bifidobacterial levels were increased only in the banana group both at 30 and 60 days of intervention, but this change did not reach a statistical significance. Analysis of the gastrointestinal symptoms records revealed significantly lower distension levels in the banana group, compared to controls, at 26-35 days (p=0.037) and 51-60 days (p=0.033). A trend for greater number of evacuations in the banana group, compared to control, was also detected at 26-35 days (p=0.080). We concluded that daily consumption of bananas is a well-tolerated eating behaviour, which may induce bifidogenesis in healthy women experiencing body weight problems.

Impact of beta-glucan on the faecal microbiota of polypectomized patients: a pilot study

K. Turunen[1], E. Tsouvelakidou[2], Tz. Nomikos[1], K. Mountzouris[3], A. Kyriacou[1]

[1] Department of Dietetics and Nutritional Science, 70 El. Venizelou str. 17671 Kallithea, Harokopio University, Kallithea, Greece
[2] Inter-university Postgraduate Study Programme (PSP) in 'Food Science and Nutrition', University of Ioannina and TEI of Athens, Greece
[3] Department of Nutritional Physiology and Feeding, Agricultural University of Athens, Athens, Greece *Corresponding author: Dr. Adamantini Kyriacou Dep. of Dietetics and Nutritional Science, Harokopio University, 70, El Venizelou str., 17671, Kallithea, Greece Tel. +30210 9549301, FAX : +30210 9577050 e-mail: mkyriacou@hua.gr

Beta-glucans are polysaccharides present in the cell walls of higher plants, in the seeds of some cereals (barley, oat), and they are also produced by certain yeasts and fungi. It is suggested that they exhibit, among many other health benefits, protective effects against carcinogenesis in the colon, but there is not enough human data to support this. The aim of the study was to determine the effect of barley-derived beta-glucan in the gut microbiota of polypectomized patients. Subjects were randomly assigned to consume 125g of bread per day with beta-glucan (3g/d), or without (placebo group), for 3 months. Thirty polypectomized men and women (mean age 57.6 years) were recruited into the study, but only 14 have completed. Subjects did not consume any probiotics or antibiotics two months prior the intervention, or during the study. Stool samples were collected at baseline, on days 30 and 90 of intervention, as well as 15 days after the intervention, for enumeration of total aerobes and anaerobes, coliforms, E. coli, enterococci, Bacteroides spp., Clostridia spp., bifidobacteria, lactobacilli and Candida spp. Faecal bacterial enzyme activity (beta-glucuronidase and beta–glucosidase), pH, stool dry weight and the concentration of volatile fatty acids in the faeces were measured. Gastrointestinal symptoms were also recorded. Mean levels of Candida spp. were decreased at 90 days of intervention in the beta-glucan group, but this change did not reach a statistical significance (p=0.061). The analysis of the other microbial counts revealed no significant differences between the beta-glucan and the placebo group.

In vitro fermentation profiles of the lean and obese faecal microbiota

S. Kolida[1], S. Sarbini[1], A.W.C. Einerhand[2], R. A. Rastall[1]

[1] University of Reading, Food and Nutritional Sciences, PO Box 226, Whiteknights, Reading RG6 6AP, UK, e-mail: s.kolida@reading.ac.uk, tel.: 0044 (0) 118 378 6218
[2] Tate & Lyle Innovation Centre, Avenue de l'Horizon, F-59650 Villeneuve d'Ascq, France

Recent studies have suggested a link between obesity and the composition of a gut microbiota with an increased capacity for salvaging energy from nutrients. Here, we have investigated the in vitro fermentation profiles of four lean and four obese individuals in 48h anaerobic, pH controlled batch culture experiments. The responses of the lean and obese faecal flora during 48 hour fermentation of resistant starch (RS75) and soluble gluco fibre (SGF) were evaluated by monitoring bacterial population changes and short chain fatty acids (SCFA). The rate of gas production was also monitored in non pH controlled cultures as a further indicator of metabolic activity. Fluorescent in situ hybridisation revealed significant increases in bifidobacteria levels in both the lean and obese cultures in response to the selected test fibres. Total SCFA, acetate and propionate concentrations in the obese cultures were significantly lower with SGF ($P<0.001$) compared to lean, whilst for RS75 cultures, only acetate was significantly lower. The rates of gas profiles were similar between lean and obese donors as was the total gas. In conclusion, the obese and lean microbiota responded in a similar manner to growth on the test fibres and the obese flora did not appear to have a higher capacity for energy salvaging. In lean individuals, SGF appeared to be more efficiently fermented into SCFA than in the obese.

A new synbiotic mixture improves allergic symptoms, gut microbiota and gut health in infants with atopic dermatitis

K. Ben Amor [1], L. van der Aa [2], H. Heymans [2], W. van Aalderen[2], H. Sillevis Smitt[3], J. Knol [1], A. Nauta[1], L. Knippels[1], J. Garssen[1], A. Sprikkelman [2]

[1] Department of Paediatrics, Emma Children's Hospital, AMC, Amsterdam, e-mail: kaouther.benamor@danone.com, tel.: 0031631047661
[2] Danone Research Centre for Specialised Nutrition, Wageningen
[3] Department of Dermatology, AMC, Amsterdam, Netherlands

We investigated the effect of a specific synbiotic mixture on allergic symptoms, gut microbiota and gut health. Ninety infants with atopic dermatitis (AD), aged < 7 months, participated in a double-blind, placebo-controlled, randomised multi-centre trial receiving either an extensively hydrolysed formula with Bifidobacterium breve M-16V and scGOS/lcFOS (9:1), or the same formula without synbiotics during 12 weeks. Severity of AD was assessed with the SCORAD index every 4 weeks. Faecal microbiota, pH and short chain fatty acids were determined at baseline, weeks 1 and 12. After 1 year parents were asked about respiratory symptoms and asthma medication use of their child, using a validated questionnaire. Although no statistically significant difference in SCORAD score between the two groups was observed at any time point, a significant improvement was observed in the subgroup of infants with elevated IgE levels in the synbiotic group compared to the placebo group (12 wks: -18.1 versus -13.5 SCORAD points, P=0.04). Furthermore, the synbiotic group showed significantly higher proportion of bifidobacteria and lower percentage of clostridia related species when compared to the control group. The synbiotic group had significantly higher proportion of acetic acid and lower proportions of butyric, isobutyric and isovaleric acid; accompanied by significantly softer stool and less reported constipation and diaper dermatitis. At the one-year follow-up, infants that had received the synbiotic mixture had a lower prevalence of asthma-like symptoms and asthma medication use. This specific synbiotic mixture showed a beneficial effect on the severity of AD in infants with IgE-associated AD and successfully modulated the composition and the metabolic activity of their intestinal microbiota, potentially explaining the beneficial effects observed for constipation and diaper dermatitis. We observed a preventive effect on asthma-like symptoms, which may beneficially affect subsequent development of asthma.

The Effect of *Quericus castanifolia* extract on pathogenic enteric bacteria

N. Bahador

Islamic Azad University, Fars Science and Research Branch, Fars, Iran
e-mail: nimabahador@yahoo.com, tel.: +989173374812

The family of Enterobacteriacea is a major group of gram negative bacteria, Some of these microorganisms are pathogen and could cause disease mainly gastroenteritis. Recently, due to drug resistant nature of these bacteria specially in developing countries treatment of the patient considered as important investigate. Qurecus castanifolia is a native plant of Yasuj province in Iran, which the people who living in this area consume the same for treatment of enteric disease. The present study was conducted to evaluate the effect of Qurecus castanifolia extract on pathogenic enteric bacteria viz., E.coli, Salmonella typhi murium, Shigella dysenteriae and Yersinia enterocolitica. Antimicrobial susceptibility and minimal inhibitory concentration (MIC) of the extracts were assessed by gel diffusion method and modification of E-test respectively. All the experiments were performed in triplicate and the statistical analysis was carried out on the results. The results obtained from this study indicated that alcoholic extract was shown antimicrobial effect on the microorganisms tested. In addition, Shigella dysentriae was more sensitive with zone of inhibition 18 mm and MIC value was 2.5 X 10-4 whereas, E.coli was less sensitive with zone of inhibition 12 mm and MIC value 1 X 10 -2 , Salmonella typhimurium and Yersinia entrocolitica showed relatively intermediate susceptibility to the extract with zone of inhibition 14 mm and MIC value 5 X10-3 . Overall, Qurecus castanifolia may be considered for treatment of the patients suffering from enteric disease. Keywords: enteric bacteria, Qurecus castanifolia, antimicrobial susceptibility.

The inhibiting activity of *Lactobacillus spp*. isolated from human milk on gastrointestinal pathogenic bacteria of nosocomial origin

S. Jara[1], M. Sánchez[1], R. Vera[1], E. Castro[1,2]

[1] Laboratorio de Bacterias Lácticas. Facultad de Ingeniería Química, Universidad de Concepción, Concepción, Chile. Casilla 160-C. Correo 3.

[2] Departamento de Obstetricia y Puericultura, Facultad de Medicina, Universidad de Concepción, Concepción, Chile. Casilla 160-C. Correo 3. E-mail: ercastro@udec.cl, tel.: 56-41-2207289

Gastrointestinal diseases are one of the leading causes of death and the most vulnerable groups are young children and the elderly. The objective of this study was to evaluate the inhibiting capacity of Lactobacillus spp. (LAB) isolated from human milk on gastrointestinal pathogenic bacteria of nosocomial origin (NI). 58 LAB with high surface hydrophobicity rates were selected, from which 51.7% were obligated homofermentative and hydrogen peroxide synthesizers. Gastrointestinal pathogens obtained in the pediatric intensive care unit were Escherichia coli (Ec43), isolated from gastric juice, Shigella spp. (Sh21) and Pseudomonas spp., the last two isolated from rectal swabs. In a second stage, the interaction between four LAB and Shigella spp. (three strains), Salmonella enteritidis (three strains) and E. coli (three strains) was evaluated. In the first part of this study, 33 out of 58 strains (56.9%) showed inhibiting activity on evaluated pathogens, and nine strains inhibited the three tested NI bacteria. In the second part of the study, S. enteritidis was sensitive to inhibiting activity from the four selected lactic strains. A similar result was obtained with Shigella spp. A lactic strain (AR2) inhibited the nine NI bacteria. The antibacterial activity of the investigated LAB strains could have a potential probiotic action in young children presenting diverse cases of diarrhea. Therefore, the use of antibiotics could be reduced, as these favour bacterial resistance and cause other side effects, such as reducing the stability of gastrointestinal microbiota.

This work was financed by FONDEF project D04i1326.

Incidence of bacteriocin types among human commensal and pathogenic *E. coli* strains

D. Šmajs[1], L. Micenková[1], J. Šmarda[1], M. Vrba[2], A. Ševčíková[2]

[1] Department of Biology, Masaryk University, Faculty of Medicine, Brno, Czech Republic, email: dsmajs@med.muni.cz
[2] Department of Clinical Microbiology, Faculty Hospital Brno, Czech Republic

The incidence of bacteriocin types among human commensal and pathogenic E. coli strains was studied in several groups of clinical strains. The control group contained commensal strains (n = 411) isolated from patients without bacterial gut infection. Additional group of commensal strains isolated from patients with bacterial gastrointestinal infection comprised 237 strains. Pathogenic E. coli strains were isolated from urinary tract infections (n = 361) and from wound infections (n = 585). In the control group, 20 different bacteriocin types were found (out of 29 tested). In the group of strains from patients without bacterial gut infection, from urinary tract infections and from patients with bacterial gut infection, 16, 16 and 25 bacteriocin types were found, respectively. E. coli strains isolated from patients with gastrointestinal infections contained lower number of solely colicinogenic strains and the increased incidence of microcin H47 producers. Colicin E1 producers and multiproducer strains were more frequently found among strains isolated from urinary tract infection when compared to control strains ($p = 0.0001$ and $p = 0.008$, respectively). Microcins (especially H47 and V) were more frequently found among producers isolated from wound infections (59,2 % and 49,2 %, $p = 0.015$). E. coli strains of phylogenetic group B2 had increased incidence in strains isolated from urinary tract infection and in strains isolated from wounds. These results showed importance of some bacteriocin types as potential virulence factors (e.g. colicin E1) or pathogenicity markers (e.g. microcin V and H47). Pathogenic strains more often belong to the phylogenetic group B2.

Isolation and Ribotyping of *Clostridium difficile* in patient stool samples

E. Kalyani

3-330A BSB, 51 Newton Road, Iowa City, IA 52242, United States
e-mail: kolie-eko@uiowa.edu, tel.: 319-354-4704

Clostridium difficile infections range from mild diarrhea to life-threatening pseudomembranous colitis. Recent outbreaks of Clostridium difficile infections are characterized by increased severity, high relapse rates, and significant mortality in North America and Europe. These infections have been related to the emergence of new hypervirulent C. difficile strain characterized as PCR – ribotype 027. More recently, another hypervirulent C. difficile strain PCR – ribotype 078 has emerged. The distribution of PCR ribotypes varies among European and North American countries. There has recently been ongoing European Clostridium difficile infection surveillance in the European Union, including Slovakia. The current situation on the presence of hypervirulent strains of C. difficile in Slovakia is unknown. Presently in hospitals, a patient's clinical symptoms and toxin positive diarrheal stool are used to diagnose C. difficile infection. This poses the problem of false cytotoxicity test results, and no isolation and further characterization of C. difficile isolates are done (e.g. ribotype, antimicrobial resistance profile). To begin collecting this data on the C. difficile ribotypes found in Slovakia, fifty samples of C. difficile toxin positive patient stools obtained from the HPL Microbiological Laboratory will be tested. If the stool sample is found to contain C. difficile, the bacteria will be isolated and the PCR ribotyping method for C. difficile will be introduced and recovered isolates of C. difficile will be characterized. The profile of these isolates from Slovakia will be compared to C. difficile isolates from other countries in the European Union as well as the United States and Canada.

Identification of inhibitors of the *Salmonella enterica* serovar *Typhimurium fimZ* gene, which regulates adherence, invasion, and motility

E. Kalyani

3-330A BSB, 51 Newton Road, Iowa City, IA 52242, United States
e-mail: kolie-eko@uiowa.edu, tel.: 319-354-4704

Salmonella species continues to be a significant health concern worldwide with millions of reported and unreported cases of salmonellosis each year. Salmonella infections are contracted by ingestion of contaminated food or water. Organisms pass through the stomach and enter the small intestine where they attach to and invade cells of the intestinal mucosa. An important source of Salmonella infections are chickens because a large percentage of birds have been found to carry Salmonella species as part of their intestinal flora. While virtually nonpathogenic for poultry, the Salmonella species are pathogenic for humans which consume them, causing gastroenteritis that ranges from mild to severe or even life-threatening. A key virulence property of Salmonella species in chickens is their ability to adhere to and colonize the intestinal epithelium of birds which is mediated by type 1 fimbriae that are produced by the bacteria. Based upon this information, we are pursuing a project to identify small cyclic peptides that inhibit production of type 1 fimbriae. Specifically, we are attempting to identify inhibitors of the type 1 fimbrial activator, FimZ, which is required for the production of these attachment proteins. In the long-term, we hope to identify small cyclic inhibitory peptides that can be used as therapeutics to reduce or inhibit the carriage of Salmonella in chickens. The hypothesis of this research is that reduction or elimination of the carriage of Salmonella species from the intestines of poultry would significantly reduce human salmonellosis resulting from consumption of chicken.

Changing pattern of antibiotic susceptibility in intensive care units: ten years experience of a university hospital

E. Alp, B. Kiran, D. Altun, G. Kalin, R. Coskun, M. Sungur, A. Akin, D. Percin, M. Doganay.

Erciyes University, Medical Faculty, Department of Infectious Diseases,Turkey
e-mail : ealp@erciyes.edu.tr, tel.: +905363146430

The purpose was to assess microorganisms and antibiotic susceptibility patterns during ten years in intensive care units (ICUs) of an University Hospital. Infection Control Committee has active prospective survellance in ICUs for thirteen years. Ten years data of ICUs was evaluated retrospectively from surveillance forms. Microoganisms and their antibiotic resistance were recorded according to the years. During ten years, gram negative microorganisms were the most frequent isolated microorganisms from clinical specimens. Acinetobacter baumannii (21.8%), Pseudomonas aerigunosa (16%), Escherichia coli (10.4%) and Klebsiella pneumoniae (8%) were the most common gram negative microorganisms. However, Stahylococcus aureus was the most prevelant gram positive microorganism, the incidence decreased from 18.6% to 4.8% during ten years (table 1). Also antibiotic susceptibility of microorganisms changed during ten years. Carbapenem susceptibility decreased from 56% to 8% in A. baumannii and ciprofloxacin susceptibility decreased in E.coli from 72% to 40% and in K.pneumoniae from 79% to 45% during ten years. However, metisilin susceptibility increased in S.aureus from 4% to 46%. Antibiotic resistance is growing problem in ICUs. Rationale antibiotic policies and infection control measures will prevent the development of resistance.

Minimum Inhibitory Concentrations of ceftriaxone and cefixime against multidrug resistant (MDR) and nalidixic acid resistant (NAR) typhoidal Salmonellae isolated from a tertiary care hospital of Pakistan

F. Kaleem, J.Usman, A. Hassan, M. Omair, A. Khalid, R. Ud Din.

National University of Sciences and Technology, Department of Microbiology, Army Medical College, Abid Majeed Road, Rawalpindi, Pakistan, tel.: 0092-322-5087234, e-mail: fatima_kaleem@hotmail.com

The timely appropriate management of typhoid fever can considerably reduce both morbidity and mortality. Since late 1980s Salmonella typhi has developed resistance simultaneously to all the drugs used in first line treatment (chloramphenicol, cotrimoxazole and ampicillin/amoxicillin) and are known as multidrug resistant (MDR). Fluoroquinolones are widely regarded as the most effective drug for the treatment of typhoid fever. Unfortunately there are reports of treatment failure with fluoroquinolones and nalidixic acid resistance is a marker of reduced susceptibility to fluoroquinolones. Recently, azithromycin is being used as an alternative agent, but sporadic reports of resistance to these antibiotics are already being reported so we are left with little options and the third generation cephalosporins prove to be a promising alternative. This study was carried out in the Department of Microbiology, Army Medical College, National University of Sciences and Technology, Pakistan, to determine the minimum inhibitory concentrations of ceftriaxone and cefixime against MDR and NAR typhoidal Salmonellae from clinical isolates of a tertiary care hospital. All clinical samples were dealt by standard microbiological methods, isolated typhoidal Salmonellae were subjected to susceptibility testing against various antibiotics by disc diffusion method as per the Clinical and Laboratory Standards Institute (CLSI) guidelines. Minimum inhibitory concentration of ceftriaxone and cefixime against MDR and NAR isolates were determined by E-test. Minimum inhibitory concentrations 50 and minimum inhibitory concentrations 90 were calculated. Results: Among 100 MDR and NAR isolates all had MICs of ceftriaxone and cefixime in highly susceptible range as per CLSI guidelines. According to our study both ceftriaxone and cefixime prove to be effective agents against MDR and NAR typhoidal Salmonellae. Ceftriaxone can be most effective on an inpatient basis and cefixime proves to be most effective on an outpatient basis, because of drug cost, convenient administration and short duration of therapy.

Influence of sexual intercourse on genital tract microflora in infertile couples

N. Borovkova[1], P. Korrovits[1,2] , K. Ausmees[2], S. Türk[1], M. Punab[2] , R. Mändar[1]

[1] Department of Microbiology, Faculty of Medicine, University of Tartu, Ravila 19, Tartu 50411, Estonia
[2] Andrology Center, Tartu University Hospital, Tartu, Estonia
e-mail: reet.mandar@ut.ee, tel.: +372 7 374 178

Vaginal microflora has been found to affect fertility. Although male genital tract microflora has direct influence on the partner's microflora, there are very scarce studies concerning microflora of couples. Our aim was to clarify the influence of the sexual intercourse on partner's genital tract microflora in infertile couples. 17 infertile couples were studied, of them 5 men had inflammatory prostatitis (IP). Semen samples were collected during menstruation of the partner. Two self-collected vaginal samples were taken 3…5 days later before and after intercourse. Quantitative anaerobic, microaerophilic and aerobic cultures were performed. Sexually transmitted diseases were detected by PCR method. Bacterial vaginosis (BV) was detected by Gram stained slides using Nugent scoring. BV was found in one woman, intermediate microflora in 3 women and normal microflora in 9 women in both samples. In 4 women, normal microflora was found in the first sample but intermediate microflora in post-intercourse sample. After the intercourse, up to 4 new species (mean 1.7, median 2) could be detected in significant counts (>10 000 CFU/swab) in vaginal microflora. This tendency was more prominent in IP patients (2.4 vs 1,4 species). Ureaplasma parvum was found from 10 women (59%), in 80% of women whose partner had IP and from 50% of women whose partner had no IP. Half of their male partners (5 out of 10) had U. parvum. Sexual intercourse causes significant shifts in vaginal microflora that are more prominent in the partners of IP patients. U. parvum is a frequent pathogen in infertile couples.

Clinical Microbial Ecology

Poster Presentations

Prevalence and genetic characterization of PER type extended-spectrum beta-lactamases among nosocomial Acinetobacter isolates collected in an intensive care unit in Iran

M. Niakan, J. Dadgar Pakdel, D.Yadegari, I.Rasouli

Microbiology Department, Medical School, Centre for Infectious Diseases, University of Shaheed Beheshti, 31-Abdolazadeh,st.Keshavarz Blvd, Tehran, Iran, tel.: 0098 21 8894792, e-mail: niakan@shahed.ac.ir

We studied the prevalence and molecular epidemiology of multiresistance plasmid PER-1-type beta- lactamases among Acinetobacter genetic environment and distributing of antimicrobial resistance genes. In community- and hospital-acquired Acinetobacter baumannii Strains isolated over a 7-month period in eight university hospitals from distinct regions of Iran. Detected among clinically relevant Enterobacteriaceae isolates from routine cultures at the Academic Hospital .A total of 102, Acinetobacter, isolates were studied (82 from urine, 20from blood cultures), respectively.Susceptibility to carbapenems was evaluated with the MicroScan system. PER type beta- lactamases Acinetobacter were identified by antibiotic susceptibility testing and blaCTX-M multiplex PCR. Beta-Lactamases were identified by PCR.The presence of blaPER was determined by the colony PCR method. We detected PER type beta-lactamases in 46% (33/72) of Acinetobacter strains. PER type enzyme producers were highly resistant to ceftazidime and gentamicin, intermediately resistant to amikacin, and susceptible or moderately susceptible to imipenem and meropenem. Among PER type-beta-lactamase-positive isolates, five Acinetobacter isolates. Our data suggest that the emergence of CTX-M-1-producing E. coli in western Austria may be attributed to multiple independent events. PER-1-type beta-lactamases appear to be restricted to Iran. However, their clonally diversity and high prevalence indicate a high spreading potential.

Keywords: Acinetobacter, PER type beta-lactamases, Prevalence, genetic characterization

Comparison between rose bengal plate test (RBPT), tube agglutination test (TAT) and 2 mercaptoethanol (2- ME) agglutination test for detection of Brucella antibodies during an outbreak of human brucellosis in Neyshabour, Iran

A.Sakhaee [1], O. Azari[2], E.Sakhaee[2]

[1]Department of Clinical Sciences, Faculty of Medicine, University of Semnan, Iran;
[2]Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Iran, tel.: 00989155512932**,** e-mail: Ehsan_Sakhaee@yahoo.com

Brucellosis is a worldwide zoonosis, particularly in Mediterranean region, Western Asia, Middle East and parts of Africa and Latin America. Despite its control in many countries, it remains endemic in Iran. This local outbreak demonstrates that Brucella melitansis in human has emerged an important public health problem in Iran. From July to September 2008, total one hundred and four sera samples were taken from suspicious cases of human Brucellosis who residing in Neyshabour and present vague clinical symptom of Brucellosis such as fever of unknown region, unexplained weight loss fatigue, etc. As results of present study showed antibodies were detected in eighty four sera (80.77 %) among one hundred and four samples by RBPT. Antibodies were detected by TAT in 84 sera (80.77%) among 104 samples at a dilution 1: 40, followed by dilution 1: 80 in 65 sera (62.5%), dilution 1: 160 in 44 sera (42.31%), dilution 1: 320 in 32 sera (30.77%), dilution 1: 640 in 18 sera (17.31%), dilution 1: 1280 in 10 sera (9.615%) and at a dilution 1: 2560 in 2 sera (1.923%). Antibodies were detected by 2-ME in 84 sera (80.77%) among 104 samples at a dilution 1: 20, followed by dilution 1: 40 in 65 sera (62.5%), dilution 1: 80 in 44 sera (42.31%), dilution 1: 160 in 32 sera (30.77%), dilution 1: 320 in 18 sera (17.31%), dilution 1: 640 in 10 sera (9.615%) and at a dilution 1: 1280 in 2 sera (1.923%). This outbreak showed that those livestock worker who are suffering with chronic fatigue, arthalgesia, depression, fever and headache without any apparent reasons should visit a physician for a medical evaluation. Results of present study confirmed that RBPT is the best screening test and TAT is the best confirmatory test for detection of Brucella antibodies.

Vancomycin against Methicillin resistant *Staphylococcus aureus* isolated from a tertiary care hospital of Pakistan

F. Kaleem, J. Usman

National University of sciences and Technology, Army Medical College, Department of Microbiology, Abid Majeed Road, Rawalpindi, Pakistan, tel.: 0092-322-5087234 e-mail: fatima_kaleem@hotmail.com

Staphylococcus aureus is a facultatively anaerobic, Gram-positive coccus. It is a major pathogen associated with serious community and hospital-acquired infections. By designation methicillin resistant Staphylococcus aureus (MRSA) is a strain of Staphylococcus aureus that is resistant to all beta-lactams, including the penicillins, cephalosporins and carbapenams. Vancomycin has a narrow spectrum of activity, restricted to most Gram-positive bacteria, and is the drug of choice for the treatment of MRSA. The rapid emergence of resistance against Vancomycin in world necessitated the study to be done in our set up to find out emergence of Vancomycin intermediate and resistant MRSA. The objective of this study was to monitor the current status of Vancomycin susceptibility for the presence of vancomycin resistant or intermediate strains of Staphylococcus aureus in our set up. This descriptive cross sectional study was carried out in the Microbiology Department, Army Medical College Rawalpindi, National University of Sciences and technology, Pakistan over a period of one year. Clinical specimens including urine, blood, pus, sputum, high vaginal swab, aspirates, central venous lines, chest tubes and catheter tips sent for culture and sensitivity to our department were inoculated on appropriate culture media and incubated at 37oC for 24 hours to get growth of bacteria. Staphylococcus aureus were identified by recommended methods like Gram staining characteristics, morphology, catalase and coagulase tests. Methicillin resistance was tested by modified Kirby-Bauer disk diffusion technique according to CLSI guidelines and minimum inhibitory concentrations (MIC) for vancomycin were detected by the use of E-strips(AB-Biodisk). The MIC results were interpreted according to criteria set by Clinical and Laboratory Standards Institute (CLSI). If the MIC of Vancomycin was < 2 μg/ml for an isolate it was considered susceptible, isolates for which the MIC was 4-8 μg/ml was intermediate and isolates with MIC >16 μg/ml was considered resistant. Minimum inhibitory concentrations 50 and minimum inhibitory concentrations 90 were calculated. Most of MRSA were isolated from Pus followed by nasobronchial lavage samples. All MRSA were sensitive to Vancomycin but majority strains showed higher MICs almost reaching break point. One isolate was found to be heterogeneous GISA (Glycopepetide intermediate Staphylococcus aureus). There is emergence of reduced susceptibility of vancomycin against MRSA. Alternatives for treatment of MRSA should be considered and indiscriminate use of Vancomycin should be avoided to decrease the chances of vancomycin intermediate and vancomycin resistant Staphylococcus aureus strains emergence.

Evaluation of tigecycline against Metallo-β-lactamase producing carbapenem resistant Gram negative rods isolated from clinical specimens of a tertiary care hospital of Pakistan

F. Kaleem, J. Usman, A.Hassan

National University of Sciences and Technology (NUST) Department of Microbiology, Army Medical College, Abid Majeed Road, Rawalpindi, Pakistan, tel.: 0092-322-5087234, e-mail: fatima_kaleem@hotmail.com

The rapid spread of acquired Metallo-β-lactamases (MBLs) among major Gram negative pathogens and their highly resistant antibiogram is an emerging threat and matter of particular concern worldwide. This descriptive study was carried out from Aug 09- Jan 10 in the department of Microbiology, Army Medical College, National University of Sciences and Technology Rawalpindi, to find out in vitro efficacy of tigecycline against metallo beta lactamase producing Gram negative rods from clinical isolates of a tertiary care Hospital. All clinical samples were dealt by standard microbiological methods, isolated Gram negative rods were subjected to susceptibility testing against various antibiotics by disc diffusion method as per the Clinical and Laboratory Standards Institute guidelines. Carbapenem resistant isolates were subjected to the detection of metallo beta lactamase production by E-test metallo beta lactamase strip method. All metallo beta lactamase producers were subjected to susceptibility testing of tigecycline by minimum inhibitory concentrations using E-strips. Minimum inhibitory concentrations 50 and minimum inhibitory concentrations 90 were calculated. Among 50 metallo beta lactamase producers, Acinetobacter baumannii were the most frequent metallo beta lactamase producers followed by Pseudomonas aeruginosa. Around 88 % of the metallo beta lactamase producers were sensitive to tigecycline. Most of the metallo beta lactamase producers were isolated from nasobronchial lavage samples. Our study showed that tigecycline is effective against metallo beta lactamase producing organisms.

Antibiogram of carbapenem resistant Acinetobacter (CRA) isolated from a tertiary care hospital

A. Hassan, J. Usman, F. kaleem

National university of sciences and technology, army medical college, Pakistan, tel.: 0092515522867, e-mail: afreenish216a@yahoo.com

Acinetobacter has emerged as a significant nosocomial pathogen. It has developed resistance against major groups of antibiotics. Acinetobacter resistance to broad spectrum antibiotics like carbapenems posing an additional threat. We have conducted this study to find out the antibiotic susceptibility pattern of carbapenem resistant Acinetobacter (CRA). This will help our clinicians in prescribing appropriate treatment against CRA. The study was conducted from June 2009 to December 2009 at the Department of Microbiology, Army Medical College Rawalpindi, affiliated with 100 bedded tertiary care hospitals. Clinical specimens were received form various wards. Acinetobacter species were identified by using standard microbiological procedures. Acinetobacter species resistant to carbapenems were identified by using Kirby Bauer disc diffusion technique according to Clinical and Laboratory Standard (CLSI) guidelines. Fourteen antibiotics were used against CRA (gentamicin, amikacin, tobramycin, tetracycline, minocycline, doxycycline, tigecycline, levofloxacin, ciprofloxacin, trimethoprim-sufmethoxazole, ceftriaxone, ampicillin-sulbactam, cefoperazone-sulbactam, piperacillin-tazobactam).antibiotic susceptibi A total of 61 carbapenem resistant Acineobacter were isolated. Majority of the isolates were sensitive to minocycline, tigecycline, tobramycin and cefoperazone -sulbactam. Doxycycline and piperacillin-tazobactam showed moderate activity against majority of CRA. Tetracycline, ciprofloxacin, ceftriaxone and ampicillin-sulbactam were least effective. Emergence of carbapenem resistant Acineobacter is a challenge for our clinicians. Antibiotics like minocycline, tigecycline, tobramycin and cefoperazone –sulbactam provide effective treatment options against CRA.

In vitro activity of aminoglycosides , β lactam-β lactamases or inhibitor combinations and tetracyclines against multi-drug resistant *Acinetobacter baumannii*, isolated from a tertiary care hospital

A. Hassan, J. Usman, F. Kaleem

National university of sciences and technology,army medical college,Pakistan
e-mail: afreenish216a@yahoo.com

Acinetobacter baumannii has emerged as a significant nosocomial pathogen, particularly in intensive care units. Isolates of A. baumannii resistant to major groups of antibiotics have been identified. These multi-drug resistant (MDR) organisms are limiting the treatment options. Objective: The study was performed to determine the in vitro activity of aminoglycosides, β lactam- β lactamase inhibitor combinations and tetracyclines against MDR Acinetobacter baumannii, isolated from a tertiary care hospital. Study Design: Descriptive cross-sectional study The study was carried out from January 2009 to August 2009, at the Department of Microbiology, Army Medical College/National University of Sciences and Technology, Rawalpindi, Pakistan looking after an 1100 bedded tertiary care hospital. Routine clinical specimens were received from various wards. Acinetobacter baumannii was identified by using standard microbiological procedures. Antimicrobial susceptibility test (gentamicin, amikacin, tobramycin, ampicillin-sulbactam, piperacillin-tazobactam, cefoperazone-sulbactam, tetracycline, doxycycline, minocycline, tigecycline,) was performed according to CLSI guidelines using Kirby-Bauer disc diffusion technique. Resistance to carbapenems, fluoroquinolones and the beta-lactams were observed in significant proportion of fifty isolates. Among the aminoglycosides, the isolates were more susceptible to tobramycin than gentamicin and amikacin. Cefoperazone-sulbactam was superior to piperacillin-tazobactam and ampicillin-sulbactam in activity against MDR A.baumannii. Both tigecycline and minocycline were the active agents against most isolates. Conclusion: Multidrug resistant Acinetobacter infections are posing an increasing threat to our population. Minocycline, tobramycin and cefoperazone-sulbactam provide an effective option against infections caused by resistant A.baumannii.

Keywords: Aminoglycosides, β -lactam/ β -lactamase inhibitor combinations, Multi-drug resistant Acinetobacter, Tetracyclines.

Minimum inhibitory concentration of colistin against multi-drug resistant *Acinetobacter baumannii* isolated from a tertiary care hospital of Pakistan

A. Hassan, J. Usman, F. Kaleem

National University of Sciences and Technology, Army medical college Pakistan, tel.: 0092515522867, e-mail: afreenish216a@yahoo.com

Acinetobacter has emerged as a significant nosocomial pathogen, especially in intensive care units. Increasing incidence of the strains resistant to major groups of antibiotics like carbapenems, glycylcyclines, aminoglycosides and fluoroquinolones have limited the treatment options. Colistin, an older antibiotic, remains sometime only option in the treatment of A.baumannii infections. To determine the in vitro efficacy of colistin against MDR A.baumannii isolated from a tertiary care hospital of Pakistan The study was carried out from September 2009 to February 2010, at the Department of Microbiology, Army Medical College/ National University of Sciences and Technology, Rawalpindi, Pakistan. Clinical specimens were received from intensive care units and various clinical wards of an 1100 bedded tertiary care hospital of Rawalpindi, Pakistan. Specimens were inoculated on appropriate culture media and incubated at 37oC for 24 hours. Acinetobacter species were identified by using standard microbiological procedures (Gram's stain appearance, colonial morphology, catalase test, cytochrome oxidase reaction, motility and by using biochemical tests). Identification up to the species level was done by using Analytical Profile index API 20NE (Biomerieux). Susceptibilities of imipenem, meropenem, ciprofloxacin, gentamicin, amikacin and tobramycin were determined by Kirby-Bauer disc diffusion technique. MDR was defined as resistance to aminoglycosides, carbapenems and fluoroquinolones. Minimum inhibitory concentration (MIC) of colistin was performed by using E-strips (AB BioDisk) for each isolate. The MIC results were interpreted according to criteria set by Clinical and Laboratory Standards Institute (CLSI). Results: A total of fifty MDR A.baumannii were isolated during the study period. Colistin exhibited excellent activity against the isolates. All the MDR A.baumannii were susceptible to colistin (MIC ≤2 μg/ml sensitive, ≥4 resistant). MDR A.baumannii associated infections are difficult to treat and colistin provides an effective treatment option against this resistant pathogen.

Keywords: Colistin, MIC, MDR A.baumannii

Evaluation of in vitro efficacy of Linezolid against methicillin resistant *Staphylococcus aureus*, vancomycin resistant enterococci and methicillin resistant *Staphylococcus epidermidis*

F.Kaleem, J. Usman, A. Hassan

National University of Sciences and Technology, Army Medical College, Department of Microbiology, Abid Majeed Road, Rawalpindi, Pakistan, tel.: +92-322-5087234,
e-mail: fatima_kaleem@hotmail.com

The incidence of nosocomial infections caused by Gram positive bacteria has increased dramatically over past few years. Methicillin resistant Staphyloccocus aureus, vancomycin resistant enterococci and coagulase negative staphylococci have been implicated in many nosocomial infections. Linezolid is one of the newer antibacterial agents with a spectrum of activity against Gram-positive bacteria. It is the first drug of a new class of antibiotics, the oxazolidinones, introduced recently to therapy. To find out in vitro efficacy of linezolid against multidrug resistant Gram positive organisms. This descriptive cross sectional study was carried out in the department of Microbiology, Army Medical College, National University of Sciences and Technology, Pakistan over a period of one year. All samples were dealt with standard microbiological methods. All isolated Gram positive organisms were subjected to the determination of minimum inhibitory concentrations of linezolid by using E strip. Minimum inhibitory concentrations 50 and minimum inhibitory concentrations 90 were calculated. Majority of the isolates were Methicillin sensitive Staphylococcus aureus followed by coagulase negative Staphylococci. 15 methicillin resistant Staphylococcus aureus and 10 vancomycin resistant Enterococci were isolated during the study period. All the Gram positive organisms were uniformly sensitive to linezolid including vancomycin resistant enterococci and methicillin resistant Staphylococci. Linezolid is highly active against Gram positive organism including multidrug resistant organisms so it can prove to be a good therapeutic option for infections caused by such bacteria.

In vitro efficacy of carbapenems against multi drug resistant and nalidixic acid resistant typhoidal Salmonellae

F. Kaleem, J. Usman, A. Hassan

National University of Sciences and Technology, Department of Microbiology, Army Medical College, 286A Lane 4, Peshawar Road, Rawalpindi, Pakistan, tel.: +92-322-5087234, e-mail: fatima_kaleem@hotmail.com

Antimicrobial resistance in Salmonella species is of grave concern, more so in quinolone-resistant and extended-spectrum beta-lactamase (ESBL)-producing isolates that cause complicated infections. The timely appropriate management of typhoid fever can considerably reduce both morbidity and mortality. Since late 1980s Salmonella typhi has developed resistance simultaneously to all the drugs used in first line treatment (chloramphenicol, cotrimoxazole and ampicillin) and are known as multidrug resistant (MDR). Fluoroquinolones are widely regarded as the most effective drugs for the treatment of typhoid fever. Unfortunately there are reports of treatment failure with fluoroquinolones and nalidixic acid resistance is a marker of reduced susceptibility to fluoroquinolones. Recently, azithromycin is being used as an alternative agent, but sporadic reports of resistance to these antibiotics are already being reported so we are left with little options and carbapenems prove to be a promising alternative. This descriptive study was carried out in the Department of Microbiology, Army Medical College, National Uuiversity of Sciences and Technology, Pakistan. All the specimens received with suspicion of typhoid fever for blood culture were dealt with standard microbiological procedures. Typhoidal salmonellae were isolated and were subjected to the determination of antimicrobial sensitivity. All typhoidal salmonellae that were resistant to first line drugs (Multi drug resistant) and nalidixic acid resistant (NAR) isolated were subjected to susceptibility testing of meropenem and imipenem using E-test method. Minimum inhibitory concentration 50 and 90 were calculated. Among 50 Multi drug resistant and nalidixic acid resistant isolates all of the isolates had minimum inhibitory concentrations well within sensitive range. Twenty six isolates were Salmonella typhi and 24 were Salmonella para tyhpi A. Our study concludes that carbapenems have good in vitro activity against MDR and NAR typhoidal salmonellae and they can be used as a last effective resort against multi drug resistant and nalidixic acid resistant salmonellae where other options lag behind.

Comparison of oral and intravenously administered third generation cephalosporins against common respiratory pathogens

F.Kaleem, J.Usman, A. Hassan

National University of Sciences and Technology, Army Medical College, 286 A, Lane 4, Peshawar Road, Rawalpindi Pakistan, tel.: 00-92-322-5087234

Respiratory tract infections (RTIs) are very common in developing countires particularly in winter months. Major pathogens associated with these infections are Streptococcus pneumoniae, Haemophilus influenzae and Moraxella cataharallis. As these infections are a major cause of morbidity and mortality proper knowledge of antimicrobial sensitivity pattern should be known to the physician so as to prescribe correct empirical therapy. The objective of this study was to find out the in vitro activity of ceftraixonea and cefixime against respiratory pathogens. Materials and Methods: This descriptive cross sectional study was carried out at the Department of Microbiology, Army Medical College, National University of Sciences and Technology, Pakistan. All respiratory samples were dealt with standard microbiological techniques. Isolated organisms were subjected to antimicrobial testing by modified Kirby Bauer disc diffusion technique and were also subjected to the determination of minimum inhibitory concentrations (MIC) of ceftriaxone and cefixime. MIC 50 and MIC 90 were calculated. Streptococcus pneumoniae was most frequently isolated followed by Haemophilus influenzae and Moraxella cataharallis. All the isolates were uniformly susceptible to both the antibiotics. Ceftraixone and cefixime both are highly effective against respiratory pathogens, however less cost of cefixime and its oral dosing option can make it a better option for treatment of respiratory infections.

In vitro assay of the antimicrobial activity of kephir

A.B. Cioaca, M. C. Chifiriuc, V. Lazar

Faculty of Biology, aleea portocalelor 1-3, sector 6, Bucharest Romania
e-mail: lazar@botanic.unibuc.ro, tel.: 0040213181576

Kephir is fermented carbonated refreshing milk, with a slightly acidic aromatic taste and creamy foam composition which contains lactobacilli, leuconostocci, acetic acid bacteria, lactostreptococci and yeasts. Recent studies have demonstrated its antibacterial, immunostimulating, antitumoral and cholesterol-lowering activities. The purpose of this study was to investigate the antimicrobial activity of kephir against Bacillus subtilis spp. spizizenii ATCC 6633, Staphylococcus aureus ATCC 6538, Enterococcus faecalis ATCC 29212, Escherichia coli ATCC 8739, Salmonella enteritidis ATCC 13076, Pseudomonas aeruginosa ATCC 9027 and Candida albicans ATCC 10231. The kephir fermented for 24 h and 48 h, as well and after 7 days preservation at 4-8°C was tested by in vitro disk diffusion method. The intensity of the antimicrobial activity was interpeted by comparison with two antibiotics, i.e. ampicillin and neomycin. Results: The antimicrobial activity of 24 h as well as 48 fermented kephir, fresh or after 7 days preservation at 4-8°C was similar and observed against B. subtilis, S. aureus, E. coli, E. faecalis and S. enteritidis. For E. coli, E. faecalis and S. enteritidis the antimicrobial activity was superior to both tested antibiotics and for B. subtilis and S. aureus superior to one antibiotic. The tested products exhibited no activity against P. aeruginosa and C. albicans. Conclusion. Kephir is exhibiting large spectrum and strong antibacterial activity probably due to the complex viable probiotic strains association producing antimicrobial substances.

Cell wall components of a *Lactobacillus brevis* strain inhibit herpes simplex virus type 2 replication

F. Cacciotti[1], A. Masci[2], D. Capobianco[1], P. Mastromarino[1]

[1] Department of Public Health Sciences, Microbiology Section
[2] Biochemical Sciences, Sapienza University of Rome, Italy
e-mail: paola.mastromarino@uniroma1.it, tel.: +39-06-49914628

Bacteria-free supernatant of Lactobacillus brevis strain CD2 grown in cell culture medium inhibits herpes simplex virus type 2 (HSV-2) replication in cell culture independently from H2O2 or lactic acid. The mechanisms of action and the bacterial factors responsible for the antiviral effect were studied using bacterial extracts obtained by sonication of L. brevis cells or by lysozyme/antibiotic treatment of the microorganism. The antiviral activity of bacterial cell wall was also studied. Bacterial extract and cell wall were not toxic to cells at the maximal concentration tested (3 mg/ml protein). Both the extract and the cell wall fraction showed a dose-dependent inhibitory activity on HSV2 multiplication when present on Vero cells before virus adsorption and during infection. The inhibition was exerted on the first phases of virus replication cycle. The inhibitory activity was resistant to a 30 minutes treatment at 100°C. DNA and lipids obtained from bacterial extract were devoid of any inhibitory effect. S-layer of bacterial cell-wall containing several heat-resistant molecules (teichoic and lipoteichoic acids, lipoglycans, teichuronic acids and other acidic or neutral polysaccharides) was removed by treatment with LiCl without affecting bacterial viability. Bacterial extract and cell-wall fragments obtained after LiCl treatment showed a dramatic reduction in the antiviral activity suggesting that cell wall components of L. brevis released in bacterial supernatant after sonication or lysozyme/antibiotic treatment are responsible for the inhibiting activity against HSV-2.

Antibiotic activity of tigecycline against clinical pathogens by the micro dilution method.

I. Mantzourani, S.Plessas, A.Alexopoulos, C.Voidarou, S. Vavias, E. Stavropoulou and E. Bezirtzoglou

Democritus University of Thrace, Faculty of Agricultural Development, Laboratory of Microbiology, Biotechnology and Hygiene. Orestiada GR68200, Greece.

Resistant pathogens are the cause of clinical infections which threatening the patients lives and challenging the health systems through their economic importance. Therefore, new antibacterial agents with a broader spectrum of activity which protects against development of resistance are required. Tigecycline (Tygacil, Wyeth) is a relatively new FDA and EMEA approved glycylcycline antimicrobial with an expanded broad-spectrum activity against pathogens involved in complicated skin and skin structure infections and complicated intra abdominal infections. In this study we evaluated the *in vitro* activity of tigecycline in comparison to 14 other antibiotics against 182 clinical pathogens by use of the micro dilution method.In overall, tigecycline exhibited the lowest Minimum Inhibitory Concentration (MIC) values in almost all bacteria with a mean of 0.57 ± 1.35 mg/L, followed by meropenem and levofloxacin (mean MIC values 1.43±2.66 and 1.61±3.26 mg/L respectively). MIC50 and MIC90 values of tigecycline were: 0.06 and 0.15 mg/L for *E. coli*, 0.12 and 1.00 mg/L for *Klebsiella sp.*, 0.12 and 0.85 mg/L for various *Enterobacter sp.*, 1.00 and 8.00 mg/L for *Pseudomonas sp.*, 0.25 and 1.00 mg/L for *Acinetobacter sp*., 0.06 and 0.12 mg/L for *Serratia sp.*, 0.12 and 0.25 mg/L for *S. aureus*, 0.03 and 5.00 mg/L for *Streptococcus sp.* The MIC values recorded were among the lowest in recent literature for *Acinetobacter sp*. (included *A. baumannii*), and comparable to those of *Klebsiella*, *Serratia* and *Enterobacter* indicating that tigecycline has a promising *in vitro* activity.

Surface-Enhanced laser desorption ionization/time-of-flight (SELDI-TOF) Mass Spectrometry (MS) as a phenotypic approach for rapid identification of antibiotic resistance.

L.Dubska [1,2] , K.Pilatova [1] , M. Dolejska [2], I. Literak [2], D. Valik[1]

[1] Department of Laboratory Medicine, Masaryk Memorial Cancer Institute, Brno, Czech Republic
[2] Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Palackeho 1-3, 612 42 Brno, Czech Republic

The determination of antimicrobial susceptibility of a clinical isolate, especially with emergence of multidrug-resistant microorganisms, is often crucial for the optimal antimicrobial therapy of infected patients. Cultivation methods for detection of antibiotic resistance are recently often amended by nucleic acid-based techniques that elucidate resistance mechanisms. These genotypic methods have some limitations for example new resistance mechanisms may be missed. We present new method for phenotyping of *Escherichia coli* based on mass spectrometry profiles. SELDI-TOF-MS is a variation of matrix-assisted laser desorption/ionization mass spectrometry method used for analysis of protein mixtures. Using this method we analyzed gram-negative bacteria; colonies were grown on agar plate, one colony was suspended in 100 µl of distilled water, frozen at -70 °C, thawed, applied on a gold chip and allowed to dry at room temperature. Measurement was performed on SELDI-TOF instrument (Ciphergen). Considering the lipopolysacharide layer of G-, we tested nonpolar and low polar extraction before profiling as well; however we have not yielded qualitatively improved result. Using SELDI-TOF MS we were clearly able to distinguish between *Salmonella typhimurium* and *E. coli*. Further, we established bacterial profiles of nonresistant *E. coli*, *E. coli* harbouring genes for antibiotic resistance (*tetA, tetB, strA, cat, aadA1, aadA1, qnrS, sul1, sul2, dhfr17*) and ESBL-producing (extended-spectrum beta-lactamases) *E. coli* and the protein phenotypes differed significantly between analyzed strains. Thus, we present MS profiling as a promising rapid and reproducible phenotypic approach for antibiotic resistance identification.

Streptococcus sanguinis-like-strains play an important role in Behcet's disease

K. Oguma[1], K. Yokota[2], S. Lianhua[1], Y. Yamamoto[1], E. Isogai[3], and F. Kaneko[4]

[1]Department of Medical Bacteriology, Graduate School of Medicine, Dentistry and pharmaceutical Sciences

[2]Graduate School of Health Science, Okayama University

[3]Department of Microbial Biotechnology, Graduate School of Agricultural Science,Tohoku University

[4] Institute of Dermato-Immunology and Allergy, Southern Tohoku Research Institute for Neuroscience, Japan

We found that proportion of *S. sanguinis* in the flora of patients of Behcet's disease (BD) was always higher than that of healthy and disease controls. The strains were identified as *S. sanguis* (previously, *S. sanguinis* was named as *S. sanguis*) by Api/Strept test (kit). The antigenicity, sugar constituents of cell wall, and DNA homology, however, were somewhat different from those of *S. sanguinis* ATCC10556. They produced the IgA protease, and bound to the patients' buccal epithelial cells more than standard strains. The antibody titers of the BD patients against these cell-lysates, especially one strain designated as 113-20, were high. In the chemiluminescence assay using whole blood, the lysates of 113-20 strongly activated the neutrophils in both patients and healthy volunteers, as compared with the lysates from several standard streptococcal strains. Furthermore, the 113-20 cell-lysates significantly increased the production of IFN-γ, IL-8 and IL-12 from the patients' peripheral blood mononuclear cells (PBMCs) than the standard *S. sanguinis* cell-lysates. These indicate that *S. sanguinis*-like strains play an important role in developing BD. The effects of heat shock protein 60 (HSP60) on BD were also investigated. The whole amino acid sequences of 113-20 HSP60 were almost identical to those of several standard streptococcal strains. The five peptides that are highly homologous to the T cell-epitope were prepared (designated as LO1, IIIa, IIIb, LO2, UK), and mixed into the PBMCs from the active BD patients, that producing some amounts of IL-8 and IL-12. The production of IL-8 and IL-12 from the PBMCs was significantly inhibited by LO1, IIIa and IIIb, and by LO1, LO2, LO3, IIIb and UK, respectively, indicating that some peptides might be used as a therapeutic agent.

Antibacterial activities of essential oils from eight Greek aromatic plants against clinical isolates of *Staphylococcus aureus*.

A. Alexopoulos[1*], A.C. Kimbaris[1], S. Plessas[1], I. Mantzourani[1], I. Theodoridou[1], M.G. Polissiou[1] and E. Bezirtzoglou[1]

[1] Democritus University of Thrace. Faculty of Agricultural Development. Department of Food Science and Technology, 193 Pandazidou Str. 68200 Orestiada, Greece
[2] Laboratory of Chemistry, Department of Science, Agricultural University of Athens, 75 Iera Odos Street, 11855 Athens, Greece
* Correspondence: alexopo@agro.duth.gr

Aromatic plants have been used widely to extend the shelf life of foods but at the same time research is undergo for their properties as antibacterial agents in clinical use. Although there are promising results for antimicrobial properties of various essential oils against *S. aureus* isolated from environmental samples or foods, there are limited studies concerning these properties against clinical isolates of the pathogen which is responsible for an increase number of nosocomial infections and at the same time exhibits an increased resistant to synthetic agents.
In this study, essential oils from eight aromatic plants indigenous to Greece were isolated by hydrodistillation, analyzed by gas chromatography (GC) and GC/mass spectrometry for their chemical components and tested for their antimicrobial activities against 24 clinical isolates of *Staphylococcus aureus*. The methods used were the Kibry-Bauer and the dilution method in order to determine the Minimum Inhibitory Concentration (MIC).
Our results showed that essential oils from *Origanum vulgare* and *Origanum dictamnus* were active against *S. aureus* when tested by disk diffusion, but even those where exhibited increased MIC values (> 1024 mg/L) with the dilution method. In contrast, the reference strain NCTC 6571 showed to be extremely sensitive in most of the oils tested (MICs 0.25 – 32.0 mg/L) and resistant only to the essential oil from *Ocimum basilicum*. Therefore, there is no evidence of a potential clinical use for those essential oils and further research is needed in order to determine if they efficiently could substitute the synthetic antibiotics or, perhaps be used in combination.

Keywords: essential oils, *Staphylococcus aureus*, Minimum Inhibitory Concentration.

Microbial Biofilms
Invited Lectures

Microbial biofilms: open issues and perspectives

G.Donelli

Istituto Superiore di Sanità, Department Technologies and Health and Microbial Biofilm Laboratory, IRCCS Fondazione Santa Lucia, Viale Regina Elena 299, Rome, Italy, tel.: +39 06 49902228, e-mail : gianfranco.donelli@gmail.com

Since the early ’80s, a series of papers by Costerton and colleagues, provided significant information on the ability of bacteria to form communities of physically and chemically interacting cells growing in a sessile mode on both biotic and abiotic surfaces. This novel view of the microbial world has led not only to the understanding that most of human chronic infections are biofilm-based but also to the awareness that new preventive and therapeutic strategies are to be designed. In spite of the great number of studies performed in the last 30 years which provided us with a large amount of data on the phases and mechanisms involved in biofilm development, a series of open issues is still to be fulfilled, including: i) the interaction mechanisms among different microorganisms in multispecies biofilms; ii) the development of non-invasive in vivo methods to sample a biofilm in a “soft” way in order to save its tridimensional structure; iii) the validation of a general model able to explain the increase in antimicrobial resistance of biofilm-growing microorganisms; iv) the investigation on natural or synthetic quorum sensing inhibitors able to interfere or compete with the signaling molecules responsible for population density; v) the study of physical and chemical agents able to disrupt mature biofilms or prevent their formation; vi) the realization of medical devices refractory to microbial colonization and biofilm formation and the finding of more advanced solutions to prevent biofilm-based device-related infections. The present knowledge on the perspectives to address these issues will be reviewed.

Laboratory modelling of oral biofilms

J.Pratten

UCL Eastman Dental Institute, 256 Grays Inn Road, London, United Kingdom, tel.: 02079152338, e-mail: j.pratten@eastman.ucl.ac.uk

There are many experimental systems available for the generation and study of in vitro dental plaque, such that a suitable system can be chosen to suit the requirements of almost any investigation. All are compromises between the reality of the in vivo ecosystem and the simplification and controllability necessary to gain meaningful, useful results. Well before the term biofilm was introduced researches were using 'Artificial mouths' to grow dental plaque in vitro, with a focus on dental caries. Artificial mouths are specifically constructed to mimic the situation in the oral cavity. Hence, dental plaque formation is studied on a human tooth inoculated with saliva and then supplied with mucin-containing artificial saliva. In order study biofilm development or perturbation, for example, the use of antimicrobial agents over a period of time, data must be comparable and reproducible from experiment to experiment. One approach to reproducibility is to develop constant depth reactors where surface growth is periodically removed to maintain a constant geometry. Such a device, the Constant Depth Film Fermentor (CDFF), employing a mechanical scraper bar has been used with great success to investigate oral biofilms. As well as being used for the study of bacterial perturbation, the model has been used in the study of endodontic microleakage, oral malodour generation and the changes in composition of plaque from health to disease. The advantage and disadvantages of several biofilm models will be discussed as well as focussing upon the CDFF as a recognised oral biofilm model.

Quorum sensing in biofilms

V. Lazar

University of Bucharest, Romania, Aleea Portocalelor nr. 1-3, Sector 6, Bucharest Romania, tel.: +0040213181576, e-mail: veronica.lazar2009@gmail.com

Biofilms or microbial communities formed by adherent and cohesive cells on cellular or inert substrata (like medical devices), are involved in ~ 60% of all infections and characterized by moderate intensity symptoms, chronic evolution and resistance to antibiotics. Biofilms' pathogenicity, even of those formed by opportunistic microorganisms, is amplified by two major biofilm characteristics: 1) the increased resistance to antimicrobials; 2) the protection of cells against the host's defense mechanisms. The studies at the molecular level shown that the biofilms formation is controlled by cell-to-cell signaling mechanisms and the gene regulation during biofilm growth is due to the accumulation of signal molecules. In this regard, quorum sensing mechanism (QS) is defined as a cell-density dependent bacterial intercellular communication, involved in gene expression (e.g. virulence genes for exoenzymes, exopolysaccharides) and the consequent changed behaviour of biofilm's cells, including the resistance to stress conditions; this resistance is different of well known antibioresistance, being named phenotypical resistance or tolerance. Considering the differences in physiology and susceptibility to antibiotics of biofilm embedded bacteria, as well as their increased power against the host defense responses new strategies for prevention and therapy of biofilm associated infections are required. The dental plaque is a typical example of biofilm, involved in the aethiology of cariogenesis and periodontal diseases associated with local chronic inflammation and cytokines production. The genetic and phenotypic versatility of the biofilm's cells represent a challenge for discovering new methods of treatment and prevention of periodontitis, this polimicrobial infection having a great incidence in world population.

Oral Microbial Ecology
Invited Lectures

Microbial ecology of the healthy oral cavity

M. Wilson

University College London, United Kingdom, e-mail: m.wilson@eastman.ucl.ac.uk
tel.: +44 (0)2079151231

Because of its complex anatomy, the mouth has many habitats for microbial colonization. Uniquely, it has non-shedding surfaces, the teeth, which enable the formation of complex biofilms (dental plaques). Mechanical forces and salivary flow hinder microbial colonization of exposed regions - successful colonizers need to be able to adhere strongly to a surface or else colonize protected sites. Most of the communities present have a high species diversity. More than 700 phylotypes have been detected and approximately 50% of these have not yet been cultivated. Culture-independent approaches are now revealing the true complexity of these communities. Most of the bacteria in the mouth are found on tooth surfaces in biofilms. The composition of these is complex and depends on their location. In supragingival plaques, streptococci and Actinomyces predominate, but anaerobes (Veillonella, Fusobacterium) are also present. Plaque composition alters with time and is affected by the host's diet. The microbiota of plaque in the gingival crevice is more diverse and the proportion of anaerobes is greater than in supragingival plaques. Anaerobic organisms frequently detected include Veillonella, Gram-positive anaerobic cocci, Prevotella, Fusobacterium, Selenomonas, Eubacterium and spirochaetes. The tongue is densely colonized and streptococci generally predominate. A variety of anaerobes are frequently present including Prevotella, Veillonella, Eubacterium, and Fusobacterium. Other mucosal surfaces are sparsely populated. The community composition varies with location, but facultative anaerobes and capnophiles predominate, e.g. streptococci, Gemella, Neisseria, Haemophilus and Capnocytophaga . However, anaerobes (Fusobacterium, Veillonella and Prevotella) are also often present.

Oral microbial diseases: Ecological perspectives

Spratt Dave

Ucl Eastman Dental Institute, 256 Gray's Inn rd, London wc1x8ld,United Kingdom,
e-mail: d.spratt@eastman.ucl.ac.uk, tel.: 02079151107

The oral cavity provides a large number of diverse surfaces on which a wide variety of complex communities are able to form. These communities consist of may taxa adhered to a surface and embedded in a matrix of polymers of bacterial and salivary origin. Ecology can be defined as the study of the distribution and abundance of living organisms, and the interactions between these organisms and their environment. Microbial driven oral diseases are amongst the most common infections in humans and are as a result of changing environmental conditions and the concomitant shifts in the proportion of microbes in the community. With the vast range of culture independent techniques now in existence the true richness, diversity and community structure of these populations can be investigated. Various microscopic techniques allow us to visualise ever more diverse facets of community structure. Molecular biology techniques like PCR Cloning and qPCR allow us to determine richness and diversity in oral communities and DGGE allows us to profile communities and track population shifts in health and disease. The advent of “meta” biology is revolutionising our understanding of the microbial world in both functional and richness terms. Metagenomic data from massively parallel sequencing has estimated that the total human oral microbiota consists of around 19,000 phylotypes. Functional metagenomic screens have been successful in detecting numerous antibiotic resistant determinants from the metagenome of oral microbial communities indeed, a novel tetracycline resistance gene, tet(37) has been isolated and characterised. In the near future these techniques will have a profound impact on our understanding of the ecology of the oral cavity in health and disease.

Clinical oral microbiology: new directions

Ready Derren

Eastman Dental Hospital, 256 Gray's Inn Road, London, WX1X8LD , United Kingdom Tel : +44(0)2079152338, e-Mail : d.ready@eastman.ucl.ac.uk

Many challenges await the oral microbiology service over the next decade, including structural changes to the delivery of pathology services and the essential implementation of molecular-based techniques into a service which has often focussed on the use of culture techniques for the isolation, identification and characterisation of clinically relevant pathogens. Specific areas to address for clinical oral microbiology include; training sufficient future staff members; the implementation of up-to-date technologies; the survival and improvement of small oral microbiology diagnostic services within larger medical and pathology laboratories; the role of privatisation and outsourcing of diagnostic tests; the development and implementation of chair-side tests within dental practices; improving our understanding of the association between oral and medical health and providing diagnostic tests to support this; fully characterising the role of the oral cavity as a reservoir of oral and medically important antibiotic-resistant bacteria (including MRSA) and finally, ensuring that high standards of infection control are implemented in the community and in specialist hospitals. These challenges will face all of us working in diagnostic oral microbiology and how these are managed will alter the future of clinical oral microbiology.

Microbial Biofilms and Oral Ecology
Oral Presentations

Detection of biofilm producing Gram positive and Gram negative bacteria isolated from clinical specimens and their antibiotic susceptibility pattern

A. Hassan, J. Usman, F. Kaleem

National University of Sciences and Technology, Army Medical College, Rawalpindi, Pakistan, tel.: 0092515522867, e-mail: afreenish216a@yahoo.com

Microorganisms adhere to non-living material or living tissue, and form biofilms made up of extracellular polymers/slime. Biofilm-associated microorganisms behave differently from planktonic organisms with respect to growth rates and ability to resist antimicrobial treatments and therefore pose a public health problem. *Objective:* To detect the prevalence of biofilm producers among Gram positive and Gram negative bacteria isolated from clinical specimens, and to study their antimicrobial susceptibility pattern. The study was carried out from November 2009 to February 2010, at the Department of Microbiology, Army Medical College/ National University of Sciences and Technology, Rawalpindi, Pakistan. *Method:* Clinical specimens were received from various wards of a tertiary care hospital. These were dealt by standard microbiological procedures. Gram positive and Gram negative bacteria isolated were subjected to biofilm detection by congo red agar method (CRA). Antimicrobial susceptibility testing of those isolates, which showed positive results (slime production), was done according to the Kirby-Bauer disc diffusion technique. A total of 100 isolates were tested for the production of biofilm/slime. Among them, 53 isolates showed positive results. From these 53, 22 were Gram positive and 31 Gram negative. All the 53 slime producers showed reduced susceptibility to majority of antibiotics. Bacterial biofilms are an important virulence factor associated with chronic nosocomial infection and antibiotic failure. Congo red agar is a method that can be successfully used to determine whether an isolate has the potential for biofilm production or not.

Key words: Antibiotic resistance, Biofilm, Congo red agar method.

Biofilm formation by *Helicobacter pylori* and its pathogenesis

S. Kamiya[1], H. Yonezawa[1], T. Woo[1], S. Kurata[1], C. Zaman[1], T. Hanawa[1], S. Kato[2] and T. Osaki[1]

[1]Department of Infectious Diseases, Kyorin University School of Medicine, 6-20-2 Shinkawa,Tokyo, Japan
[2]Department of Cell Transplantation and Regenerative Medicine, Tokai University School of Medicine, Kanagawa, Japan, tel.: 81-422-47-5511, e-mail: skamiya@ks.kyorin-u.ac.jp

Helicobacter pylori forms biofilms on glass surfaces at the air-liquid interface in *in vitro* batch cultures; however, biofilms of *H. pylori* have not been well characterized. In the present study, we analyzed the ability of *H. pylori* strains to form biofilms and characterized the underlying mechanisms of *H. pylori* biofilm formation.
Strain TK1402 showed strong biofilm forming ability relative to the other strains in Brucella broth supplemented with 7% FCS. The strong biofilm forming ability of TK1402 is reflected the relative thickness of the biofilms. In addition, outer membrane vesicles (OMV) were detected within the matrix of only the TK1402 biofilms. Biofilm formation was strongly correlated with the production of OMV in this strain. We further observed that strain TK1402 did not form thick biofilms in Brucella broth supplemented with 0.2 % □-cyclodextrin. However, the addition of the OMV-fraction collected from TK1402 could enhance biofilm formation.
The results suggested that OMV produced from TK1402 play an important role in biofilm formation in strain TK1402. Updated data on correlation between urease activity and biofilm formation will be presented and discussed.

Lectins (glycoconjugate recognizing proteins and their complexes) of living organisms

V. Lakhtin

G.N. Gabrichevsky Epidemiology and Microbiology Research Institute, Miklukho-Maklay Street 65-4-72, Moscow 117342, Russia, tel.: 903 503 1180, e-mail: lakhtinv@yandex.ru

The author and coauthors are studying lectins (L) since 1983 year (first publications) and their experimental materials have been published in more than 200 books, articles, patents, abstracts, etc. The main aspects of their study include the isolation and characterization of L and glycoconjugates from higher and lower plants, micromycetes, symbiotic and probiotic bacteria, arthropods, other living organisms. Besides much attention is directed to the following aspects of L application in biotechnology, enzymology, microecology and pharmacology: carriers of biologically active effectors; antimicrobials, mitogens, blasttransformants, hormones, enzyme and metabolome regulators; microassays, etc. Report summarizes approaches of authors to solve problems of current lectinology. Authors offer and base term of L, their classification and structure-function principles (1986, 1987, 1989, 1992, 1994, 2004, 2006-2010), organization and functioning of L systems (2009, 2010) on examples of L of symbiotic microorganisms (for "bacteria - eukaryotic host" systems). It will be discussed fundamental and applied prospects of human probiotic bacteria L: L as metabiotics, auto- and antimicrobial modulators of microbiocenosis in health and disease conditions; as drug delivery carriers; in construction of predictable biofilms, biomatrices, replicas and scaffolding as tools for microassays, chips and biosensors.

Pattern differentiation of mixed-species biofilms formed by *Staphylococcus aureus* with *Pseudomonas aeruginosa* mutants

L.Yang , Y.Liang , M.Søren

Department of Systems Biology, Technical University of Denmark, Denmark, Building 301, Room 112, tel.: 45252511

Microbial lung infection is the leading cause of morbidity and mortality in cystic fibrosis (CF) patients. Chronically colonized CF airways represent a complex ecosystem and the interspecies interactions of different organisms remain largely unelucidated. Here, we have investigated interactions of two of the major bacterial species of the CF lung microflora - Pseudomonas aeruginosa and Staphylococcus aureus – when grown in co-culture biofilms. By growing mixed-species biofilms formed by S. aureus and P. aeruginosa mutants with different CF adapted phenotypes in the standard flow chamber system and observing them with confocal laser scanning microscopy, we show that wild-type P. aeruginosa PAO1 strain associates with S. aureus and forms two-species microcolonies. In contrast, we find that P. aeruginosa mucA mutant and rpoN mutant only weakly associate with S. aureus and tend to out compete S. aureus in mixed-species biofilms.

Biofilms of anaerobic bacterial species isolated from clogged biliary stents

C. Vuotto[1,2], E.Guaglianone[1,2], I.Francolini[3], R. Cardines[4], P. Mastrantonio[4], G. Donelli[1,2]

[1]Department of Technologies and Health, Istituto Superiore di Sanità
[2]Microbial Biofilm Laboratory, IRCCS Fondazione Santa Lucia, Viale Regina Elena 299, Italy, tel.: +39 0649903506, e-mail: claudia.vuotto@iss.it
[3]Department of Chemistry, Sapienza University of Rome; [4]Department of Infectious, Parasitic and Immune-mediated Diseases, Istituto Superiore di Sanità; Rome, Italy,

Biofilm growth of anaerobic bacteria from the human intestinal tract has been so far poorly investigated while a large amount of data are available on the sessile growth of anaerobes colonizing the oral cavity.
One of the main limitations in the investigation of most anaerobes is represented by their cultivation techniques requiring the complete absence of oxygen only obtainable through the use of appropriate anaerobic cabinets. In fact, are few the facultative anaerobic species that can tolerate small levels of oxygen concentration.
Recent studies from our group (Guaglianone et al., 2008) have strictly associated the clogging of biliary stents to the development in their lumen of a polymicrobial biofilm constituted by aerobic and anaerobic bacterial species other than some fungal species. The aim of this study was to test the ability to form biofilm in vitro by some anaerobic bacterial strains isolated from clogged biliary stents.
The ability of strains to form biofilm has been tested by using the microplate biofilm assay while scanning electron microscopy has been employed to evaluate the typical tridimensional structure of mature biofilms.
Further, preliminary experiments on the biofilm disaggregating activity of Dispersin B, an enzyme purified from *Aggregatibacter actynomicetemcomitans* and active on the β-1,6-N-acetyl-D-glucosamine, have been also carried out. Particularly, positive results have been obtained on mature biofilms formed by a strong biofilm-producer strain of *Peptostreptococcus magnus*, while other isolates belonging to different species are under investigation.

Aknowledgements

Dispersin B was kindly supplied by Kane Biotech in the framework of the Material Transfer Agreement signed with our Institute in the year 2006.

Novel intrinsically antimicrobial polymers to control biofilm formation on medical devices

I. Francolini[1], L. D'Ilario[1], E. Guaglianone[2], C. Vuotto[2], G. Donelli[2], A. Martinelli[1], A. Piozzi[1]

[1]University of Rome Sapienza, Department of Chemistry, Rome, Piazzale Aldo Moro 5 - 00185, Rome Italy, tei.: +390649913162, e-mail: iolanda.francolini@uniroma1.it;
[2]Department of Technology and Health, Istituto Superiore di Sanità and Microbial Biofilm Laboratory, IRCCS – Fondazione Santa Lucia, Rome, Italy.

Device coating with antimicrobial agents able to inhibit microbial colonisation and biofilm formation represents a pivotal approach in the prevention of medical device-associated infections. Existing antiseptic or antibiotic loaded devices mainly suffer from a relatively short persistence of antimicrobial action as consequence of an early and rapid drug release. On the other hand, the most frequently implicated microrganisms in device colonisation, such as Staphylococcus, Pseudomonas and Candida spp, are known to develop a largely increased antibiotic resistance due to their sessile mode of growth to form a biofilm. These issues are both critical for the management of patients in clinical settings and need the development of innovative and safer medical devices refractory to microbial adhesion and biofilm formation. To this aim, we developed and tested in vitro novel intrinsically antimicrobial polymers based on functionalized polyurethanes able to coordinate metal ions, including silver, zinc, copper, aluminium and iron. With the exception of the aluminium-containing polymer, all the other experimented polymers showed satisfactory antimicrobial properties. The best antibacterial effect was obtained by the polyurethane functionalized with silver ions which showed an ability to inhibit the S. epidermidis growth up to 16 days. The further adsorption of ciprofloxacin on this polymer allowed to obtain a long-lasting antibacterial synergistic activity against Gram-positives and Gram-negatives. In conclusion, the combined treatment of our functionalised polyurethanes with antibiotic/antifungal drugs and silver ions seems to offer promising perspectives in prevention of bacterial colonisation, biofilm formation and control of drug resistance.

Different microbial biofilm formation on Polymethylmethacrylate (PMMA) bone cement loaded with gentamicin and vancomycin

E. Bertazzoni Minelli, T. Della Bora, A. Benini

Department of Public Health and Community Medicine, Pharmacology Section, University of Verona, Policlinico GB Rossi-37134 Verona, Italy, tel.: +39 045 8027603, e-mail : elisa.bertazzoni@univr.it

PMMA bone cement used for the fixation of joint prostheses and orthopaedic devices may be colonised by gram-positive cocci. The use of antibiotic-loaded PMMA should prevent bacterial adhesion to cement surfaces. Aim. To evaluate the in vitro effects of gentamicin(G)- and vancomycin(V)-loaded PMMA cement on the bacterial adhesion of multiresistant (Met-R and Gent-R, and VRSA) clinical isolate staphylococci (S. aureus and CoNS). Materials and Methods. The PMMA specimens (discs) loaded with G (1.9%) or V (1.9%) or their combination were placed in MH Broth inoculated with bacterial strains. After incubation, bacterial growth was determined by optical density (OD540) and subcultures. The biofilm PMMA-associated dye (crystal violet) was measured. Antibiotic concentrations in broth were determined by FPIA. Results. Antibiotic-loaded specimens released high and inhibitory concentrations of G and V. The Met-R and Gent-R CoNS showed no adhesion to G-loaded specimens for 24 h; strains with G-intermediate-susceptibility showed delayed growth after 48 h and reduced adhesion. VRSA strain only was able to adhere to V-loaded specimens after 24 h. The G-V-loaded cements inhibited growth and adhesion of all strains for the duration of the experiments (>144 hours). Conclusions. G and V alone reduced bacterial adhesion to PMMA of susceptible and intermediate-resistant Staphylococci. The combination G-V inhibited both growth and adhesion of all strains, inclusive VRSA. The anti-adhesion effect of the antibiotic-loaded cement depends on the amount of drug eluted, characteristics of the microorganism and its capacity of adhering to antibiotic-loaded bone cement surfaces.

Probiotic characterization of *Lactobacillus spp*. strains isolated from individuals without caries records

FJ. Salazar[1] , D. San Martín[1], K.Sossa[2], L.Vergara[3], M.Sánchez[1], R.Vera[1], S.Jara[1], E.Castro [1,4]

[1] Laboratorio de Bacterias Lácticas, Facultad de Ingeniería Química, Universidad de Concepción, Concepción, Chile. Casilla 160-C. Correo 3. e-mail: gbacteriaslacticas@gmail.com
[2] Centro de Biotecnología, Universidad de Concepción
[3] Departamento de Odontología Restauradora. Facultad de Odontología, Universidad de Concepción, Concepción, Chile
[4] Facultad de Medicina, Universidad de Concepción, Concepción, Chile. Casilla 160-C. E-mail: ercastro@udec.cl**,** tel.: 56-41-2207289

Los resultados encontrados son promisorios para continuar estudios de modelos cariogénicos y la posibilidad de obtener un LAB con capacidad de restablecer el desbalance de una microbiota alterada de personas con caries y/o prevenir el desarrollo de un ambiente favorable para el desarrollo de esta patología. Buccal pathologies are one of the major public health problems, as caries have been recognized as having the biggest disease burden and being the main cause of buccal morbidity. Several researches have proved the presence of lactic acid bacteria as a part of the resident oral microbiota, as well as their probiotic properties. The objective of this study was to characterize Lactobacillus spp. (LAB) strains isolated from individuals without caries records. Out of 72 available strains, 34 LAB were selected due to their high hydrophobicity rates (over 70%). The items evaluated in these strains were the self-aggregation capability, adhesion to three protein types (keratin, collagen IV and bovine serum albumin), presence of exopolysaccharides (EPS) through adherence to Congo red, and interaction with Streptococcus mutans and Streptococcus sanguis. In the evaluated strains, 26 out of 34 presented high autoaddition rates, 30 out of 34 inhibited the growth of Streptococcus mutans, and 27 LAB inhibited S. Sanguis. All strains presented a medium adherence to queratin and produced exopolysaccharides. These promising results allow continuing studies of cariogenic models, and the chance of obtaining a LAB with the capacity to restore balance altered microbiota presented by individuals with caries and/or to prevent a favourable environment for the development of this pathology.

This work was financed by FONDEF project D04i1326.

Microbial Biofilms and Oral Ecology
Poster Presentations

The comparison of antibacterial effect of green and black tea on streptococcus mutans in dental caries

M. Niakan

31-Abdolazadeh,st.Keshavarz Blvd, Iran
e-mail: niakan@shahed.ac.ir, tel.: 0098 21 8894792

Introduction: Tea is old traditional drink that prepare from leaf of Camellia snensis plant. Based on the processing method, teas are divided in three types; Green tea is a non fermented type of teas, semi - fermented is Oolong tea and fermented type is Black tea .Several different health benefits such as antioxidant, anti carcinogenic and antibacterial effects have been reported to green tea. Dental caries is a most common infectious disease. Without a proper treatment, it can be a troublesome process. Streptococcus mutans are caries producing microorganisms. Material and methods: by an experimental study of aerial parts of camellia sinensis were collected fro Lahijan Province, Iran. The methanol and aquatic extracts of green and black tea were examined on streptococcus mutans (ATCC 35668). By serial dilution of extract were tested by using Disk diffusion and well assay methods. MIC and MBC were determined by using macro broth dilution method. The agar dilution method recommended by CLSI standards was used. The 3-way ANOVA was used for statistical data. Results: The Iranian green tea and black tea had antibacterial effects on 100 to 400 mg/mL concentration. The anti streptococcus mutans activity of methanol extract of black tea was more than effects of green tea. The difference was significant ($P<0.05$). Conclusion: the Iranian green tea and black tea have anti streptococcus mutans effects. The anti streptococcus activity of black tea is greater than green tea.

Key words: Green tea, Black tea, Antibacterial effects, Streptococcus mutans.

Whole genome fingerprinting of 8 strains from the genus Treponema

L. Mikalová[1], M. Strouhal [1,3], D. Čejková[1,3], M. Zobaníková[1], P. Pospíšilová[1], S. J. Norris[3], E. Sodergren [3], G. M. Weinstock[1], D. Šmajs[1]

[1] Department of Biology, Faculty of Medicine, Masaryk University, Kamenice 5, Building A6, 62500 Brno, Czech Republic CZ
[2] Department of Pathology and Laboratory Medicine, University of Texas-Houston Medical School, Houston, USA (3)The Genome Center, Department of Genetics, Washington University School of Medicine, St. Louis, USA, e-mail: lmikal@med.muni.cz, tel.: 00420549492366

Treponema pallidum subsp. pallidum (TPA) causes sexually transmitted disease syphilis, T. p. pertenue (TPE) causes yaws and T. p. endemicum (TEN) is the causative agent of endemic syphilis. The different degree of invasiveness and pathogenicity of these spirochetes reflects differences in their genomes. The goal of this work was to identify these genome differences. Eight Treponema pallidum strains were compared by whole genome fingerprinting (WGF). We used 4 TPA strains – Nichols, DAL-1, Mexico A, SS14; 3 TPE strains - Samoa D, CDC-2, Gauthier and one unclassified strain Fribourg-Blanc isolated from baboon. Based on WGF results, genome sizes and genetic relatedness of studied treponemal strains were estimated. The genome size varied between 1039.2 – 1040.9 kb with genome sequence identity of 99,5% and higher. Although TPA and TPE strains are highly similar, the genomes differ in 6 chromosomal regions including 4 deletions (33 – 377 bp) and 2 insertions (52 bp and 377 bp). The simian strain Fribourg-Blanc was similar to TPE strains suggesting its relatedness to TPE strains. Genes TP0433-434 and TP0470 contained repetitive sequence with strain specific number of repetitions. Five strains (Nichols, Samoa D, DAL-1, Gauthier and Fribourg-Blanc) showed unique genome differences, which can be useful in diagnostics and epidemiology. Most of the observed differences were localized in tpr loci and in the vicinity of these loci, suggesting their possible role in the pathogenicity of Treponema pallidum.

This work was supported by grants 310/07/0321 from the Grant Agency of the Czech Republic.

Clinicopathological and immunological studies on Toxoid Vaccine as a successful alternative in controlling clostridial infection in broilers.

S.Fathalla [1], N. Saleh [2], R. Nabil [3] and A. Mosaad [2]

[1] Department of Physiology, Faculty of Veterinary Medicine, Menufiya University, El-Sadat Branch, Egypt;
[2] Department of Clinical Pathology,
[3] Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Menufiya University, El-Sadat Branch, Egypt.
e-mail: saidfathalla@yahoo.com, tel.: (+20)1655-45680

Three vaccination regimes based on Clostridium perfringens type A,C or combined AC toxoids were evaluated to detect if toxoid vaccines can prevent necrtotic enteritis (NE) caused by clostridial infection in broilers. The vaccines were administered two times at two weeks interval, then the birds were challenged with virulent strains of C. perfringens type A, C or combined AC. Evaluating parameters included clinical signs, gross intestinal lesions, hemogram ,serum biochemical assays [total protein(TP), albumin(Alb), globulin(Glob), albumin globulin ratio(A/G), serum activities of alanine amino transferase(ALT), aspartate amino transferase(AST), alkaline phosphatase(ALP) and uric acid(UA) values] and ELIZA test for detecting serum antibody titers. Results revealed that affected birds showed marked depression, anorexia, reluctance to move, ruffled feathers and diarrhea. Numbers of chickens with intestinal lesions in immunized challenged groups were greatly fewer than the infected non immunized ones. There was an increase in RBCs, PCV and Hb. TLC decreased in infected non immunized birds and increased in vaccinated ones. Heterophils were increased in infected groups while, lymphocytes decreased. Prominent lymphocytosis was observed in immunized birds. There was a significant increase in TP,Alb,Glob,ALP,ALT,AST and UA and decrease in A/G. Results of ELIZA test showed that there was a significant increase in antibody titer after immunization paricularly after the second dose of vaccination. The combined AC toxoid provided the greater antibody titer and best protection followed by toxoidA and finally toxoidC. We concluded that results provide an evidence that immunization of broilers with toxoid vaccines paricularly the combined type AC is safe, well-tolerated and can protect broiler chickens against NE after the second booster dose of the vaccine.

Modulation of virulence and antibiotic susceptibility of enteropathogenic *E. coli* strains by *Enterococcus faecium* probiotic strain culture fractions

L.-M. Ditu[1], M.C. Chifiriuc[1],E. Bezirtzoglou[2], C. Voltsi[2], C. Bleotu[3], D. Pelinescu[1], I. Sarbu[1], G. Mihaescu[1], V. Lazar[1]

[1]University of Bucharest, Faulty of Biology, Microbiology Department, Aleea Portocalelor nr. 1-3, Sector 6, Bucharest, Romania
[2] Democritus University of Thrace, Department of Agricurtural Development;
[3]Stefan Nicolau" Institute of Virology,e-mail: lia_mara_d@yahoo.com, tel.: +0040213181576

The increasing rate of antimicrobial resistance drastically reduced the efficiency of conventional antibiotics and led to the reconsideration of the interspecies interactions in influencing bacterial virulence and response to therapy. The aim was to investigate the influence of the soluble and cellular fractions of Enterococcus faecium CMGB 16 probiotic culture on the virulence and antibiotic resistance markers expression in clinical enteropathogenic E. coli strains. The 7 enteropathogenic E. coli clinical, one standard E. coli ATCC 25922 and one Bacillus cereus strains were cultivated in nutrient broth, aerobically at 37oC, for 24 h. The Enterococcus faecium CMGB16 probiotic strain was cultivated in anaerobic conditions, at 37oC in MRS (Man Rogosa Sharpe) broth, and co-cultivated with different pathogenic strains (Bacillus cereus and Escherichia coli O28) culture fractions for 6 hours, at 37^{o}C. After co-cultivation, the soluble and cellular fractions were separated by centrifugation (6000 rpm, 10 min.), heat-inactivated (15 min., 100oC) and co-cultivated with the clinical entheropatogenic E. coli strains in McConkey broth, for 24 h, at 37^{0}C, in order to investigate the influence of probiotic fractions on the adherence capacity and antibiotic susceptibility. All tested probiotic combinations influenced the adherence pattern of E. coli tested strains. Susceptibility to aminoglycosides, beta-lactams and quinolones antibiotics was increased by all probiotic combinations and decreased only for amoxicillin-clavulanic acid. This study demonstrates that the plurifactorial anti-infective action of probiotics is also due to the modulation of virulence factors and antibiotic susceptibility expression in E. coli pathogenic strains.

Screening for lactobacilli with probiotic properties in the infant gut microbiota

K. Kirtzalidou[1], P. Pramateftaki[2], M. Kotsou[1], A. Kyriacou[1]

[1]Department of Dietetics and Nutritional Science, Harokopio University, 70 El. Venizelou str., 17671 Kallithea, Greece
[2] Wine Institute of Athens, National Agricultural Research Foundation (N.AG.RE.F.), 1 Sofokli Venizelou Str., 14123 Lykovrissi, Greece, e-mail: mkyriacou@hua.gr,: Kyriacou Adamantini, tel.: +302109549142

Lactobacilli are believed to be beneficial for the human hosts and are currently being evaluated as potentially probiotic bacteria. In this study, Lactobacillus strains were isolated from infant faeces and were examined in vitro for potential probiotic properties. Faecal specimens from 63 healthy, full-term infants were collected at 4, 30 and 90 days after delivery. Seventy-four Lactobacillus strains were isolated and one or more different phenotypes from each infant (n=44) were selected for further testing. The isolated strains were identified mainly as L. johnsonii, L. crispatus, L. paracasei, L. salivarius, L. fermentum after amplification of 16rDNA and sequencing. The strains were examined for acid and bile tolerance, adhesion to Caco-2 cells, antibiotic susceptibility and antimicrobial activity against selected enteric pathogens. The great majority of the isolated lactobacilli were susceptible to ampicillin, amoxicillin/clavulanic acid, tetracycline, erythromycin, cephalothin, chloramphenicol and rifampicin. Resistance to vancomycin or bacitracin was detected to 41% of the strains. Twenty-two strains out of forty-four exhibited significant tolerance to bile salts. Those strains were subsequently tested for resistance to low pH conditions (pH2 and 3). Interestingly, 96% (21 strains) of the tested lactobacilli remained unaffected at pH 3 after three hours of incubation, 21 strains were found resistant at pH 2 after 1.5 hours and only 10 strains remained viable after three hours of incubation. Approximately 40% of the strains were able to adhere to Caco-2 cells. In conclusion, at least two isolates fulfilled the in vitro probiotic criteria and are good candidates for further in vivo evaluation.

Antibodies to *Toxoplasma gondii* in Schizophrenia Patients

A. Daryani, S.H. Hosseini, M. Sharif, S.A. Karimi

Mazandaran University of Medical Sciences, 18th Km of Khazar abad Road, Sari, Iran, e-mail: daryanii@yahoo.com, tel.: 0098-9111587234

Schizophrenia is a severe neuropsychiatric disorder of unknown etiology. As there is few information about the epidemiology of Toxoplasma gondii and schizophrenia in Iran, we investigated the seroprevalence of T. gondii in these patients and compared with that obtained in control individuals in Sari city, Iran. Eighty schizophrenia patients and 99 healthy people were examined for the presence of IgG and IgM antibodies to T. gondii by Enzyme linked immunosorbent assay (ELISA). Prevalence rates of anti- T. gondii antibodies (IgG/IgM) in case and control groups were 72.5% and 61.6%, respectively (P>0.05). IgG antibodies indicating chronic form of toxoplasmosis were found in 28 (35%) and 25 (25.3%) of case and control people (P>0.05). IgM antibodies (acute form) were also seen in 9 (11.2%) and 11 (11.1%) of case and control individuals (P>0.05). The highest 10th percentile of IgG titers in schizophrenia individuals (18.8%) was significantly higher than control people (6.1%, P=0.02). Conclusion: As prevalence rate of T. gondii antibodies in patients with schizophrenia was high, it seems that designing a cohort study will determine the causative relation between Toxoplasma infection and schizophrenia disease.

Prevalence and susceptibility of *Saccharomyces cerevisiae* causing vaginitis in Greek women.

V. Papaemmanouil [1], N. Georgogiannis [2], M. Plega [1], E. Lambrou [1], D. Lidakis [1], M. Dimitriou [2], A. Papadimitriou [2]

[1] Microbiological Laboratory of ANTICANCER HOSPITAL, Elikonos 18 Piraeus 18540, Greece;
[2] Obstetrics and Gynecology ,GENERAL HOSPITAL OF PIRAEUS,
e-mail: vpapaemmanouil@yahoo.gr, tel.: 6974903549

Saccharomyces cerevisiae is an ascomycetous yeast, that is traditionally used in wine bread and beer production . Vaginitis caused by Saccharomyces cerevisiae is rare. The aim of this study was to evaluate the frequency of Saccharomyces cerevisiae isolation from the vagina in two groups of women and determined the in vitro susceptibility of this fungus. Vaginal samples were collected from a total of 262 (asymptomatic and symptomatic) women with vaginitis attending the centre of family planning of General hospital of Piraeus. All blastomycetes that isolated from the vaginal samples were examined for microscopic morphological tests and identified by conventional methods : By API 20 C AUX and ID 32 C (Biomerieux). Antifungal susceptility testing for amphotericin B,fluconazole itraconazole ,voriconazole, posaconazole and caspofungin was performed by E –test (Ab BIODIKS SWEDEN) against saccharomyces cerevisiae. A total of 16 isolates of Saccharomyces cerevisiae derived from vaginal sample of the referrd women, average 6,10 %. Susceptibility of 16 isolates of Saccharomyces cerevisiae to a variety of antimycotic agents were obtained. So all isolates of Saccharomyces carevisiae were resistant to fluconazole, posaconazole and intraconazole, but they were sensitive to voriconazole caspofungin and Amphotericin B wiche were found sensitive (except 1/16 strains). None of the 16 patients had a history of occupational domestic use of baker's yeast. Vaginitis caused by Saccharomyces cerevisiae occure, is rising and can not be ignored. Treatment of Saccharomyces vaginitis constitutes a major challenge and may require selected and often prolonged therapy.

Study of the relationships between infectious agents of cardiovascular diseases, prosthetic devices and the eukaryotic cell

O. Banu, C. Chifiriuc, C.Bleotu, V. Lazar, T. Sesan, L. M. Ditu, L. Marutescu, M. Bucur

Faculty of biology, aleea portocalelor nr. 1-3, sector 6, Bucharest, Romania
e-mail: otiliabanu@gmail.com, tel.: 0040722265365

Purpose: To identify the relationships between some infectious agents of cardiovascular diseases with the cellular substrate and prosthetic devices in the presence of antibiotics. Specific objectives: strains isolation and identification, study of antibiotic resistance of bacterial cells in suspension (disk diffusion, E-test, automatic systems) or adhered to the substrate (using original experimental models for monospecific biofilm in vitro development) and virulence assay (adherence and invasion of HeLa cells, slime test, soluble virulence factors expression); study the dynamics of biofilm formation on inert substrates, under the influence of antibiotics; the study of bacterial fractions and HeLa cells interactions (by CLSM, TEM, fluorescence microscopy, flow cytometry, real-time PCR). The identified strains were isolated from diverse sources, the etiology being dominated by Gram-negative non-fermentative bacilli, Gram-positive cocci and yeasts, expressing invasion enzymes that could explain their ability of producing systemic infections. The isolated strains exhibited a high level of antibiotic resistance to beta-lactams, aminoglycosides and quinolones, regardless of the analyzed species and a marked tendency to colonize the cellular and inert substrate, the degree of colonization depending on the physico-chemical nature of the substrate. Bacterial strains grown in biofilms expressed a changed profile of antibiotic resistance, this aspect being very important in addressing treatment and prevention of infections associated with prosthetic devices. In vitro experiments showed that different fractions of S. aureus bacterial cultures triggers thr release of proinflammatory (TNF-α, IL-1b, IL-6) and anti-inflammatory (IL-8) cytokines in HeLa cells and induced eukariotic cell apoptosis.

Alternative Approaches to Combat Endogenous Hospital Infections by Modulating of Host Microbiota

Invited Lectures

Colonization resistance: Reality or myth

Tore Midtvedt

Department of Microbiology, Tumor and Cell Biology (MTC)
Karolinska Institute, Stockholm, Sweden

Simplified - all ecosystems can be defined as co-operations between various species or strains present in a defined area. An ecosystem is never in balance, but always in some sort of a balanced unbalance between its member, and a variable degree of resistance to a new-comer seems to be a general feature.
Together with its host, the intestinal microbiota in exert a long variety of functions; among those also a capability to resist establishment of q new comer. Over the years, this capability has been called ”natural resistance, ”control function”, colonization resistance”, etc.; the latter being the one most commonly used. In spite of thousands of publications in PUB-Med, there is no agreement for a definition of this capability.
Summarizing previous attempts – and in order to generalize – the following two definitions are to be discussed: *Colonization resistance” (CR)* is a function to be found in all ecosystems, representing a sum of factors inhibiting a new comer to be established.
Colonization conductance” (CC) is a function to be found in all ecosystems, representing a sum of factors allowing a new comer to be established.
Over the years, there have been several attempts to establish convenient biomarkers for measurement of CR, but so far, with little, if any, success. Attempts have also been made for demonstration of increased intestinal CR against pathogens following ingestion of probiotics and so far with little success.

In summary: agreement should be made for definition of CR and CC. Proper biomarkers for measurement of CR and CR should be worked out. Priobiotics should be screened for their capability to influence upon CR and CC.

Human microbial ecology: environmental, nutritional and medical factors

M. Mikelsaar

Department of Microbiology, Medical Faculty, University of Tartu. Ravila 19, Tartu 50411, Estonia
e-mail: marika.mikelsaar@ut.ee, tel.: +3727374179

Evidence based understanding of human microbial ecology, particularly the variability of lactic acid bacteria has rapidly widened due to applied bacteriological, biochemical and molecular methods. The studies on environmental factors bound to life-style diseases (allergy, metabolic syndrome, overweight) have shown a close association with formation of infants´ gut microbiota, incl. lactic acid bacteria. The revealed differences in species composition of Lactobacillus in children, adults and seniors are clearly connected on one hand with geographical and socio-economic influences, yet from the other hand with particular diet and nutrition. Recently the beneficial impact of consumption of fermented with lactobacilli products on the higher count of intestinal lactobacilli and the lower values of blood atherogenicity indices (ox-LDL) of elderly has been proved in our laboratory. In microbial ecology variable environmental factors like food contamination with some pathogens, high load of antibiotics in soil and animal feed, application of broad-spectrum antimicrobial preparations by medical doctors are damaging the microbial ecology of humans from medical standpoint. To correct the imbalance of microbiota composition and the tightly associated with it health markers, the application of functional food, incl. probiotics have been suggested. However, in probiotic treatment the large differences of human microbial ecology caused by environmental, nutritional and medical issues need the evidence based efficacy approvement using well designed clinical trials. Moreover, in selection of probiotic Lactobacillus species for different age groups with particular health status their interconnection with some blood indices (glucose content, WBC count) of host should be considered.

Probiotic impact of the newborn and young children intestinal microflora

E. Bezirtzoglou and E. Stavropoulou

Democritus University of Thrace, Faculty of Agricultural Development, Department of Food Science and Technology,193, Pantazidou St, 68200 Orestiada,Greece,
e-mail: empezirt@agro.duth.gr

Human body has developed a holistic defense system, which mission is either to recognize and destroy the aggressive invaders or to evolve mechanisms permitting to minimize or restore the consequences of harmful actions.The host immune system keeps the capital role to preserve the microbial intestinal balance *via* the barrier effect. Specifically, pathogenic invaders such as, bacteria, parasites, viruses and other xenobiotic invaders are rejected out of the body *via* barriers formed by the skin, mucosa and intestinal flora. In case physical barriers are breached, the immune system with its many components comes into action in order to fence infection. The intestine itself is considered as an "active organ" due to its abundant bacterial flora and to its large metabolic activity.The variation among different species or even among different strains within a species reflects the complexity of the genetic polymorphism which regulate the immune system functions. Additionally factors such as ,gender, particular habits, smoking, alcool consumption, diet ,religion ,age ,gender, precedent infections and vaccinations must be involved. Hormonal profile and stress seems to be associated to the integrity microbiota and inducing immune system alterations.Which bacterial species are needed for inducing a proper barrier effect is not known, but it is generally accepted that this barrier function can be strongly supported by providing benefic alimentary supplements called functional foods. In this vein it is stressed the fact that early intestinal colonization with organisms such as lactobacilli and bifidobacteria and possibly subsequent protection from many different types of diseases .Moreover, this benefic microflora dominated but Bifidobacteria and Lactobacilli supports the concept of their ability to modify the gut microbiota by reducing the risk of cancer following their capacity to decrease β-glucoronidase and carcinogen levels.Because of their beneficial roles in the human gastrointestinal tract, LAB are referred to as "probiotics," and efforts are underway to employ them in modern nutrition habits with so-called functional foods.Members of *Lactobacillus* and *Bifidobacterium* genera are normal residents of the microbiota in the human gastrointestinal tract, in which they developed soon after birth. But, whether such probiotic strains derived from the human gut should be commercially employed in the so-called functional foods is a matter of debate between scientists and the industrial world .Within a few hours from birth the newborn develops its normal bacterial flora.Indeed human milk frequently contains low amounts of non-pathogenic bacteria like *Streptococcus, Micrococcus, Lactobacillus, Staphylococcus, Corynebacterium* and *Bifidobacterium*. In general, bacteria start to appear in feces within a few hours after birth. Colonization by *Bifidobacterium* occurs generally within 4 days of life. Claims have been made for positive effects of *Bifidobacterium* on infant growth and health. The effect of certain bacteria having a benefic action on the intestinal ecosystem is largely discussed during the last years by many authors. *Bifidobacterium* is reported to be a probiotic bacterium, exercising a

beneficial effect on the intestinal flora.An antagonism has been reported between *B. bifidum* and *C. perfringens* in the intestine of newborns delivered by cesarian section. The aim of the probiotic approach is to repair the deficiencies in the gut flora and restore the protective effect.However, the possible ways in which the gut microbiota is being influenced by probiotics is yet unknown.

Probiotics as biotherapeutics: how normal are our "normal" control laboratory animals?

E. Norin, PhD

Dept Microbiology Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden

A Swedish laboratory animal breeder experienced that their animals were infected with some pathogen bacteria occurring at the FELSA "black list"; How to handle this issue.

- Kill the animals and start a new breeding colony
- Give all the animals antibiotics
- Give all animals probiotics – and in this case – which probiotic strain(s) should be inoculated

We chosed to administer all females during the impedance measuring with a *Lactobacillus reuteri* strain and the males were inoculated on all genital areas with the same microbe, and at the same time all animals at the breeding unit were given these microbes in the drinking water. This handling was repeated once after four weeks. Thereafter, samples from animals were investigated in a blind fashion and the non-desired microbes were eliminated.

However, how normal are laboratory animals in use to-day, when kept under strict barrier conditions – are the SPF (specific pathogen free) conditions within the laboratory animal production units producing animals with "normal" functional intestinal microflora. Our findings call for a re-considering of the SPF concept.Over the years, we have worked with functional aspects of host-microbe interactions(s) in man and animals, and our aim has been to investigatethe activity of some intestinal microbial biochemical activities in laboratory mice harboring an ASF flora. Presence of different functions should be taken into consideration when rodents are used in the biomedical research. We have shown that SPF animals harbor an abnormal gut microbiome. Given the importance of mice in biomedical research and the dependence of host responses on the types of organisms present, a call for a re-considering of the SPF concept is needed. The time might have come for AALAS and FELASA to take a closer look into their SPF and ASF concepts. An adequate intestinal microbiota is far more than just freedom from some pathogens.

Important Questions on Food Hygiene plus Probiotics, Prebiotics, Symbiotic Approaches

Invited Lectures

Recent investigations and updated criteria for the assessment of antibiotic resistance in food lactic acid bacteria

L. Aquilanti and F. Clementi

Dipartimento di Scienze Alimentari, agro-Ingegneristiche, Fisiche, Economico-agrarie e del Territorio (SAIFET), Università Politecnica delle Marche, via Brecce Bianche, 60131, Ancona (Italy) f.clementi@univpm.it
e-mail: l.aquilanti@univpm.it, tel.: +390712204959

Food is generally recognized as the most important vector for spreading antibiotic resistance between man and animals. The greatest risk is associated with food-borne zoonotic agents, although viable micro-organisms used as active agents in feed and food additives may represent a risk for consumer health, as well. Among these latter, lactic acid bacteria (LAB) are Gram-positive micro-organisms, which play a key role in the production of a multitude of fermented food products, such as fermented sausages, sourdough leavened goods, dairy products, etc. Data from various studies demonstrate the existence of intergenus and interspecies differences in both the mechanism of resistance and the likelihood of antibiotic resistance transfer. As a general rule, lactobacilli, pediococci and leuconostocs show an intrinsic (or natural) resistance to some antibiotics of human or veterinary importance (f.i. vancomycin) whereas a minority of strains, even used as starter, adjunctive or probiotic cultures, exhibit transferable resistances to other antimicrobial agents (f.i. tetracycline and erythromycin). Since mobilization and exchange of transferable resistances genes from LAB to human bacteria is a safety concern, antibiotic-susceptibility assays relying on the use of internationally recognised and standardised methods have to be performed onto strains of technological interest, in accordance with the guidelines recently updated by EFSA (http://www.efsa.europa.eu/en/scdocs/scdoc/732.htm). As a basic requirement, the Minimum Inhibitory Concentration (MIC) of the antimicrobial should be preferably determined by microdilution tests and additional information about the genetic basis of the antimicrobial resistance provided.

Probiotics from an industrial perspective

S. Forssten.

Danisco Health and Nutrition, Kantvik, Finland

e-Mail : sofia.forssten@danisco.com

Probiotics defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO 2002), have been used for a long time as natural components in supplements and functional foods, mainly in fermented dairy products such as milk drinks and yoghurts. Most of the strains used as probiotics are from the genera Lactobacillus and Bifidobacterium and a strain has to have documented health benefits, in order to be called a probiotic. Although each bacterial strain is unique, there are some points that are essential when selecting a probiotic regarding the genetic stability, survival, and technical properties of a strain. Proper components and food matrices need to be selected since the matrices may affect the viability of the strain in the intestine, and survival is considered as a precondition for the beneficial effects of probiotics.

Probiotic potential of Lactobacillus casei ATCC 393: Gastrointestinal survival and modulation of intestinal microbial flora in Wistar rats

Marianthi Sidira[1], Alex Galanis[1], Petros Ypsilantis[2], Constantinos Simopoulos[2] and Yiannis Kourkoutas[1*]

[1]Applied Microbiology and Molecular Biotechnology Research Group, Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupolis, GR-68100, Greece

[2]Laboratory of Experimental Surgery and Surgical Research, School of Medicine, Democritus University of Thrace, Alexandroupolis, GR-68100, Greece

*E-mail: ikourkou@mbg.duth.gr

Nowadays an upsurge of interest in developing novel foods containing probiotic microorganisms, such as lactic acid bacteria (LAB), is observed. Such functional foods have a great potential in promoting human health, as a probiotic-rich diet is linked with the prevention and potential treatment of several severe enteropathogenic-derived diseases. In order to deliver the health benefits, probiotics need to contain an adequate amount of live bacteria (at least 106 cfu/g), able to survive the acidic conditions of the upper gastrointestinal (GI) tract and proliferate in the intestine, a requirement that is not always fulfilled. Since it is well established that cell immobilization enhances the viability of cultures, the aim of the present study was to assess the survival of both free and immobilized L. casei ATCC 393 on apple pieces, contained in probiotic fermented milk, after gastrointestinal (GI) transit and to investigate the potential regulation of intestinal microbial flora in a rat model. In in vitro GI stress tolerance tests, immobilized L. casei ATCC 393 exhibited significantly higher survival rates compared to free cells. At a second stage, probiotic fermented milk produced by either free or immobilized cells was administered orally at a single dose or daily for 9 days in Wistar rats. By 12h after single dose administration, both free and immobilized cells were detected by microbiological and molecular analysis at levels ≥ 6 logCFU/g of feces. Moreover, daily administration led to significant reduction of staphylococci, enterobacteria, coliforms and streptococci counts. In conclusion, L. casei ATCC 393 contained in fermented milk survived GI transit and modulated intestinal microbiota.

Microbial ecology and quality assurance in food fermentation systems. The case of kefir grains application.

S. Plessas [1]. , A. Alexopoulos[1] , C. Voidarou[1] , I. Mantzourani, S. Vavias, A Koutinas[2], E. Stavropoulou[1] and E.Bezirtzoglou[1]

[1]Democritus University of Thrace, Faculty of Agriculture Development, Laboratory of Microbiology, Biotechnology and Hygiene, 193 Pantazidou str. GR-68200, Orestiada, Greece, e-mail: splessas@agro.duth.gr

[2] Food Biotechnology Group, Department of Chemistry, University of Patras, Greece

Fermentation technology has become a modern method for food production the last decades as a process for enhancing product stability, safety and sensory standards. The main reason for this development is the increasing consumers' demand for safe and high quality food products. The above has led the scientific community to the thorough study for the appropriate selection of specific microorganisms with desirable properties such as bacteriocin production, and probiotic properties. The main food products produced through fermentation activity are bread, wine, beer cheese and other dairy products. The microorganisms conducting the above processes are mainly yeasts and lactic acid bacteria. The end-products of carbohydrate catabolism by these microorganisms contribute not only to preservation as it was believed years ago, but also to the flavour, aroma and texture and to the increase of the nutritional quality by thereby helping determine unique product characteristics. Thus, controlling the function of specific microorganisms or the succession of microorganisms that dominate the microflora is therefore advantageous, because it can increase product quality, product functionality and product value. Throughout the process of the discovery of microbiological diversity in various fermented food systems, the development of starter culture technology has gained more scientific attention, and it could be used for the control of the manufacturing operation, and management of product quality. In the frame of this review the presentation of the quality enhancement of most consumed fermented food products around the world is attempted and the new trends in production of fermented food products are discussed. The review is focused in kefir grains application in various fermented food production.

Keywords: fermentation, food, quality, microbiology, kefir grains

Food Microbial Ecology - Veterinary

Oral Presentations

Ecology of avian pathogenic Escherichia coli in food

V.Kmet and M. Kmetova

Institute of Animal Physiology, Slovak Academy of Sciences, Soltesovej 4, 040 01 Kosice, Slovakia, Email: kmetv@saske.sk; *Institute of Medical Microbiology and Clinical Microbiology, P. J. Safarik University, Faculty of Medicine, Kosice, Slovakia

Virulence factors from avianpathogenic E.coli (APEC) might be important as a carrier for humanuropathogenic E.coli. APEC ussually contain the various virulence factors e.g. iut-receptor for aerobactin, iss-increased serum survival, cvaC-colicin V, kps-capsular polysialic acid virulence factor, tsh-temperature sensitive haemagglutinin and ibeA-invasive factor responsible for neonatal meningitis.
The aim of study was to examine the presence of virulence factors and antibiotic resistance in 41 ciprofloxacin resistant Escherichia coli isolated from poultry meat, during one year. Two combinations of five APEC virulence genes iutA, iss, cvaC, tsh, papC or iutA, iss, kpsII, tsh, ibeA were recorded. Ciprofloxacin resistant strains had also the high occurrence of resistance to ampicillin (87%), ceftiofur (45%), gentamicin (22%) and cotrimoxazol (79%). CTX-M15 and CMY-2 betalactamases were recorded by PCR and DNA sequencing, also. Plasmid mediated quinolone resistance was not detected. Results shows that virulent and antibiotic resistant Escherichia coli, may represent a newly recognized group of medically significant foodborne pathogens.

This study was supported by Slovak grants APVV-0028-07 and VEGA 1/0495/10.

The influence of mannan oligosaccharides, acidifiers and their combination on caecal microflora of Japanese quail (Coturnix japonica)

E.Bonos [1], E. Christaki[1], A. Abrahim[2] , N.Soultos [2], P. Florou-Paneri[1]

[1] Laboratory of Animal Nutrition, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece, e-Mail : ppaneri@vet.auth.gr

[2] Laboratory of Hygiene of Foods of Animal Origin, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece

The aim of this study was to investigate the effects of the dietary supplementation of mannan oligosaccharides (MOS) extracted from yeast Saccharomyces cerevisiae, acidifiers - calcium formate (CF), calcium propionate (CP)- and their combination on the caecal microflora of Japanese quail (Coturnix japonica). Four hundred fifty 1-day old quail where divided in six groups with three replicates each. One group that served as control received the basal diet. The five experimental diets consisted of the basal diet to which either 1 g MOS/kg, or 6 g CF/kg, or 6 g CP/kg, or 1 g MOS plus 6 g CF/kg or 1 g MOS plus 6 g CP/kg were added. The body weight was examined at weekly intervals and mortality was recorded daily. At days 21 and 42 of age, the total count of aerobic bacteria, lactic acid bacteria, enterobacteriaceae and coliforms in the caecal content of one bird of each replicate was determined. Also, at day 42 of age, two birds of each replicate were slaughtered and their carcass weight was determined. The results showed that MOS significantly ($P \leq 0.050$) increased the total aerobic plate and lactic acid bacteria counts on day 21. Furthermore, CP significantly ($P \leq 0.050$) decreased the total aerobic plate and lactic acid bacteria counts compared to controls on day 21. Significant interaction between MOS and acidifiers was noticed on total aerobic plate count on day 21. No significant ($P > 0.050$) difference was found in the caecal microflora on day 42. Finally, no significant ($P > 0.050$) difference was noticed on mortality, body and carcass weight.

Keywords: quail, mannan oligosaccharides, acidifiers, calcium formate, calcium propionate, caecal microflora.

Chemical and microbiological characterization of artisan inoculants used for the fermentation of traditional dairy products in Epirus area, Greece

C. Voidarou[1], A.Tzora[1], K.Demertzi- Akrida[2], D. Vassos[1], G. Rozos[3], A. Alexopoulos[3], S. Plessas[3], K. Fotou[1], E. Stavropoulou[3], J. Skoufos[1] and E. Bezirtzoglou[3]

[1]Technological Institute of Epirus, Department of Microbiology, Arta and Public Health School of Athens
[2]University of Ioannina, Department of Chemistry
[3]Democritus University of Thrace and Faculty of Agricultural Development, Department of Food Science and Technology, Laboratory of Microbiology, Biotechnology and Hygiene, Orestiada, Greece

Traditional dairy products in Epirus area, Greece, have been the pride of culinary tradition for centuries. It is evident that these products have played a major role in the diet of the local rural communities. Starter cultures play an important role in the fermentation process of raw milk and highly affect the sensory profile, the nutritional value and the physical properties of these products.
This study was concerned with the chemical composition, flavor components and microbiological parameters of dried artisan inoculants used in the fermentation of traditional dairy products in Epirus area, Greece. The dehydrated rumen of young lambs, which is called “pytia” (rennet), is used commonly for fermention of dairy products, because of its microbial load on beneficial bacteria and its content of certain enzymes (e.g. rennin).
In this study we analysed 50 samples of «rennet» («pytia») which is a traditional Greek product prepared from the dried stomach’s wall of small ruminants and used as a coagulant in cheese manufacture from raw milk with natural microflora.
We also analyzed another 50 samples of artisan made rennet from thermophile yoghurt culture consisting of a mixture (1:1) of Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus (Chr. Hansen’s Laboratorium, Copenhagen, Denmark). The culture was reactivated in raw milk and used at a level of 0.75% (w/w).
The viable numbers of the total (mesophilic) microflora, enterococci, lactococci, lactobacilli, enterobacteria, staphylococci, pseudomonads, yeasts and moulds were determined by the standard plate counting method on specific media.
Solid residue, NaCl, total protein and acidity, pH was determined. Nitrogen content and dry matter were also evaluated.
Lactic acid bacteria (LAB) and yeasts present in traditional “pytia” were responsible for lactic acid fermentation and aroma development. The LAB and yeast counts in traditional “pytia” were higher than that in the yoghurt derived rennet.
Samples from the traditional rennet were found to have a high variation in their chemical composition. The microbiological tests revealed that there were high amounts of total bacteria, yeast and moulds, lactic acid bacteria, lipolytic bacteria, proteolytic bacteria and also coliforms in comparison to the yoghurt derived rennet.

Physical, chemical and microbiological quality of ice used to cool drinks and foods in Greece and its public health implications

Gerokomou[1], C.Voidarou[1], A. Vatopoulos[1], E.Velonakis[1], A.Tzora[1], J.Skoufos, G.Rozos[3], A. Alexopoulos[3], S. Plessas[3], E. Stavropoulou[3], E.Bezirtzoglou[3], P.G. Demertzis[2] and K.Demertzi-Akrida[2]

[1]Technological Institute of Epirus, Department of Microbiology, Arta and Public Health School of Athens
[2]Section of Industrial and Food Chemistry, Department of Chemistry, University of Ioannina, Panepistimioupolis, GR-45110 Ioannina, Greece
[3]Democritus University of Thrace and Faculty of Agricultural Development, Department of Food Science and Technology, Laboratory of Microbiology, Biotechnology and Hygiene, Orestiada, Greece

Ice used for direct human consumption or to refrigerate foods and drinks can be contaminated with pathogenic microorganisms and may potentially become a vehicle for consumer's infection. To evaluate physical, chemical and microbiological quality of commercial ice and ice used to refrigerate fish and seafood, 100 ice samples collected at 10 deferent retail points in the region of Epirus were studied. Sampling took place during the first five months of 2009. The following microbiological parameters were determined: Total coliforms, fecal coliforms, Salmonella spp., Shigella spp., Yersinia spp., E. coli, Vibrio cholerae and Aeromonas spp.. Pseudomonas aeruginosa, Clostridium perfringens.
E.coli was detected in 11 % and coliforms were detected in 31% of samples.
Samples in which coliforms were detected fail to meet the microbiological criteria specified by the drinking water legislation.
Aeromonas spp., Shigella spp. and *V. cholerae* were not detected. Spore forms of *C. perfringens* were persistent at 35% and the psychotropic bacteriums *Pseudomonas aeruginosa* and *Yersinia* spp. were found only at three samples each.
The presence of high numbers of coliforms as well as of other pathogenic strains suggested that commercial ice and ice used to make cool drinks and used to refrigerate fish and seafood may represent a potential hazard to the consumer. In view of the results herein reported, it is highly recommended that national regulatory guidelines should be established for the production of ice as long as regular inspections.

Analysis of bio-availability of vitamin K2 in fresh cheese

S. Forssten[1], P.Fourcassié[2], A. Ouwehand[1]

[1]Danisco Finland, Sokeritehtaantie 20, Kantvik, Finland
[2]Danisco France, Dangé Saint Romain, France, e-mail: sofia.forssten@danisco.com, tel.: +358405367810

Vitamin K is an essential cofactor involved in blood clotting functions, cell growth, and bone mineralization. Vitamin K recently received a positive opinion from EFSA concerning bone health. Vitamin K occurs naturally in two forms; plants synthesize phylloquinone, also known as vitamin K1, while bacteria synthesize menaquinones (MK-n), referred to as vitamin K2. Vitamin K2 is produced by the intestinal microbiota, but is poorly absorbed from the colon. Vitamin K2 is furthermore found in fermented foods like cheese and curd. The aim of the study was to determine the bio-availability of vitamin K2, produced by Lactococcus lactis in fresh cheese, for the host. A daily dose (100 g) of fresh cheese underwent simulated digestion in the upper gastrointestinal tract. The digestion and absorption in the upper gastrointestinal tract was simulated with different digestive enzymes and acids, incubation and centrifugation. Samples were collected from the different steps and subjected to vitamin K2 analysis. The in vitro simulation showed that the pellet after the centrifugation step in the simulation contained most of the vitamin K2, while less than 0.05% of the vitamin K2 amount was detected in the supernatant and hence it can be concluded that vitamin K2 was available to the host in the colon after simulated passage through the upper gastrointestinal tract.

Effect of diet quality on the gut bacterial communities of the Norway lobster

A. Meziti, E.Mente and K.Kormas

School of Agricultural Sciences, Department of Ichthyology and Aquatic Environment, University of Thessaly, Fytoko, GR-38446, Nea Ionia, Greece
e-mail: ameziti@uth.gr, tel.: 0030-24210-93237

Knowledge on the gut microbiome of aquatic animals has progressed with the use of molecular analysis in recent years. Although gut microbial diversity could be influenced by nutrition or environmental factors few data is available that experimentally confirm that. The aim of this study was to examine the gut bacterial communities of the Norway lobster Nephrops norvegicus, kept in laboratory conditions, using 16S rRNA gene diversity analysis. N. norvegicus specimens were collected from Pagasitikos Gulf (Greece) and were kept in captivity in tanks for 6 months. Water temperature, salinity and photoperiod were maintained at 11±1°C, 330 ppm and 24 h darkness, respectively, reflecting in situ conditions. After a period of a 15-day acclimatization period, the animals were divided into three groups. Groups I and II were fed frozen mussels (natural feed) and fish pellets (synthetic feed), respectively, three times per week, while Group III was fasted. Samples for 16S rRNA gut bacteria diversity analysis were collected at the sampling day (natural population, t0), 15 days (end of acclimatization period, t1), three months (t2) and six months (t3). Water samples from the tanks were collected for 16S rRNA diversity analysis two days before the above sampling points. Samples collected at t2 and t3 showed similar gut bacterial community composition in mussels fed and starved animals. Only few phylotypes were common with the ones detected at the initial time points (t0 and t1), showing a significant shift in the gut bacterial communities between natural and cultured populations.

A double-blind, placebo-controlled, crossover study to establish the bifidogenic effect of a very long chain inulin extracted from globe artichoke in healthy humans

A. Costabile, S. Kolida, A.Klinder, E.Gietl, M. Bäuerlein, C. Frohberg, V. Landschütze and Glenn R. Gibson

Food Microbial Sciences, School of Food and Nutritional Sciences, University of Reading, Reading, United Kingdom
e-mail: a.costabile@reading.ac.uk, tel.: 0044(0)1183786218

There is growing interest in the use of inulins as substrates for the selective growth of beneficial gut bacteria such as bifidobacteria and lactobacilli because recent studies have established that their prebiotic effect is linked to several health benefits. In the present study, the impact of a very long chain inulin, derived from globe artichoke (VLCI), on the human intestinal microbiota compared to maltodextrin was determined. A double-blind, crossover study was carried out in thirty-two healthy adults who were randomised into two groups and consumed 10 g/d of either VLCI or maltodextrin, for two 3-week study periods, separated by a 3-week washout period. Numbers of faecal bifidobacteria and lactobacilli were significantly higher upon VLCI ingestion compared to the placebo. Additionally, levels of Atopobium group significantly increased, while Bacteroides-Prevotella numbers were significantly reduced. No significant changes in faecal SCFA concentrations were observed. There were no adverse gastrointestinal symptoms apart from a significant increase in mild and moderate bloating upon VLCI ingestion. These observations were also confirmed by in vitro gas production measurements. In conclusion, daily consumption of VLCI extracted from globe artichoke exerted a pronounced prebiotic effect on the human faecal microbiota composition and was well tolerated by all volunteers.

In vitro evaluation of the fermentation properties and potential prebiotic activity of *Agave fructans*

A. Klinder, G.R. Gibson and A. Costabile
Food Microbial Sciences, School of Food and Nutritional Sciences, University of Reading, Reading, UK e-Mail : a.costabile@reading.ac.uk

Agave is a crassulacean plant and in adaptation to its arid or semi-arid environment uses crassulacean acid metabolism to fix CO2 during photosynthesis. Agave tequilana is grown mainly in the state of Jalisco, Mexico and exhibits a higher carbohydrate content (mostly fructans) compared to that reported for chicory. The aim of this study was to evaluate Agave as a potential source of prebiotics by testing the bifidogenic properties of fructans extracted from Agave tequilana Weber var. azul (Predilife) in batch culture fermentations systems. We compared the ability of Agave fructans to selectively increase the number of bifidobacteria and alter colonic metabolic output to four different commercial prebiotic brands and cellulose as a control. Measurement of prebiotic efficacy was obtained by comparing bacterial changes, and the production of short-chain fatty acids (SCFA) was also determined. The work presented here is the first in vitro study to investigate the influence of the fermentation of Agave fructans on a complex faecal microbiota. Inulin derived from Agave significantly increased the growth of bifidobacteria and lactobacilli. The magnitude of this effect was similar to the ones observed for established inulin-type prebiotics derived from chicory root thus justifying the potential of Agave as a prebiotic.

Antimicrobial Resistance Patterns of *E.coli* Strains Isolated from Bloodstream Infections in Erciyes University Hospital

S. Durmaz[1], A.Kiraz[1], D.Percin[1] M.Doganay[2]

[1] Department of Microbiology
[2] Department of Infectious Diseases, Faculty of Medicine, Erciyes University, Kayseri, Turkey, e-mail: sdurmaz@erciyes.edu.tr, tel.: +905054283173

Background: Nosocomial bacteremia is a major cause of hospital- acquired infections and Escherichia coli strains, especially extended spectrum β-lactamase (ESBL) producing ones, are frequently isolated from hospitalized patients. The presence of bacterial resistance is a problem for treatment and control of infections. Throughout the world, increasing attention is being focused on the growing involvement of ESBL producing strains of E.coli. The aim of this study is to compare the in vitro antibiotic resistance patterns of ESBL producing and non-producing E.coli. Methods : Blood cultures collected from adult patients in several intensive care units were studied with BacT / Alert (BioMerieux, France) automatised blood culture system in Erciyes University Hospital, between 2007 –2010. Only one positive blood sample of every patient were included in the study. All positive blood samples were cultured in blood agar, EMB agar and chocolate agar plates. Bacteria isolated from blood specimens were identified by using routine microbiological methods. The susceptibility to various antibiotics of the strains and presence of ESBL were investigated using the Kirby–Bauer disk diffusion method. Results were interpreted according to CLSI breakpoints. Results : Eighty one E.coli strains were isolated from blood samples in three years. Among these, 44(% 54.3) strains were ESBL-producing E.coli. Among ESBL-producing E.coli strains, resistance to amikacin found in 7 strains (% 16), to ciprofloxacin in 36 strains (% 81.8), to ampicillin in 44 strains (% 100), to piperacillin-tazobactam in 14 strains (% 31.8). All ESBL-producing E.coli strains were susceptible to imipenem. Among ESBL-non producing E.coli, resistance to amikacin found only in one strain (% 2.7), to ciprofloxacin in 10 strains (% 27), to ampicillin in 25 strains (% 67.5), to piperacillin-tazobactam in 8 strains (% 21.6). All of the ESBL-non producing E.coli strains were susceptible to cefotaxime and imipenem. Conclusions: More than half of the E. coli strains isolated from ICUs are ESBL producing strains. ESBL producing strains are more resistant to amikacin, ciprofloxacin and piperacillin-tazobactam than ESBL non producing ones. All strains are susceptible to imipenem.

A consideration of fecal streptococcus contamination of hamburgers presented in Tbariz market

M.Golabi[1], A. Namvaran[1], A. Javadi[2], S. Meshkini[3]

[1] Student of Veterinary medicine, Islamic Azad University of Tabriz, member of young researcher club
[2] Assistant prof.of Food Hygiene Department, Veterinary faculty, Islamic Azad University of Tabriz
[3] Expert of food Hygiene Department, Veterinary Faculty, Islamic Azad University of Tabriz, e-mail: dr.m.golabi@gmail.com, tel.: +984113344674

Nowadays, health of food is one of important aspects of social health and bacterial evaluate of food is one of the health indexes. Fecal streptococcus is one of the important indications of food health. The aim of this study is recognition the fecal streptococcus in presented hamburgers in Tabriz market. Method: 20 samples (hamburger) collected randomly from 4 regions of Tabriz. Samples diluted until 10-3 then the two end dilutions cultured orderly in KF- streptococcus agar (37°c-24h), BHI (37°c-24h), BHI (44.5°c-24h), salty BHI (37°c-24h) and finally Bile Scolin Agar (37°c-24h). Results: The results showed that 15 samples were contaminated, and in 5 samples the fecal streptococcus was not detected. Totally the average contamination of 20 samples that were considerate in this study was . Conclusion: This result indicates that hygiene standards are not observed during producing and preparing the hamburgers.

Occurrence of microorganisms of public health significance in fruit juices sold in retail markets

P.Kokkinos, M.Affifi, M.Papapetropoulou, A.Vantarakis

Environmental Microbiology Unit, Department of Public Health, Medical School, University of Patras, GR 26500 Rion, Patras, Greece Tel: +30-2610-969875, Fax: +30-2610-969875, e-mail: avantar@med.upatras.gr**,** e-mail: pkokkin@med.upatras.gr, tel.: +30-2610-997935

Fruit juices are an important part of the modern diet in many countries. However, few data are available concerning the microbiological quality of the foods sold in Greece, and particularly fruit juices. Using standard microbiological procedures, we conducted a bacteriological survey of commercially sold, pasteurized fruit juices from retail markets. A total of 120 samples of fruit juices sold in various retail markets were examined for bacteriological quality. The pH of the tested juices was 2.4 - 4.8. Bacteria were isolated from 60 samples (50%) and fungi from 88 samples (73%). Escherichia coli O157:H7 was detected in four of the analyzed samples (3.34%), and Staphylococcus aureus was detected in four different cases. In 11 samples (9.1%), the total number of microorganisms detected was as high as 125 colony-forming units (CFUs). Acidophilic bacteria were isolated from 15 samples (12.5%) and acidophilic fungi from 6 other samples (5%). Blastomyces was detected in 77 samples (64.17%). All samples were negative for Lactobacillus, Clostridium perfrigens, Salmonella spp., Bacillus cereus, total coliforms, E. coli, and Listeria monocytogenes. Many of the microorganisms detected can cause disease in humans; thus, a number of the tested samples did not meet the Greek guidelines for the microbiological quality of juices. Use of a Hazard Analysis and Critical Control Point (HACCP) system should be generally introduced into the food industry sector to improve the quality of fruit juices, as well as other manufactured foods.

Comparison between the *Yersinia enterocolitica* contamination of bulk milk in milk collecting center of Garmsar and the ram milk distributed in Garmsar shops in summer

Moosavi Tannaz[1], Karbalaee Hasan [2]

[1] Food Hygiene Department, Islamic Azad University, Garmsar Branch
[2] Veterinary student, e-mail: tannazmoosavi@yahoo.com, tel.: +989123018259

Yersinia enterocolitica is a ubiquitous microorganism isolating from GI tract of many different mammalian species, therefore milk and dairy products may contain the bacterium and in the case of insufficient pasteurization or cross contamination the possibility of the presence of yersinia will increase. The study was conducted to determine the contamination of raw milk in Garmsar to Y. enterocolitica during summer. In this research 100 samples of raw milk gathered from milk collecting center of Garmsar and 100 samples gathered from milk distributed in shops of Garmsar and were sent to microbiology lab of veterinary faculty under cool and sterile condition. Samples were enriched in PSB and cultured in CIN medium. The results showed that Yersinia enterocolitica wasn't detected in any of 200 samples. Key words: Garmsar, raw milk, Yersinia enterocolitica.

Isolation and Identification of *Helicobacter pullorum* in Chickens flocks in Mashhad-Iran

A. Jamshidi [1], M. R. Bassami [2]

[1] Department of Food Hygiene, Veterinary Faculty, Ferdowsi University of Mashhad, Mashhad, Iran
[2] Department of Clinical science, Veterinary Faculty, Ferdowsi University of Mashhad, Mashhad, Iran, e-mail: ajamshid@um.ac.ir, tel.: +989155186386

Helicobacter pullorum was originally isolated from the feces and damaged livers of broilers and laying hens. It was defined as a new species in 1994. This organism has also been isolated from humans with gastroenteritis. In this study a total of 100 intestinal content of broilers carcasses from 10 flocks at a poultry abattoir in mashhad suburb were tested for the presence of Helicobacter pullorum by conventional culture method using a combination of supplemented brain heart infusion agar and a modified filter technique and for identification were performed using a polymerase chain reaction amplifying a 447-bp fragment of the 16S rRNA gene of H. pullorum; fore isolates from intestinal samples from 4 different flocks were isolated. The results show the presence of H. pullorum live chickens in this aria of Iran and may represent a risk to human health.

Key words: *Helicobacter pullorum*, Poultry, PCR

Food Microbial Ecology - Veterinary

Poster Presentations

Listeria monocytogenes in live *Mytillis galloprovincialis* collected from butrinti lagoon located in south part of Albania

K. Sulaj[1], H. Memoci[2]

[1] Faculty of Veterinary Medicine, Agriculture University of Tirana, Kamza, Tirana
[2] Institute of Food Safety and Veterinary, Street. "A. Moisiu", no. 82, Tirana, Albania e_mail: k_sulaj@yahoo.com, tel.: 00355684037736

Live bivalve mollusk (Mytillus galloprovincialis) samples collected from three stations in the lagoon of Butrinti were analyzed for the presence e of Listeria monocytogenes on 2008 and 2009. On 2008 and on 2009 were analyzed respectively 78 and 111 mussel samples (M. galloprovincialis) originated from three stations of Butrinti lagoon located in northern, southern and western parts of it. From analytical control carried out in the Institute of Veterinary and Food Safety was concluded that during 2008, 4 (5.1%) of 78 live bivalve mollusks samples (M. galloprovincialis) were positive for presence of L. monocytogenes and on 2009, 8 (7,2%) out of 98 collected samples were positive for this pathogen. The identified strains of Listeria monocytogenes (12) represents 6, 1 % of total number of samples analyzed in two years and 7 of them were isolated from the station in northern part of lagoon confirming that this part as more polluted by microbiological contamination regarding to pathogen; L. monocytogenes.

Key words: L. monocytogenes, M. galloprovincialis, Butrinti lagoon, Albania

The effect of modified atmosphere packaging on extending shelf life of the traditional Greek pastry "touloumpaki"

I.S.Arvanitoyannis[1*], K.P.Bossinas[2a], A.D.Bouletis[2a], E.A.Papa[2a], D.C.Gkagtzis[2a], C. Papaloucas[3] A.Alexopoulos[4], E. Bezirtzoglou[4], C. Chatzichristodoulou[2].

[1]School of Agricultural Sciences, Department of Agriculture, Ichthyology and Aquatic Environment, University of Thessaly, Fytoko St, 38446 Nea Ionia Magnesias, Volos, Hellas, Greece.
[2]School of Health Sciences, Department of Medicine, Laboratory of Epidemiology and Public Health, Lapithon 6, Larissa, Hellas (Greece)
[3]Medical School, Laboratory of Anatomy, Democritus University of Thrace, 68100-Alexandroupolis, 68100-Greece
[4]Democritus University of Thrace, Faculty of Agricultural Development, Department of Food Science and Technology, Laboratoty of Microbiology, Biotechnology and Hygiene, Orestiada, Greece
[a]Postgraduate Programme on "Food and Water Quality and Safety on Public Health"
e-mail: parmenion@uth.gr

The aim of the present work was to evaluate the effect of atmosphere modification on microbial (mesophiles, yeast and moulds) qualities, colour, pH, texture and water activity of the Greek bakery product "touloumpaki". Samples were stored under MAP (60% CO_2) either alone or with the addition of honey for 16 days at room temperature (22-24^{o}C). Texture was better maintained under MAP and the addition of honey prevented any increase in required shear force for penetration (1.498 and 3.20 for samples with and without honey, respecitvely). Honey inhibited the growth of yeasts on samples stored under MAP (1.6 and 2.02 log CFU/g for samples under MAP with and without honey, respectively) while multivariate analysis showed that MAP and honey acted synergistically in keeping down the yeasts. In samples with pH≤6.59 an increase in yeast content was recorded. By viewing the pattern of mesophile growth it was clear that the presence of honey restrained growth until the end of storage period (5.21 and 4.29 log CFU/g for MAP and control samples, respectively) while MAP did not have any beneficial effect. The use of multivariate analysis revealed that mesophile growth is mainly affected by the applied treatment; the addition of honey resulted in the lowest counts whereas low water activity (Wa<0.754) was strongly associated with reduced mesophile growth also. Lightness values showed a significant decrease during time with no significant changes among treatments in both internal layers and external surface of the product.

Microbial and sensory quality of "*Lollo Verde*" lettuce and rocket salad with the addition of olive oil and "*Aceto balsamico di Modena*" wine vinegar stored under active atmosphere packaging.

I.S. Arvanitoyannis[1], A.D. Bouletis[2a], E.A. Papa[2a], C. Papaloucas[3], D.C. Gkagtzis[2a], S. Plessas[4], C. Voidarou[4], C. Chatzichristodoulou[2]

[1]School of Agricultural Sciences, Department of Agriculture, Ichthyology and Aquatic Environment, University of Thessaly, Fytokou St, 38446 Nea Ionia Magnesias, Volos, Hellas
[2]School of Health Sciences, Department of Medicine, Laboratory of Epidemiology and Public Health, Lapithon 6, Larissa, Hellas
[3]Medical School, Laboratory of Anatomy, Democritus University of Thrace, 68100-Alexandroupolis,Greece
[4]Democritus University of Thrace, Faculty of Agricultural Development, Department of Food Science and Technology, Laboratoty of Microbiology, Biotechnology and Hygiene, Orestiada, Greece
[a]Postgraduate Programme on "Food and Water Quality and Safety on Public Health"
e-mail: parmenion@uth.gr

Fresh processed rocket *"Eruca Sativa"* and lettuce '*Lollo Verde"* leaves were stored with the addition of olive oil and wine vinegar *"Aceto balsamico di Modena"* under modified atmosphere packaging (5% O_2 and 10% CO_2 for MAP 1 and 2% O_2 and 5% CO_2 for MAP 2). The microbial (mesophilic, psychrotrophic bacteria and *Enterobacteriacae*), physical (colour and firmness) and sensory parameters of samples were studied in relation to storage time (up to 10 days at 5±1^{o}C). Both atmosphere modifications led to oxygen depletion (10 and 6 days for MAP 1 and 2, respectively), a phenomenon that was delayed by the presence of wine vinegar. The combination of acidic environment, due to wine vinegar addition, and atmosphere modification had a detrimental effect on the mesophile growth rate reaching 5.78 and 6 log CFU/g for atmospheres 1 and 2, respectively by the end of storage. Psychrotroph numbers were almost 1 log lower for samples with olive oil stored under MAP than those which did not contain any oil, having a significant difference ($p<0.05$) regarding the control populations. Firmness was negatively affected by wine vinegar while samples with olive oil under MAP 1 (113.5±4.8gr/mm) maintained firmness close to normal (124±1gr/mm). Colour attributes were sustained well under MAP while lightness was negatively affected by vinegar, especially in samples stored under MAP 1 that did not reach the initial fresh levels (51±1.2) even at the end of storage. Samples with olive oil under MAP 1 gave the best score for overall impression while the addition of vinegar limited sensory shelf-life to 3 days.

Microbial and sensory quality of "*Lollo Verde*" lettuce and rocket salad stored under active atmosphere packaging.

Ioannis S. Arvanitoyannis[1*], A. D. Bouletis[2a], E. A. Papa[2a], D.C. Gkagtzis[2a], C. Papaloucas[3], A. Alexopoulos[4], E. Bezirtzoglou[4], C.Chatzichristodoulou[2]

[1]School of Agricultural Sciences, Department of Agriculture, Ichthyology and Aquatic Environment, University of Thessaly, Fytoko St, 38446 Nea Ionia Magnesias, Volos, Hellas, Greece.
[2]School of Health Sciences, Department of Medicine, Laboratory of Epidemiology and Public Health, Lapithon 6, Larissa, Hellas (Greece)
[3]Medical School, Laboratory of Anatomy, Democritus University of Thrace, 68100-Alexandroupolis,Greece
[4]Democritus University of Thrace, Faculty of Agricultural Development, Department of Food Science and Technology, Laboratoty of Microbiology, Biotechnology and Hygiene, Orestiada, Greece
[a]Postgraduate Programme on "Food and Water Quality and Safety on Public Health"
e-mail: parmenion@uth.gr

Samples of fresh rocket *"Eruca Sativa"* were stored either alone or with the addition of lettuce "*Lollo verde*" leaves under two different atmosphere modifications (5% O_2 and 10% CO_2 for MAP 1 and 2% O_2 and 5% CO_2 for MAP 2). Throughout the storage period of 10 days, the microbial (mesophilic, psychrotrophic bacteria and *Enterobacteriacae*) populations, firmness, colour and organoleptic parameters were monitored. In both packages under MAP 2 anoxic conditions (O_2<0.5%) were created on day 6. The survival of mesophiles was significantly restrained by both atmosphere modifications (7.08 and 6.89 for rocket and 7.12 and 7.01 for mixed salad under MAP 1 and 2, respectively) compared to control samples (7.67 and 7.74 for rocket and mixed samples, respectively) with no differences between the two treatments. A decrease of 0.5 log CFU/g in *Enterobacteriacae* was evident on day 10 for both treated samples. The addition of lettuce helped the maintenance of texture of rocket leaves (122.3±4.1 and 134.3±5.2 g/mm for MAP 1 and 2, respectively). While MAP 1 proved to be better in maintaining the L* value in rocket leaves, the conditions created in packages under MAP 2 prevented rocket yellowing. The evaluation of sensory parameters revealed that products under MAP 1 are better preserved than under MAP 2 and the addition of lettuce affected negatively the quality of the product. The shelf life of rocket leaves was extended to 4 days under MAP 1, while mixed salads shelf life was limited to 9 days.

Incidence and susceptibility to antimicrobial agents of Listeria, Salmonella and *Campylobacter spp*. isolated from "souvlaki" in Northern Greece

N. Soultos, E. Iossifidou, E.Psomas, Z. Tzikas and T. Lazou

Department of Hygiene and Technology of Foods of Animal Origin, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, 54 124 Thessaloniki, Greece,
e-mail: soultos@vet.auth.gr, tel.: +30 2310 999807

Souvlaki is a popular Greek meat product consisting of small pieces of pork meat threaded in a small wooden or metal skewer. In the present study, 105 samples of raw souvlaki obtained from retail shops and butcher shops in Thessaloniki (Northern Greece) were screened for the incidence of Listeria, Salmonella and Campylobacter spp. and their susceptibility to various antimicrobial agents. Of the samples tested, 31,4% proved positive for Listeria spp. with 7,6% yielding L. monocytogenes; 1,9% were positive and yielded 3 Salmonella serovars (S. Typhimurium, S. Saint Paul and S. Fyris). Campylobacter spp. were not detected in any of the samples tested. The antimicrobial susceptibility to various antimicrobial agents of 11 Salmonella strains and 7 L. monocytogenes strains was also determined by disk diffusion method. Salmonella spp. were susceptible to a panel of eleven antibiotics but displayed intermediate resistance to tetracycline. L. monocytogenes isolates were resistant to nalidixic acid and ceftriaxone, partly resistant to clindamycin and cefotaxime, but sensitive to all antibiotics commonly used in veterinary and human listeriosis. Our findings indicate that souvlaki could be a potential vehicle of food borne infections due to strains of L. monocytogenes and Salmonella spp. and therefore appropriate safeguards must be taken to avoid consequent human infection.

Effect of MAP conditions on shelf life of shrimps *(Melicertus kerathurus)*

I.S. Arvanitoyannis[1], V. Konstantellia[1], A. Bouletis[2], E. Papa[2], C. Papaloucas[3], A. Alexopoulos[4], S. Plessas[4], C. Voidarou[4], E. Bezirtzoglou[4]

[1]University of Thessaly, School of Medicine, Postgraduate course on Public Hygiene, e-mail: parmenion@uth.gr, School of Agricultural Sciences, Volos, Hellas
[2]School of Health Sciences, Department of Medicine, Laboratory of Epidemiology and Public Health, Lapithon 6, Larissa, Hellas (Greece).
[3]Medical School, Laboratory of Anatomy, Democritus University of Thrace, 68100-Alexandroupolis, 68100-Greece
[4]Democritus University of Thrace, Faculty of Agricultural Development, Department of Food Science and Technology, Laboratoty of Microbiology, Biotechnology and Hygiene, Orestiada, Greece

Fish products were always a significant source of proteins of high biological value for humans. This research aimed at getting an insight into the *Melicertus kerathurus* quality and safety during its preservation. Within this framework a quality evaluation took place by studying the organoleptic and microbiological features in conjunction with the physico-chemical properties of texture and colour of the product. After the removal of the cephalothorax and the exoskeleton, under aseptic conditions, the samples were packaged and stored either in air or in modified atmosphere package (60% CO_2: 40% N_2 και 92.9%N_2:5.1% CO_2:2% O_2) for 5 days at 3^{o}C. Their organoleptic features were assessed by a trained taste panel whereas their microbiological analysis (MA) was carried out as soon as shrimps were removed from preservation facilities and before the boiling. MA included the assessment of: i) the total viable count, ii) H_2S- producing bacteria (mainly *Shewanella putrefaciens*), iii) *Pseudomonas* spp., and iv) *Brochothrix thermosphacta*. Furthermore, as regards the texture analysis, a compression test was performed in order to measure the hardness of the shrimps. The colour parameters (L*,a* and b*) were also estimated by means of a Hunterlab chromatometer. The shelf-life of the product as determined by the organoleptic and microbiological analysis was 4 days for the shrimps stored under MAP in comparison to the control, which reached a shelf-life of 3 days, at 3^{o}C. The statistic analysis was carried out by using the Principal Component Analysis (PCA), Cluster Analysis and Discriminant Analysis. The results of those statistic analysis showed that grouping of various parameters studied was feasible and the MAP composition of 60%CO_2/40%N_2 should be preferred to that of 5.1%CO_2/92.9%N_2/2%O_2). *Pseudomonas* sp. count was above 4.2 log CFU/g in the initial packages. It was observed that Pseudomonas count was by far above that limit, which is 7 log CFU/g, after the 3rd day in control samples. The samples were packaged in first gas mixture had lower level of this microorganism than the samples in the second gas mixture. Particularly, the levels of Pseudomonas in MAP_1 samples were 6 log CFU/g and in MAP_2 samples were 6.2 log CFU/g. Total viable counts (TVB) growth was lower when the CO_2 concentration increased (6.2 log CFU/g in 60% CO_2 :40% N_2, 6.8 log CFU/g in 5.1% CO_2:92.9% N_2:2% O_2. The results obtained with 2nd gas mixture and vacuum were very similar exhibiting an ascending trend. The number of colonies of *Shewanella putrefaciens* in samples packaged under modified atmosphere remained the same after 4 days.

Effect of various MAP compositions on the shelf life of Graviera

I. S. Arvanitoyannis[1], G. Kargaki[1], A. Alexopoulos[2], S. Plessas[2], C. Voidarou[2], E. Bezirtzoglou[2], Ch. Hadjichristodoulou[1] and A. Mavromatis[1]

[1]University of Thessaly, School of Medicine, Postgraduate course on Public Hygiene, e-mail: parmenion@uth.gr, School of Agricultural Sciences, Volos, Hellas
[2]Democritus University of Thrace, Faculty of Agricultural Development, Department of Food Science and Technology, Laboratoty of Microbiology, Biotechnology and Hygiene, Orestiada, Greece

In this study the shelf life of graviera cheese (a full fat cheese produced in Crete), Greece was investigated. Graviera cheese was stored at 4°C for up to 87 days in polyamide packages under three different modified atmosphere (MA) conditions. Control cheeses were packaged in air. MAP mixtures were MAP_1:40% CO_2, 55% N_2, 5% O_2, MAP_2: 60% CO_2, 40% N_2, and MAP_3: 50% CO_2,50% N_2. The product was evaluated periodically to investigate its sensory quality and microbiological characteristics. The sensory characteristics of graviera cheese were assessed by a panel composed of ten trained panelists from the cheese industry and its laboratory was used to evaluate the cheeses for external appearance (colour, texture), taste, flavour and texture on a point scale from 2 to 10 (2 very poor, 10 very good). After 11 days of storage, the sensory characteristics of the control cheeses were found to be unacceptable for all the parameters studied. The lowest microbial counts were at 60% CO_2/40% N_2. *Staplylococcus aureus* and *Listeria monocytogenes* were not detected in any of the analysed samples. *Escherichia coli* increased during the experiment, particularly in control samples and in 40% CO_2/55% N_2/ 5% O_2 atmosphere. The most effective mixtures for inhibiting the growth of *E.coli* were the MAP_1 and MAP_2 mixtures. Moreover, the 40% CO_2/55% N_2/ 5% O_2 and the control samples had a very negative effect on sensory quality.
The mean total viable counts (TVC) of the samples stored in air increased rapidly, and were higher than 7 log CFU/g after 28 days of storage at 4°C. However, cheeses packaged under modified atmospheres reached populations above 7 log cfu/g only on day 52 of storage depending on the conditions, with the exception of cheeses packaged under 60% CO_2, 40% N_2. *E. coli* growth was lower when the CO_2 concentration increased after 5 days in the control batches, and after 52 days in cheeses under modified atmosphere.
The statistical analysis of organoleptic characteristics was carried out by applying the Principal Component Analysis (PCA) and Cluster Analysis, using the JMP program for windows.

Effect of various MAP compositions on the physical and microbial properties of anthotyros

I. S. Arvanitoyannis[1], G. Kargaki[1], A. Alexopoulos[2], S. Plessas[2], C. Voidarou[2], E. Bezirtzoglou[2], Ch. Hadjichristodoulou[1] and A. Mavromatis[1]

[1]University of Thessaly, School of Medicine, Postgraduate course on Public Hygiene, e-mail: parmenion@uth.gr, School of Agricultural Sciences, Volos, Hellas
[2]Democritus University of Thrace, Faculty of Agricultural Development, Department of Food Science and Technology, Laboratoty of Microbiology, Biotechnology and Hygiene, Orestiada, Greece

Both myzithra and anthotyros cheese in Crete are produced from the whey of hard cheeses such as kefalotyri and graviera. Anthotyros is a cheese coming from the myzithra dehydration. The dehydrated anthotyros is a traditional Cretan cheese. The physicochemical characteristics of anthotyros cheese are: moisture content; 35%, fat content in dry matter; 55%, and low concentration of salt. The anthotyros cheese was packaged under three different atmospheres; MAP_1: 40% CO_2, 55% N_2, 5% O_2, MAP_2: 60% CO_2, 40% N_2 , and MAP_3: 50% CO_2, 50% N_2. The control samples were packaged in air. All cheese samples were kept under refrigeration (4±0.5^{o}C) for 55 days. Of the three applied atmospheres, the MAP_2 and MAP_3 mixtures showed to be the most effective in inhibiting total mesophilic microorganisms and *E. coli*. Neither *Staphylococcus aureus* nor *Listeria monocytogenes* were detected over the experiment. With reference to sensory analysis, good total acceptability of the control samples was reported until the 7th day and of packaged samples until the 30th day. The most effective gas mixtures in regard to sensory analysis were the MAP_1 and MAP_2. Packaging of anthotyros cheese with 60% CO_2, 40% N_2 inhibited the growth of *E.coli* and TVC. The statistic analysis of organoleptic characteristics was carried out by using the Principal Component Analysis (PCA) and Cluster Analysis, using the JMP program for windows.

Natural microflora of coffee beans : *Aspergillus ocrhraceus* OTA production and selection of Lactic acid bacteria to control mould contamination.

O. Djossou[1], I., Perraud-Gaime[1], A.G.T. Karou[2] , S. Niamke[2] & S.Roussos[1]

[1] IRD-IMEP UMR-193 ; Equipe Ecologie Microbienne Biotechnologie, Case 441, Université Paul Cézanne, 13397 Marseille cedex 20 ; France

[2] Laboratoire de Microbiologie ; Université de Cocody ; Abidjian, Côte d'Ivoire

Coffee beans are susceptible to fungal attack during post harvest both treatment and storage. These fungi may produce mycotoxins as secondary metabolite. Mycotoxins may render food unsafe for humans, are very dangerous because of the diversity of their toxic effects and their high thermal stability. The recurrent contamination of coffee by ochratoxin A (OTA) affect both coffee quality and coffee bean price with a strong consequence to the economy of the exporter countries who made their main sources of income. Recent studies put in evidence that, Aspergillus Nigri group are responsible for the post harvest contamination of coffee by OTA. The work present here describes (1) the isolation and identification of mesophilic fungi from 31 samples of coffee robusta from different Ivory Coast areas during the 2008 and 2009 coffee harvest campaigns, (2) the isolation of Lactic Acid Bacteria (LAB) from fresh pulp of coffee berries colected also in Ivory Coast at the same area. The study had a triple objective : first, to identify natural microflora present in coffee pulp and coffee beans ; then, to demonstrate the toxinogenic capacity of wild strains isolated from these specific biotopes ; and last to isolate LAB, able to inhibit the *Aspergillus carbonarius* germination and growth. 218 strains of filamentous fungi were isolated on Potato Dextrose Agar (PDA) culture medium and identified as : black Aspergilli (52%), green Aspegilli (13%), *Mucor* (16%), *Penicillium* (10%), *Fusarium* 4%), other one (5%). Also, 44 strains of LAB were isolated on agar MRS culture medium at 30°C in aerobic conditions, then purified on MRS medium and stored in glycerol at –20°C. Only two LAB strains demonstrate a strong antifungal effect against *A.carbonarius* OTA producer. The results obtained from harmful moulds and useful LAB antagonistic against mycotoxinogenic mould, in spoiled coffee beans, contribute toward the evaluation and prevention of their proliferation in order to obtain quality coffee beans for a sustainable development of coffee industry in Ivory Coast.

Key words : Coffee robusta, Ochratoxin A, Aspergillus Nigri, *Aspergillus carbonarius*, Lactic Acid Bacteria, Ivory Coast.

Serologic and bacteriologic diagnosis of bovine leptospirosis in Tehran suburb dairy farms

E.Sakhaee, [1], G.R.Abdollapour, [2], M.Bolourchi, [2], S.Sattari, [2], A.Sakhaee, [3]

[1] Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Iran
[2]Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Iran
[3] Department of Clinical Sciences, Faculty of Medicine, University of Semnan, Iran
e-mail : Ehsan_Sakhaee@yahoo.com, tel: 00989132952830

Leptospirosis is a worldwide zoonosis caused by Leptospira interrogans. This study was conducted to evaluate serologic and bacteriologic findings of leptospirosis in clinically suspected cows. Three hundred and eighty sera and thirty three urine samples were collected from 6 industrial dairy farms in Tehran suburb. The prevalence of disease was determined by microscopic agglutination test (MAT), enzyme linked immunosorbent assay (ELISA), direct dark-field microscopic (DFM) examination, indirect fluorescent antibody test (IFAT), microbiologic cultural isolation technique and polymerase chain reaction (PCR). Antibodies were detected by MAT at least against one serovar of Leptospira interrogans in 55 sera (14.47%) among 380 samples at a dilution 1:100 or greater, and L. icterohaemorrhagiae was the most prevalent serovar. Leptospiral antibodies were detected by ELISA in 85 sera (22.37%) among 380 samples. Four samples (12.12%) among 33 urine samples were suspected by DFM examination and no positive sample by IFAT was observed. Leptospires could be isolated from none of the 33 samples taken from industrial farms, but leptospires were isolated from urine samples taken from two clinically affected calves in a traditional farm. In this study, positive controls were detected only at a dilution equal to or greater than 2000 leptospires per each ml of urine sample by PCR, therefore, no DNA from serum and urine samples were collected from 6 industrial dairy farms, could be detected by this method.

Relationship between bovine Leptospirosis and reproductive disorders in Iran

E. Sakhaee[1] , G.R.Abdollapour[2], M.Bolourchi [2], S. Sattari [2], A.Sakhaee [3]

[1] Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Iran
[2] Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Iran
[3] Department of Clinical Sciences, Faculty of Medicine, University of Semnan, Iran
e-mail: Ehsan_Sakhaee@yahoo.com, tel.: 00989132952830

Leptospirosis is a worldwide zoonosis caused by Leptospira interrogans. This study was conducted to evaluate serological findings of leptospirosis in cattle with reproductive problems. Three hundred and eighty sera samples were collected from six commercial dairy farms with reproductive disorders in Tehran province, Iran. Microscopic agglutination test (MAT) and enzyme linked immunosorbent assay (ELISA) were used to identify specific antibodies against Leptospira interrogans in dairy cattle. Antibodies were detected by MAT at least against one serovar of Leptospira interrogans in 55 sera (14.47%) among 380 samples at a dilution 1:100 or greater, and L. icterohaemorrhagiae (42.52%) was the most prevalent serovar, followed by serovar hardjo (35.63%), grippotyphosa (9.2%), pomona (8.05%), canicola (3.45%) and ballum (1.15%). Leptospiral antibodies were detected by ELISA in 85 sera (22.37 %) among 380 samples. The highest prevalence of positive sera by ELISA was found in farm 1 (10.53 %), followed by farm 3 (7.63 %), farm 6 (2.10 %), farm 4 and 2 (0.79 %) and farm 5 (0.53 %). Positive titers against more than one serovar were detected in 27 sera of the positive samples. It seems that L. icterohaemorrhagiae and hardjo had the furthest rule in reproductive disorders in cattle. Present study showed that the highest antibody titer is detected during 3 to 6 months after abortion and the titer decreases after seventh month.

Comparison of Microscopic Agglutination test (MAT) and enzyme-linked immunosorbent assay (ELISA) for detection of leptospiral antibodies in cattle

E. Sakhaee[1] , G.R.Abdollapour[2], M.Bolourchi [2], S. Sattari [2], A.Sakhaee [3]

[1] Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Iran
[2] Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Iran
[3] Department of Clinical Sciences, Faculty of Medicine, University of Semnan, Iran
e-mail: Ehsan_Sakhaee@yahoo.com, tel.: 00989132952830

Leptospirosis is a worldwide zoonosis caused by Leptospira interrogans. This study was conducted to evaluate two serological techniques, the Microscopic Agglutination test (MAT) and enzyme-linked immunosorbent assay (ELISA), for detection of leptospiral antibodies in cattle. Three hundred and eighty serum samples were collected from 6 industrial dairy farms in Tehran suburbs. Antibodies were detected by MAT at least against one serovar of Leptospira interrogans in 55 sera (14.47%) among 380 samples at a dilution 1:100 or greater, L. icterohaemorrhagiae was the most (42.52 %) and L. ballum the least (1.15 %) prevalent serovar among 87 positive reactions against different serovar of Leptospira interrogans. Leptospiral antibodies were detected by ELISA in 85 sera (22.37%) among 380 samples. The results of present study showed that 2.25 % of the sera that reacted positive in the MAT were found negative in the ELISA and 8.45 % of the MAT-negative sera were positive in the ELISA. The Microscopic Agglutination test, the most commonly used in the serodiagnosis of leptospira, was adopted as reference that for the IgG ELISA kit evaluation. Comparison of the two techniques showed that there was a high correlation ($0.61 < \text{kappa} = 0.649 < 0.8$) between the IgG – ELISA and MAT, for detection of leptospiral antibodies in cattle.

A case report of listeric Septicemia in goat

O. Azari[1], E. Sakhaee[1] , A. Sakhaee, [2]

[1] Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Iran
[2] Department of Clinical Sciences, Faculty of Medicine, University of Semnan, Iran
e-mail: Omidazari@yahoo.com, tel.: 00989123185781

Listeria monocytogenes is a gram-positive, non-sporulating rod, which produces encephalitis, septicemia, and abortion in sheep. Livestock are also susceptible to listeric infection, with a large proportion of healthy asymptomatic animals shedding L. monocytogenes in their feces. Ovine listeriosis is usually classified as 1) encephalitis, which is the most common form, 2) placentitis with abortions occurring in the last trimester, and 3) septicemia. In humans, L. monocytogenes has been implicated in major food-borne epidemics. In June 2009, a one-month-old male goat kid (15 lbs.) from a small flock of 20 sheep and goats with traditional management in Kerman suburbs were referred to the Teaching and Research Hospital of the Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Iran. The kid was exhibited severe depression to the point of somnolence, nystagmus, mild opisthotonos, incoordination, head deviation, walking in circles, ataxia, Corneal opacity, dyspnea, high fever 40°C (104°F), anorexia and passing loose, green-colored feces. Death follows in about twenty-four hours. Tissue samples (brain, ileum, duodenum, liver, and kidney), whole blood, and serum were obtained from the kid. For isolation of Listeria, brain tissues were plated to tryptose blood agar base supplemented with 5% defibrinated bovine blood and incubated in aerobic and anaerobic, environments (up to 48 hr, 37º C). This suggests either environmental contamination on farms due to L. monocytogenes in bovine feces or feeding of contaminated ensilage shared by goats and cattle. goats may be more susceptible to clinical listeriosis than cattle.

The first serologic study of Q fever in sheep in Iran

E.Sakhaee[1], M.Khalili[2], A.Sakhaee[3] ,O. Azari[1]

[1] Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Iran
[2] Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Iran
[3] Department of Clinical Sciences, Faculty of Medicine, University of Semnan, Iran
e-mail: Ehsan_Sakhaee@yahoo.com, tel.: 00989132952830

Coxiella burnetii is an obligate intracellular microorganism that causes Q fever in humans and animals. In ewes, C. burnetii infections are generally asymptomatic, but they can lead to abortions, stillbirths and delivery of weak and unviable lambs. Serological assays are suitable for screening herds. ELISA technique has a high sensitivity and a good specificity. Purpose: The aim of this study was to investigate the presence of anti-C. burnetii antibodies among sheep in southeast Iran. Methods: A total of 85 serum samples were collected from ten sheep flocks from April to September 2009. Serum samples were tested for Q fever antibodies using a commercial indirect ELISA kit. Results: Antibodies were detected in 25 sera (29.42 %) of 85 samples. Sixteen female (18.82 %) and nine male (10.58 %) cases had antibodies specific to C. burnetii. There is significant difference in seropositivity between male and female groups ($P < 0.05$). Conclusions: This first study of C. burnetii seroprevalence in sheep in southeast Iran has indicated that seropositive animals can be found throughout the country. Further work is now required to characterize the epidemiology of the infection more thoroughly.

Evaluation of the immune response to *Clostridium perfringens* vaccine in broiler chicken

A.A. Mosaad[1], S.I.Fathalla[2], A.M.Atwa[1]

[1]Department of Bacteriology,Immunology and Mycology; Fac. of vet. Med. Mnf. Univ.
[2]Department of Physiology; Fac. of Vet. Med. Mnf. Univ.

A total of 160 broiler chickens were vaccinated by toxoid or bacterin against *C.perfringens* type ''A'' and "C" at 12 days of age and boostered after one week, this regime gave a protective immunization against experimentally infected necrotic enteritis (NE).It's shown that coccidia can be proceed *C.perfringens* infections. Also using of prebiotics and levamisole helps in controlling the disease. It's noted that the toxoid A&C vaccine was give a high titer of antibodies measured by ELISA. It is concluded that using of toxoid give maximum titer of antibodies still for two weeks. In spite of prebiotic's role in immunity the usage of both prebiotics and vaccines may be have a possible interference. Coccidia play an important role in occurrence of NE.

Key words: Broiler, Clostridia , vaccine, prebiotics, Coccidia

Microbiota Profile in Feces of Breast- and Formula-Fed Newborns by Using Fluorescence *in situ* Hybridization (FISH)

Eugenia Bezirtzoglou[1*], Arsenis Tsiotsias[1] and Gjalt W. Welling[2]

[1]Democritus University of Thrace, Faculty of Agricultural Development, Department of Food Science and Technology, Laboratory of Microbiology, Biotechnology and Hygiene, GR68200, Orestiada, Greece.

[2]Department of Medical Microbiology, University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands.

The development of the gut is controlled and modulated by different interacting mechanisms such as, genetic endowment, intrinsic biological regulatory functions, environment influences and last but no least, the diet influence. Considered together with other endogenous and exogenous factors the type of feeding may interfere greatly in the regulation of the intestinal microbiota. During the last years molecular methods offer a complementarity to the classic culture-based knowledge. FISH has been applied for molecular evaluation of the microbiota in newborns delivered by vaginal delivery. Eleven probes/probe combinations for specific groups of fecal bacteria were used to determine the bacterial composition in fecal samples of newborns infants under different types of feeding. Breast-fed infants harbor a fecal microbiota by more than two times increased in numbers of *Bifidobacterium* cells when compared to formula-fed infants. After formula-feeding, *Atopobium* was found in significant counts and the numbers of *Bifidobacterium* dropped followed by increasing numbers in *Bacteroides* population. Moreover, under formula feeding the infants microbiota was more diverse.

Keywords: newborn; breast-fed; fecal microbiota; FISH technique

Microbiological quality and related factors of sheep milk produced in farms of NE Greece.

Tzatzimakis G[1]., Abas Z[1]., Alexopoulos A[2]., Plessas S[2]., Voltsi C[2]., Konstandaki V[2]. Anastasiadis E[2]., E. Stavropoulou[2], Sinapis, E[3]., and Bezirtzoglou E[2].

[1]Democritus University of Thrace. Faculty of Agricultural Development. Laboratory of Animal Production. Orestiada-Greece.

[2]Democritus University of Thrace. Faculty of Agricultural Development. Laboratory of Microbiology, Biotechnology and Hygiene. Orestiada-Greece.

[3]Aristotle University of Thessaloniki, School of Agriculture, Department of Animal Production. Thessaloniki-Greece.

Sheep herbs are a significant capital of dairy industry in Greece. Sheep's milk has a higher content of essential vitamins and minerals than cow's milk and could be used to cater to consumers' appetite for healthy and safer products. However the task of producing quality milk and maintaining animal with low incidence of mastitis is a management challenge for all dairy producers. As dairy farming becomes more complex and intense the need to provide assistance to dairy producers on milk quality is critical. Our purpose was to survey the raw milk produced in sheep farms of NE Greece and exploring the role of various factors on its quality.

In Total, 21 dairy ewe's farms from the regions of Xanthi and Evros, in the north-eastern Greece were monitored. Milk samples were sampled after the morning milking every 15 days throughout the dairy period (March – June). For the study, a questionnaire was also filled by personal interview with the owners in order to collect information about flock characteristics, health status, handling practices etc. From each farm, air was also sampled for microbiological analysis (Surface Air System at a flow rate of 3 L/sec). Milk samples were examined for chemical components: fat content, protein, lactose, non-fat dry matter (NFDM) and somatic cells count (SCC). Microbiological examination involved the estimation of Total Bacterial Counts (TBC), coliform count, *Staphylococcus aureus*, *Streptococcus sp.*, and preliminary incubation count (PIC). The possible correlation among different bacterial species and their interaction with SCC and chemical components of milk was also considered. It was examined whether farm management practices could influence the hygiene and the quality of milk.

Our results show that as an average TBCs were 5.48 log cfu/ml, SCC: 6.05 log cells/ml, coliforms: 4.49 log cfu/ml, Staphylococcus aureus: 3.94 log cfu/ml, environmental streptococcal counts: 4,95 log cfu/ml and PIC: 5.7 log cfu/ml. The mean fat, protein, lactose and NFDM were 6.17%, 5.28%, 4.73% and 10.95% respectively. The study revealed significant positive correlation between TBC and PIC (0.825), while SCC was marginal positive correlated with protein and NFDM. No statistically significant correlations observed among SCC with any of the bacterial species. Herd size and farm management practices had considerable influence on SCC and bacterial species.

Key Words: Dairy, Sheep, milk quality.

Effect of non-thermal processing by High Hydrostatic Pressure on the survival of probiotic microorganisms: study on *Bifidobacteria* spp.

M. S. Tsevdou and P. S. Taoukis

Laboratory of Food Chemistry & Technology, School of Chemical Engineering, National Technical University of Athens, 5 Iroon Polytechniou Str., Zografou 15780, Athens

mtsevdou@chemeng.ntua.gr

The global market focuses on functional foods in order to satisfy consumer's demand for products that promote health. The dairy industry is increasingly introducing to the market products (milks, yoghurts, yoghurt-like products and cheeses) containing probiotic microorganisms, promoting their beneficial effects on the function of gastrointestinal tract due to their relation to the natural gut microflora. High Hydrostatic Pressure (HP) processing has been suggested as a method that could contribute to texture improvement of dairy products. HP can be applied either to raw milk which is used for the production of dairy products, or to the final product.

The effect of HP on the viability of *Bifidobacterium lactis* BB12 was studied. HP inactivation experiments were conducted at various combinations of pressure (100-400 MPa) and temperature (20-40°C) for appropriate time periods. High pressure treatments were achieved using laboratory scale HP equipment consisting of six vessels of 42 ml capacity each. The medium used for the inoculation of the bacterium was MRS broth (Merck, 1.10661) and its pH value was adjusted either to 6.50 (the pH value of milk) or to 4.80 (common pH value of fermented milk products). The bacterium was inoculated at a level of 10^8 in the growth medium and subjected to HP treatment. Enumeration of remaining cells was conducted with the appropriate plate methodology.The D-values for *Bifidobacterium lactis* BB12 were calculated at all pressure-temperature combinations examined. The effects of temperature (Z_T), as well as the effect of pressure (Z_P) were estimated at all pressures and temperatures respectively and for all growth media used. At pressures up to 200 MPa, moderate temperatures (20-25ºC) and at both growth media no significant reduction was observed even for prolonged process times. The bacterium exhibited greater viability in high pH value when subjected in HP processing at pressures higher than 200MPa and temperatures higher than 35ºC. The D-value was 36.5 min at 300MPa and 35ºC and pH value of the medium equal to 6.50, while it was decreased to 1.43 minutes at pH value of the medium equal to 4.80. The effect of pressure and temperature on the inactivation kinetics of *Bifidobacterium lactis* BB12 was mathematically modelled and the parameters of the model were estimated. The predicted D-values from the model were very well correlated with the corresponding D-values received from the experimental data (R^2=0.98). In conclusion, pressure and temperature appeared to act synergistically in probiotic microorganisms' inactivation. The developed models for the inactivation of *Bifidobacterium lactis* BB12 enable the proper design of HP processing to achieve improved textural properties while maintaining adequate viability of the probiotic strain necessary for functional dairy products.

Microbial challenges of poultry meat production

D. Vassos, E. Mauromati, G.Rozos, I. Anagnostopoulou, A. Alexopoulos, S. Plessas, J.Skoufos, A. Tsinas, C.Voidarou, E. Stavropoulou and E.Bezirtzoglou

Technological Institute of Epirus, Department of Microbiology, Arta and Public Health School of Athens
University of Ioannina, Department of Chemistry
Democritus University of Thrace and Faculty of Agricultural Development, Department of Food Science and Technology, Laboratory of Microbiology, Biotechnology and Hygiene, Orestiada, Greece

Food safety and shelf-life are both important microbial concerns in relation to broiler meat production. Focus is mainly placed on the absence or control of potentially pathogenic microbes such as Salmonella spp and Campylobacter spp but, from the commercial point of view, other spoilage bacteria also play a role as potential threats. Regarding food safety, the primary target should be the production of pathogen-free live animals, thus allowing slaughter plants to keep the processing line free of those microorganisms.

Consumers believe that quality of foods from organic production is superior to foods from conventional production. The aim of the present study was to evaluate and compare the bacterial quality of chicken meat from organic and conventional production on the basis of traditional meat quality criteria. Fresh free grazing broiler carcasses were purchased directly from rural households (n:=40) and fresh retail chicken parts (legs, wings and giblets) from conventional broiler carcasses from the local supermarkets in the region of Epirus (Poultry Producers Association. Arta) (n:100).

The samples were microbiologically tested for the presence of bacteria such as: Salmonella spp., Listeria monocytogenes, Staphylococcus aureus, Enterobacteriaceae, Escherichia coli, Campylobacter spp., and , Clostridium perfringens. Total count of aerobic mesophilic bacteria was also determined. Bacteriological tests were performed by means of standard methods of isolation and identification of individual species of bacteria according to ISO requirements. API-tests (Biomerieux) and Vitek 2 Identification System (Biomerieux) were used for biochemical determination. With respect to microbiological quality and contamination of chicken meat, of importance are the findings for the conventional poultry meat: S. aureus (4%), enterobacteria (14%) and Clostridium perfringens (1.50%). Salmonella spp., L. monocytogenes and Campylobacter spp. were not found in any of the analyzed samples. For the samples of fresh free grazing broiler carcasses: Salmonella spp.() S. aureus (4%), enterobacteria (14%), Clostridium perfringens (1.50%). Salmonella spp., L. monocytogenes, Campylobacter spp. These high levels of microbial contamination and occurrence of pathogenic bacteria reflect the poor hygienic quality of the slaughter conditions in the rural households.

Isolation and Identification Of Lactic Acid Bacteria From Raw Poultry Meat

C.Voidarou, D. Vassos, E. Mauromati, G.Rozos, I. Anagnostopoulou, A. Alexopoulos, S. Plessas, J.Skoufos, A. Tsinas, E. Stavropoulou, P.G. Demertzis, K. Demertzi- Akrida and E.Bezirtzoglou

Technological Institute of Epirus, Department of Microbiology, Arta and Public Health School of Athens
Section of Industrial and Food Chemistry, Department of Chemistry, University of Ioannina, Panepistimioupolis, GR-45110 Ioannina, Greece
Democritus University of Thrace and Faculty of Agricultural Development, Department of Food Science and Technology, Laboratory of Microbiology, Biotechnology and Hygiene, Orestiada, Greece

There is a global tendency in the market to use milder preservation methods in foods. This tendency derives from the consumer's desire for the least possible treatment of foods either because of energy-saving, or the desire for having more 'fresh' meat products, or because of an aversion to the use of preservatives. Lactic acid bacteria produce various compounds such as organic acids, diacetyl, hydrogen peroxide, and bacteriocins or bactericidal proteins during lactic acid fermentations. The aim of this work was to isolate and identify lactic acid bacteria from raw poultry meat. Fresh retail chicken parts (legs, wings and giblets) from conventional broiler carcasses from the local supermarkets in the region of Epirus (Poultry Producers Association. Arta)) (n=100) were tested for the presence of LAB.

The influence of antimicrobial activities were obtained by using the agar well diffusion method (Muller Hinton Agar) against some members of gram positive and gram negative pathogenic bacteria genii commonly involved in meat spoilage and foodborne infections (Escherichia coli, Bacillus cereus, Klebsiella pneumoniae, Salmonella spp. and Staphylococcus aureus). The results show that the LAB inhibit the growth of these bacteria by developing an inhibiting zone around the wells which contain these LAB. Hence the natural LAB microflora of the chicken carcass contributes largely to the self-life and self preservation of the product and so meets the demands of the consumers.

Environmental Microbial Ecology

Environmental Flora and Foodborne Pathogens

Invited Lectures

Biogical control strategy to protect foodstuffs from post harvest moulds and producing mycotoxins

S.Roussos[1] ; N. Belkacem[1], A. O. Djossou[1] , Efire-Nguema[1] , Y.Labrousse[1], I. Perraud-Gaime[1] and E. Bezirtzoglou[2]

[1] Institut Méditerranéen d'Ecologie et de Paléoécologie , IRD UMR-193, IMEP Case 441, FST Saint Jérôme, Université Paul Cézanne, 13397 Marseille Cedex 20, France

[2] Democritus University of Thrace, Faculty of Agricultural Development, Department of Food Science and Technology, Laboratory of Microbiology ,Biotechnology and Hygiene,68200-Orestiada,Greece

E-mail: s.roussos@univ-cezanne.fr

Foodstuffs quality is essential for the consumer health. Moulds and bacteria are frequently present as natural microflora of common food such as dried fruits, cereals, olives, coffee and grapes. However, these foodstuffs have a significant change in their microflora after harvesting, during storage, as well as during the fermentation processes.

After harvest their natural microflora drops in numbers and fungi like *Penicillium* and *Aspergillus* which appears, can produce carcinogenic metabolites (aflatoxins, ochratoxins). The purpose of this work is to contribute to the development of the new methods of fight against fungal post harvest contaminants. Based on the antagonism produced between moulds producing mycotoxins and lactic acid bacteria (LAB) producing acid metabolites, implementation of a biological control strategy was applied. Our study focused on the following experimentation (i) to characterize the present fungal and bacterial microflora on spoiled cereal, coffee, olives and tomatoes (ii) to study and evaluate their capacity to produce aflatoxin or ochratoxin when grown on starch-based culture media, iii) to identify the main mycotoxinogenic moulds as well as the main lactic acid producing bacteria (LAB), and (iv) to select active LAB against *Aspergillus carbonarius*. In this vein, LAB strains isolated from coffee pulp, table olive and other fruits were tested for their antifungal activity in a dual-culture agar plate assay. Ten LAB strains which demonstrated an antifungal effect against *A.carbonarius* OTA producer were selected for testing of antimould activity of the LAB cell-free supernatant and identification of antimould compounds. A particular attention will be carried out in the process of fermentation, to answer the standards of food quality and safety required by the national and international market low acidity and low rate of mycotoxines.

Key words: Coffee, table olive, Ochratoxin A, *Aspergillus carbonarius*, Lactic Acid Bacteria, Antagonism.

Streptomyces as bioactive compound producers: an important genus in microbial community

A.D. Karagouni

National and Kapodistrian University of Athens, Faculty of Biology, Department of Botany, Microbiology Group, 15781 Athens, Greece Email: akar@biol.uoa.gr
e-mail: akar@biol.uoa.gr, tel.: +30 210 7274526

Almost 80% of the known bioactive compounds are produced from Streptomyces, a very interesting genus of the Actinobacteria family. The need for more natural products is profound and amongst the microbiologists is clear that the answer is the extensive study of the microbial community in the planet. Companies and scientists collect samples from particular habitats, from different parts of the world in order to screen for antibiotic/or other production. The Microbiology group of the University of Athens has studied the Streptomyces diversity for over 20 years and a collection of very important isolates have been selected from ecosystems which display either typical Mediterranean climatic conditions or extreme conditions like volcanoes or polluted areas. The isolates were identified at the strain level and studied phenotypically, physiologically and genetically. The obtained results showed that Greek strains were the most active ones compared with other isolates from European countries; also there were well adopted in the environment of origin, their behavior differed respectively to the type of soil when they examined for new bioactive compounds production they exhibited a variety of some new interesting properties.

Keywords: Actinobacteria, Streptomyces, Mediterranean ecosystem, bioactive compounds

Do leafy green vegetables and their ready-to-eat (rte) salads carry a risk of foodborne pathogens?

B. Mercanoglu Taban[1], A. K. Halkman[2]

[1] Gazi University, Vocational School of Health Services, Golbasi, Ankara, TURKEY;
[2] Ankara University, Faculty of Engineering, Food Engineering Department, Dıskapi, Ankara, TURKEY, e-mail: birce@gazi.edu.tr, tel.: +90-532-3270957

Over the past 10 years, there is an increasing demand for leafy green vegetables and their ready-to-eat (RTE) salads since people changed their eating habits because of healthier lifestyle interest. Nevertheless fresh leafy green vegetables and their RTE salads are recognised as a source of food poisoning outbreaks in many parts of the world. However, this increased proportion of outbreaks cannot be completely explained by increased consumption and enhanced surveillance of them. Both in Europe and in the USA, recent foodborne illness outbreaks have revealed links between some pathogens and some leafy green vegetables such as mostly lettuces and spinachs and their RTE salads since fresh leafy green vegetables carry the potential risk of microbiological contamination due to the usage of untreated irrigation water, inappropriate organic fertilizers, wildlife or other sources that can occur anywhere from the farm to the fork such as failure during harvesting, handling, processing and packaging. Among a wide range of pathogens causing food-borne illnesses, Escherichia coli O157:H7, Salmonella spp., and Listeria monocytogenes are the most common pathogens that contaminate leafy green vegetables. Children, the elderly, pregnant women and immunocompromised people are the most at risk for developing complications from foodborne illness as a result of eating contaminated leafy greens or their RTE salads. These outbreaks are mostly restaurant associated or they sometimes spread across several countries by international trade routes. This presentation summarizes current observations concerning the contaminated leafy green vegetables and their RTE salads as important vehicles for the transmission of some foodborne pathogens to humans.

Water Microbial Ecology – Faecal and Waste Bacteria in Marine Environments

Invited Lectures

Environmental factors affecting the survival of faecal bacteria in marine waters - Mechanisms of the creation of viable but non culturable forms of bacteria in watery environment

M. Parapetropoulou[1] and A. Mavridou[2].

[1]University of Patras, Medical School, Laboratory of Public Health, Patras, Greece

[2]Technological Institute of Athens, Department of Medical Laboratories, Athens, Greece

The factors affecting the survival of enteric bacteria in the marine environment were driven by public health concern and the need to expand our understanding of their responses to environmental stress. Lymatic bacteria are exposed to sunlight, predation mostly by protozoa, osmotic upchock, nutrient deprivation, low temperatures and elevated pH. For many studies the survival rates are dependent on their history preceding the seawater chock. Previous growth history plays a major part in pre-adaptation of the cells and stationary phase cells are generally more resistant that exponentially growing ones. Metabolic and compositional changes occur in the bacterial cells starved in seawater. In a study, *E.coli* cells lost β-galactosidase activity gradually with time and acquired the ability to degrade gelatine. A small number of genes with most predominant the rpoS, were found that, when mutated, affected the seawater sensitivity of the bacterium. In many studies die-off rates based on colony formation capacity were the main parameter used to characterize the bacterial responses to the seawater upchock. On resuscitation from the "viable but non culturable" (VBNC) state, the bacterial cells, including a number of human pathogens, regain culturability and the renewed ability to cause infection. It has been recognized that H_2O_2 might pay a significant role in inducing VBNC state in a variety of bacteria, including *E.coli.* An interesting range of factors induce the VBNC state, like food preservatives and heavy metals. Cells in the VBNC state are detected using reagents designed to demonstrate through microscopic examination the presence of an intact cytoplasmic membrane. The cells exhibit typical dwarfing, they retain high levels of rRNA and they show modifications in the outer membrane. Several antibiotics acting on peptidoglycane or β-lactams are blocking resuscitation of VBNC cells. Other studies detected the pathogenic potential of toxin producing *V. vulnificus* when entering VBNC state. It seems certain that the survival mechanisms differ from bacterium to bacterium. It has been suggested that "the VBNC state may be an intermediate in an altruistic death process that is part of a survival strategy" or that " dormancy and waking up from this state could be a method analogous to sending scouts to test the environment for suitability for growth of the entire population.

The role of bacteria in the source tracking of aquatic contamination

A. Vantarakis

Environmental Microbiology Unit, Department of Public Health, Medical School, University of Patras, Greece

Waters contaminated with human feces are generally regarded as a greater risk to human health, as they are likely to contain human enteric pathogens, including such as Shigella spp., Hepatitis A and Noroviruses. Animals can also serve as reservoirs for a varietry of enteric pathogens (e.g.Salmonella, Escherichia coli, and Cryptosporidium spp.). Understanding the origin of fecal pollution is important in assessing associated health risks as well as the actions necessary to solve the problem while it still exists. Bacterial Source Tracking (BST) is a methodology that is being used to determine the sources of fecal bacteria (e.g. from human, livestock, or wildlife origins) in environmental samples. BST methodology has been described as having the ability to turn nonpoint sources into point sources. BST continues to evolve rapidly and promising developments are emerging. A number of culture-independent and library-independent methods based on molecular techniques have been gaining popularity among source trackers. Molecular methods may offer the most precise identification of specific types of sources, but are limited by high per-isolate costs, detailed and time-consuming procedures, and are not yet suitable for assaying large numbers of samples in a reasonable time frame. All methods have different viewpoints associated with the practical use of BST to identify critical research gaps, propose a priority-based timeline to address them, and outline emerging technologies that will likely impact the future of source tracking. Maintenance of the microbiological quality and safety of water systems used for drinking, for recreating, and in the harvesting of seafood is imperative, as contamination of these systems can exact high risks to human health as well as result in significant economic losses due to closures of beaches and shellfish harvesting areas.

Dispersion of antibiotic resistance in aquatic ecosystems bacterial flora.

A. Alexopoulos, S. Plessas , C. Voidarou, E. Stravropoulou and E.Bezirtzoglou

Democritus University of Thrace, Faculty of Agricultural Development, Laboratory of Microbiology, Biotechnology and Hygiene. 193 Pantazidou Str. GR-68200, Orestiada, Greece, e-mail: alexopo@agro.duth.gr

Antibiotics are widely used in large quantities for human and animal treatment of infections or, until recently, as growth promoters for the livestock. But, despite their use, the occurrence and fate of these chemicals in the environment and particularly in water has received little notice. Although, bacteria have the ability to adapt to environmental changes and survive in unfavorable conditions, aquatic environments mostly enhance microbial activities and the dispersion of antibacterial agents. Also in aquatic environments bacteria have access to a large reservoir of transferable genes contributing through the horizontal and vertical transfer in developing an inherent, intrinsic or acquired resistance. The transfer of resistant bacteria to humans could occur via irrigation, drinking or recreational waters.

In the past, due to the lack of experimental data it was only speculated that resistance was connected to low concentrations of antibiotics. In the mean time a significance emergence of bacterial resistance was recorded and efforts are under way to comprehend the complexity of the process but with little progress even for more favorable environments as the medicinals. Although, in vitro toxicity tests concerning the antibiotics exists only for a small battery of aquatic organisms, there are evidence of adverse effects in algae, plants and fish that may affect the balance in an aquatic system. Bacteria that are resistant to antibiotics are present in surface waters like rivers and lakes and there are numerous studies which correlated them with corresponding urban inputs. Additionally, antimicrobial resistance especially for tetracyclines and sulphonamides, has also been found in marine bacteria and bacteria living in estuaries or coastal waters polluted with sewage water. In ground water, antibiotics are rarely found and even then, they occur at very small concentrations. Given the negligible microbial load of those waters it is assumed that only in the case of leaching from fields fertilized with animal manure or sludge application combined with a geological favorability might be a source of pollution.Antibiotic resistant bacteria have been found in drinking water probably as a result of the treatment process and distribution. Additionally, increase resistance rates were also detected at drinking water sampling points indicating the importance of source quality.

Drinking water and wastewater treatment processes need continued research to develop effective remediation methods that restrict introduction of antibiotic compounds to the environment. Those methods should be simple in order to be facilitated in all over the world, and when effectively combined with other intervention actions (prudent use, drug degradability, risk assessment studies) could significantly contribute to the controlling of resistant dispersion in the environment.

Key words: Antibiotics, Environment, Water, Resistant bacteria

Waterborne parasitic diseases and their importance

N. Altintas

Ege University, School of Medicine, Department of Parasitology, Izmir-TURKEY
e-mail: nazmiye.altintas@ege.edu.tr

Parasite infections represent important public health problems with great economic impact most of the countries. Water is a major tool for parasites, and contaminated water is an important source of human infection. Waterborne diseases are caused by pathogens spread through contaminated drinking water or recreational water. People can ingest these microbial agents by drinking contaminated water, or by eating seafood from contaminated waters, or by eating fresh produce irrigated or processed with contaminated water. Most of parasites that play a role in waterborne diseases have a zoonotic origin. The faeces-water-food connection for parasite zoonoses is also important. Because contaminated water transports transmissible stages into drinking water supplies, recreational sites, including fresh and marine waters. Contamination can also occur when foods are rinsed in parasite-contaminated portable water in the household. Ethnicity, culture, religion and different eating habits all play important roles in waterborne diseases as much as the other parasitic diseases. Parasitic protozoa have been recognised as having great potential to cause foodborne and waterborne disease. At least 325 water-associated outbreaks of parasitic protozoan disease have been reported worldwide. North American and European outbreaks accounted for 93% of all reports and nearly two-thirds of outbreaks occurred in North America. *Cryptosporidium*, *Isospora belli*, *Cyclospora cayetanensis*, *Giardia intestinalis, Entamoeba histolytica/E.dispar*, *Blastocystis hominis*, *Acanthamoeba* and *Naegleria fowleri* can contaminate food and water. The most significant zoonotic waterborne helminthic diseases are either snail-mediated (*Schistosoma* spp., *Fasciola hepatica*, *Fasciola gigantica*, *Fasciolopsis buski*), copepod-mediated (*Gnathostoma*, *Spirometra, Dracunculus medinensis*) or transmitted by faecal-contaminated water *(Taenia solium, Taenia saginata, E. granulosus and E. multilocularis).*

Water Microbial Ecology – Faecal and Waste Bacteria in Marine Environments

Oral Presentations

Differential colonization of three Greek olive cultivars' root system by two AMF genus: Plant growth and mineral nutrition of olive plants

Th. Chatzistathis[1], M. Orfanoudakis [2], D. Alifragis[2] and I. Therios[1]

[1] Laboratory of Pomology, School of Agriculture, Aristotle University, 54124, Thessaloniki, Greece
[2] Laboratory of Forest Soils, School of Forestry and Natural Environment, Aristotle University, 54124, Thessaloniki, Greece
E-mail : ChChatzi@in.gr, tel.: 00306934375680

Rooted leafy cuttings of three Greek olive cultivars ('Koroneiki', 'Kothreiki' and 'Chondrolia Chalkidikis') were grown for 6 months in three soils, in an experimental greenhouse, in order to investigate: i) if their root system was colonized by different genus of arbuscular mycorrhiza (AMF), and, ii) the growth and mineral nutrition of olive plants. We found that two different AMF genus (Glomus sp. and Gigaspora sp.) were colonizing the root system of the three cultivars studied, and that this colonization covered different percentage (%) of their root system. Hence, Glomus sp. colonized only the root system of 'Koroneiki' and not that of the other two cultivars. The greatest colonization of root system (by Gigaspora sp.), in all the soils, was found in 'Chondrolia Chalkidikis' (between 58% and 73%), compared to colonization recorded in the other two cultivars (between 45% and 57%). In all the soils, the ratio leaves d.w./ root d.w. of 'Chondrolia Chalkidikis' was significantly greater (1.75-1.79), compared to those ones of the other two cultivars (0.66-1.11). This could be possibly ascribed to the significantly greater colonization percentage of the root system of that cultivar by AMF. Concerning leaf concentrations of seven nutrients (Mn, Fe, Zn, Ca, Mg, K and P), for most of them significant differences were found between cultivars, between soils, as well as between soil type and olive cultivar (interaction effect). Those differences could be possibly ascribed to the differential colonization of olive plants' root system by AMF Glomus sp. and Gigaspora sp.

Some physical, chemical and bacteriological properties of the drinking water in fountain of the streets in Canakkale city

E.Altıparmak, N.N. D. Zorba

Çanakkale Onsekiz Mart University, Food Engineering Department, 17020, Çanakkale, Turkey, e-mail : dnukhet@hotmail.com, tel.:+902862180018-2260

The risk of spread of infections by contaminated water supply in the community increases the significant of water sanitation. Thus in this study, the physical, chemical and bacteriological properties of drinking water in the street fountains in Çanakkale was investigated. Water samples, pH, turbidity, total hardness, total and coliform bacteria values for the samples were established. According to the results; pH, turbidity, and total hardness were found between 6.25 – 7.30, 1 – 12 NTU and 124 – 472 mg/L CaCO3, respectively. All of 25 samples searched for total bacteria at 37ºC and 22ºC were found to be suitable for drinking water quality (0 – 19 cfu/ml at 37ºC, 1 – 34 cfu/ml at 22°C), however coliform bacteria was detected in one sample (2 unit/ml).

Decolorization of synthetic silk dye by biological treatment using white rot fungus, *Lentinus squarrosulus* Mont. LS-YA and *Lentinus polychrous* Lev. LP-PT-1

S. Booncharoen, P. Saejung and C. Pukahuta

Faculty of Science, Ubon Ratchathani Univ.,Ubon Ratchathani 34190 Thailand.
e-mail: phira88@gmail.com, tel.: +66-86-688-0234

Lentinus squarrosulus Mont. LS-YA and Lentinus polychrous Lev. LP-PT-1 are saprophytic white rot fungi that can produce lignocellulolytic and peroxidases enzymes. Their optimal temperature is 30 degree celcius which is benefit to the reduced cooling cost in fermentation. In this study, these fungi were cultivated in Fahraeus liquid medium supplemented with an azodye, blue or golden yellow at the same concentration of 100 ppm under shaking at 150 rpm at 30 degree celcius. It was found that in 24 hours, L.squarrosulus Mont.LS-YA reduced the blue dye concentration to 0 ppm at pH 6.44 while L. polychrous Lev. LP-PT-1 reduced to 20.46 ppm at pH 6.29. L.squarrosulus Mont. LS-YA reduced the golden yellow dye concentration at to 81.58 ppm at pH 6.54 while L. polychrous Lev. LP-PT-1 reduced to 84.25 ppm at pH 6.40. The result shown that both the type of azodye and the original concentration effected the ability on decolorization of the white rot fungi. Application will be made to biologically treat on the waste of synthetic silk dyeing by these fungi in the future.

Serological study of bovine viral respiratory diseases in dairy herds in Kerman province, Iran

E.Sakhaee[1], M.Khalili [2], A.Sakhaee[3], O. Azari [1]

[1] Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Iran
[2] Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Iran
[3] Department of Clinical Sciences, Faculty of Medicine, University of Semnan, Iran
e-mail: Ehsan_Sakhaee@yahoo.com, tel.: 00989132952830

Respiratory disorders are major concern for Cattle, given the frequency of such infections and the high number of animals affected. Viruses and bacteria combined with stress play a key role in triggering acute respiratory infections. The most important viral agents are bovine viral diarrhea virus (BVDV), bovine herpes virus type 1 (BHV-1), bovine respiratory syncytial virus (BRSV), parainfluenza virus type 3 (PIV-3) and bovine adenovirus (BAV). This cross-sectional study, the first master plan in the field of bovine viral respiratory diseases in Iran, was conducted to evaluate serologic findings of BVDV, BHV-1, BRSV, PIV-3 and BAV in dairy herds in Kerman province, Iran. One hundred and eighty one serum samples were collected from 1–3 years old cattle originating from 15 industrial dairy farms in Kerman province, Iran, from June to November 2007. The samples were tested by commercial indirect ELISA kits. Antibodies were detected against; BVDV, BHV-1, BRSV, PIV-3 and BAV in 77.90%, 30.39%, 100%, 100% and 100% of serum samples, respectively. Antibodies against all of 5 viruses were detected in 4 herds (26.66 %) among 15 dairy farms and there are no serologically negative farms against all of 5 viruses. According to serological findings, BVDV, BRSV, PIV-3 and BAV are common viruses in dairy herds in Kerman province, Iran.

Fate and survival of Campylobacter coli in swine manure at different temperatures

X. T. Bui[1], A. Wolff[2], D. D. Bang[1]

[1] Laboratory of Applied Micro and Nanotechnology, National Veterinary Institute, Technical University of Denmark (DTU-VET);
[2] Biolabchip Laboratory, Department of Micro and Nanotechnology, Technical University of Denmark (DTU-Nanotech)
E-mail: thxb@vet.dtu.dk, tel.: +45 3588 6895

Introduction: Thermophilic Campylobacter are recognized as a leading cause of human foodborne infection worldwide. Campylobacter coli is mainly reported in pig (95%), but its survival times in swine manure environments and transmission to human enteric illness are poorly understood. We present a new method to detect and quantify C. coli in swine manure at different temperatures by using messenger RNA of C. coli as a marker of viable cells. Materials and Methods: Eighty-five fresh and aged manure samples were collected from seven different pig farms in Jutland (Denmark) and transported immediately on ice to the laboratory. Campylobacter-negative manure samples were spiked with Campylobacter coli (5x108 CFU/ml) and incubated at 40C, 140C, 200C, 420C, and 520C. Then C. coli was detected and quantified at various times after inoculation (day 1, day 2, day 3…day 45) by DNA/RNA quantitative real time PCR and plate counting. C. coli glyA house-keeping gene was used to detect viable cells. Results and conclusion: Comparable results were obtained by the different methods. RNA-based quantitative PCR assay detected only live C. coli cells, thus avoiding the dangers of false positive result from non-viable cells. Our data indicated that C. coli could survive in swine manure up to 24 days at 40C. However, they lived no longer than 7 days at 140C and 200C. At higher temperatures (420C and 520C), the half life of C. coli was extremely short (less than 4 hours). The results from mRNA-based PCR were very consistent with the data of the plate counting method. In contrast, DNA-based quantitative real time PCR could detect C. coli in all samples even more than 45 days after inoculation at all temperatures. Thus, the mRNA-based PCR is much more reliable than DNA-based PCR and other traditional methods to detect active bacteria in swine manure.
Keywords: real-time PCR, Real-time RT-PCR, Campylobacter, mRNA, glyA gene.

Superficial bacterial contamination of bovine carcasses in one the slaughter houses around Tehran

M.Tannaz[1], F.Peyman[2], Y. B.Majid[3]

[1] Department of Food Hygiene, Faculty of Veterinary Medicine, Islamic Azad university, Garmsar Branch, Garmsar, Iran;
[2] Veterinary Student, Faculty of Veterinary Medicine, Islamic Azad university, Garmsar Branch, Garmsar, Iran
[3] Department of Food Hygiene, Faculty of Veterinary Medicine, Islamic Azad university, Garmsar Branch, Garmsar, Iran *Corresponding author: tannazmoosavi@yahoo.com, tel.: +989123018259

Food borne diseases often follow the consumption of contaminated food-stuffs especially from animal products such as meat from infected animals or carcasses contaminated with pathogenic bacteria as Salmonella spp., and Escherichia coli O157: H7.The majority of these germs result from contamination occurring at the slaughterhouse , where conventional veterinary inspection cannot detect the presence of these bacteria on apparently healthy carcasses .The different stages of the conversion from live animals into meat make the microbial contamination of carcasses an unavoidable and undesirable result. During the slaughtering process, main sources of contamination are the slaughtered animals themselves, the staff and the work environment. The contamination of equipment, material, and workers' hands can spread pathogenic bacteria to non-contaminated carcasses. The purpose of our work is to study the degree of superficial bacterial contamination of bovine carcasses at a slaughterhouse; quantitatively by counting the total viable and fecal coliform counts, and qualitatively by the research for Salmonella spp. at 3 different bovine carcasses sites. Carcasses were examined just after eviscerating. Wet-dry double swab sampling was used. The results showed that the brisket area and the posterior side of the foreleg were the most contaminated areas. Salmonella wasn't isolated from carcasses and Escherichia coli O157: H7 was isolated from one sample.Our results reflect poor conditions of slaughtering and handling of carcasses

Detection of antibody against infectious bovine rhinotracheitis glycoprotein gE in aborted cattle in Mashhad, Iran

H.Tabar [1,3] , G. R. M. Rad[1], Z. Naseri[3], M Azizzadeh[2]

[1] Department of Pathobiology, Faculty of Veterinary Medicine,
[2] Department of Clinical Sciences, Faculty of Veterinary Medicine,
[3] Center of Excellence in Ruminant Abortion and Neonatal Mortality, Ferdowsi University of Mashhad, Mashhad, 91775-1793, Iran
e-Mail : hashemit@um.ac.ir, τel.: +98 9153138954

Infectious Bovine Rhinotracheitis is a highly contagious disease caused by the bovine herpes virus-1 (BoHV-1), resulting in significant losses to livestock around the world. BoHV-1 is a major pathogen of cattle, primarily associated with respiratory/genital tract infections and abortion. In the present study, we determined the presence of antibodies in 120 serum samples of cattle with the history of abortion in different period of pregnancy from different industrial dairy herds in Mashhad. Also we tested 30 samples from normal cattle with no history of abortion as negative control. The presence of antibodies against infectious bovine rhinotracheitis was investigated by enzyme-linked immunosorbent assay (ELISA). The results showed that seroprevalence of IBR in aborted cattle were 70% (84 samples). From these positive samples, 11 (13.09%), 42 (50.00%) and 31 (36.91%) samples were associated to the first, second and third trimester of pregnancy, respectively. From these seropositive cattle (84 samples), 12 (14.28%) samples were associated with stillbirth and 7 (8.33%) samples were related to mummified fetus. From 84 positive samples, 59 (70.1%) were related to cattle between 2-5 years old and 25 (30.9%) were associated to cattle more than 5 years old. In negative control group, 5 samples showed antibody against IBR antigen. Based on our results, we can not establish the relationship between abortion and IBR. So interpretation is difficult as the abortion is commonly found so long after the IBR infections occurred. However, configuration of disease by serological means must be demonstrated by either seroconversion or significant rise in antibody levels. Keywords: Abortion, Antibody, Cattle, ELISA, Infectious bovine rhinotracheitis.

Campylobacteriosis as a zoonotic disease!

G. R.Hashemi Tabar

Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, 91775-1793, Iran

Campylobacteriosis is caused by *Campylobacter* organisms. This is most commonly caused by *C. jejuni*, It is among the most common bacterial infections of humans, often a foodborne illness. It produces an inflammatory, sometimes bloody, diarrhea or dysentery syndrome, mostly including cramps, fever and pain. It is found in cattle, swine, and birds, where it is non-pathogenic. But the illness can also be caused by *C. coli* (also found in cattle, swine, and birds) *C. upsaliensis* (found in cats and dogs) and *C. lari* (present in seabirds in particular).
Infection with a *Campylobacter* species is one of the most common causes of human bacterial gastroenteritis. For instance, an estimated 2 million cases of *Campylobacter* enteritis occur annually in the U.S., accounting for 5-7% of cases of gastroenteritis. Furthermore, in the United Kingdom during 2000 campylobacter jejuni was involved in 77.3% in all cases of foodborne illness. 15 out of every 100,000 people are diagnosed with campylobacteriosis every year, and with many cases going unreported, up to 0.5% of the general population may unknowingly harbor *Campylobacter* in their gut annually. A large animal reservoir is present as well, with up to 100% of poultry, including chickens, turkeys, and waterfowl, having asymptomatic infections in their intestinal tracts. An infected chicken may contain up to 10^9 bacteria per 25 grams, and due to the installations, the bacteria is rapidly spread to other chicken. This vastly exceeds the infectious dose of 1000-10,000 bacteria for humans. In this article this disease is fully discussed in human and animals.
Key words: Campylobacteriosis, Human, Animal, Zoonosis.

Modeling the influence of Bunium persicum Boiss. Essential oil, pH and temperature on growth of Listeria monocytogenes

S. Khanzadi[1], A. Jamshidi[1], H. Seifi[2], Z. Noori[3]

[1] Department of Food Hygiene, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad;
[2] Graduated Student, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad;
[3] Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad. Corresponding author's email: khanzadi@um.ac.ir, tel.: +989155028981

Listeriosis is known as a major food-borne disease throughout the world caused by Listeria monocytogenes. Different factors can affect the growth of food borne microbial pathogens. Knowing the precise boundary for the growth-no growth interface of L. Monocytogenes and also determining the period of time needed for bacterial growth initiation is necessary for food safety risk assessment. This study was designed to examine the combined effects of different levels of Bunium persicum essential oil (0%, 0.08%, 0.16%, 0.24%), temperature(35 ˚C, 25 ˚C, 4 ˚C), pH(5, 6, 7) and inoculum (103, 105 cfu ml -1) on the growth of L. Monocytogenes in brain heart infusion broth. Growth was monitored by visible turbidity over a 30 days period. The measured data points showed significant effects for selected parameters on growth of L. Monocytogenes ($P<0.05$). Stepwise Multiple Regression Program was used to predict the growth initiation. For obtaining a boundary model the logistic Regression Program was used. The models adequately predicted the growth initiation and growth inhibition of L. Monocytogenes. Keywords: Listeria Monocytogenes, predictive model, Bunium persicum

Chemotaxis behavior of *Campylobacter spp*. in function of different temperatures (37^{o}C and 42^{o}C)

M. Baserisalehi

Islamic Azad University, Kazeroun branch, Kazeroun, Iran
e-mail : majidbaseri@hotmail.com, tel.:+989173043055

Chemotaxis response of Campylobacter spp. toward different amino acids at two temperatures (37oC and 42oC) was major purpose of this study. Two strains of catalase positive and negative Campylobacter were isolated from river water in tonkabon, Iran. The isolates were identified based on phenotyping and Gene sequence of 16srRNA methods and they were recognized as Campylobacter jejuni and Campylobacter gravis. Chemotactic responses of the isolates toward a variety of amino acids viz., cystine, histidine, aspartic acid, serine, phenylalanine, leucine and tryptophan at two temperatures 37oC and 42oC were assessed by disc and capillary methods. However, positive chemotaxis for Campylobacter jejuni was observed towards cystine, tryptophan, phenylalanine and leucine at both temperatures, but it was greater at 37oC. Campylobacter gravis revealed negative or weak chemotaxis response toward all of the amino acids. The results of capillary assay indicated that chemotaxis behavior of Campylobacter jejuni was greater at 37oC than at 42oC. Overall, chemotaxis response of Campylobacter jejuni was vigorous than that of Campylobacter gravis and its expression was greater at 37oC . Hence, the human body temperature (37oC) well induces expression of chemotaxis as a virulence factor of Campylobacter jejuni in compared to bird with 42oC body temperature. On the other hand, chemotaxis response of nonpathogenic Campylobacter such as Campylobacter gravis is fewer than that of pathogenic Campylobacter, such as Campylobacter jejuni. Key words: chemotaxis, Campylobacter, temperatures, aminoacids

Microbial Community in Process of Fermentative Biohydrogen Production from Organic-rich Waste

P. Nipon

Division of Environmental Technology, Department of Agro-Industrial Technology, Faculty of Applied Science, King Mongkut's University of Science and Technology North Bangkok, Bangkok 10800, Thailand
e-mail: npisutpaisal@gmail.com, tel.:66 86 314 7829

The current study was to investigate the microbial ecology in a process of fermentative biohydrogen production from organich-rich waste in batch reactor operated at pH 6, and 37oC. Microbial seed obtained from an upflow anaerobic sludge blanket reactor treating food processing factor wastewater was used for the startup. During the experiment, hydrogen, carbon dioxide and methane gas evolved from the reactor, the reduction of organic content and the generation of the biodegradation byproducts in the reactor content was monitiored simultaneouly. At the optimal reactor performance with respect to the hydrogen percent compositon, hydrogen production potential and hydrogen gas yield, microbial community was analyzed in order to identify the dominant microorganisms. Denaturing Gradient Gel Electrophoresis (DGGE) technique and the analysis of the DNA sequences of the five selected major bacteria showed that the community was dominated by Firmicutes, mainly clostridia and weissella and actinomycetes. Phylogenetics of microbial community was further investigated in details by sequencing of 16S rDNA clonal library. In total, 96 clones were randomly selected for sequencing of the full-length 16S rDNA sequences (approx. 1.5 kb). Restriction Fragment Length Polymorphism (RFLP) analysis of the inserts resulted in 25 RFLP patterns and their DNA sequences were then compared to the GenBank database for phylogenetic identification. Based on 16S rDNA clonal library, the biohydrogen producing microbial community was dominated by Firmicutes (50%) and Proteobacteria (40%) with Actinobacteria and Chloroflexi as the minor bacterial phyla. The composite microbes were closely related to several hydrogen producing bacteria in the phylum Firmicutes, including class Bacillales and Clostridia. Bacilli and clostridia are metabolically versatile bacteria capable of using complex polysaccharide as carbon and energy sources by production of a wide range of polysaccharide degrading enzymes together with other hydrolytic enzymes targeted on other substrates. The presence of Proteobacteria and Actinobacteria suggested their roles on degradation of the complex substrates used as feedstock in the fermentation process and production of metabolites or intermediates for hydrogenogenesis, which might metabolically linked to the hydrogen producing bacteria. The result thus suggested the co-existence of few bacterial phyla in this complex hydrogen producing community.

Actinomycetes from coastal marine sediments: A potential source for antimicrobial/ anticancer antibiotics

S. Rattanaporn[1], S. Thitinat[2] S. Chantarawan[3]

[1] Institute of Marine Science, Burapha University, Chonburi. 20131. Thailand
2 Faculty of Medical Science, Naresuan University, Phitsanulok, 65000. Thailand
[3] Faculty of Allied Health Sciences, Burapha University, Chonburi. 20131. Thailand
e-mail: rattanap@buu.ac.th, tel.:6638391671-3

Actinomycetes have a great potential for producing a wide range of biologically active compounds, especially antibiotics for clinical use and therapeutic agents: antibacterial, antifungal, anticancer, immunomodulating, cholesterol-lowering drugs etc. The purpose of the research was to search for actinomycete antimicrobial/anticancer producing strains from marine sediments. Sampling sites were in the mangrove, Chantaburi, and shallow coastal areas in Chonburi, Thailand.

Twenty sediment samples were serially diluted with natural seawater before inoculation on various kinds of selective isolation media. All isolation media were added with 50% of natural seawater and supplemented with 25 µg/ml of novobiocin and 50 µg/ml of nystatin to prevent growth of other bacteria and fungi. Isolation plates were incubated at 30^{o} C and were observed up to 4 weeks. Out of 166 isolates, 85 from the mangrove areas and 81 from the coastal sediments; 92 isolates were antibiotic producing strains, 61 from the mangrove and 31 from the coastal sediments. Ethyl acetate crude extract of four promising active strains isolated from the mangrove sediments, FK 1-7, FK1-12, FK2-6, FK2-7 were chosen to investigate for apoptosis of HeLa cells and found that FK 2-6 crude extract gave the best result of 28.28 µg IC50 comparing to the others. The active strains were identified by chemical analysis of diaminopimelic acid and sugar patterns in cell wall and whole- cell hydrolysates, some were confirmed by 16S RNA gene sequencing analysis. The result showed actinomycetes from the mangrove sediments were rather diverse in which *Streptomyces, Actinomadura, Saccharomonospora, Nocardia, Streptoalloteichus, Kibdelosporangium, Micromonospora* including some unidentified genera were found, while those from coastal sediments were less diverse in which *Micromonospora* were dominant and *Streptomyces griseus*, *Streptomyces, Salinispora* and *Rhodococcus* were minor groups. Antimicrobial antibiotic/anticancer producing strains recovered from our research reveal that actinomycetes from marine sediments are clearly a potential source for novel antimicrobial/ anticancer agents. The rest of active strains are to be studied further.

Energy generation from toluene degrading - *Pseudomonas putida* F1 using "Bio-Fuel Cells"

H. Friman[1,2], R. Cahan[1], A. Schechter[1] Y. Nitzan[2]

[1] The Ariel University Center in Samaria, Ariel, ISRAEL,
[2] Bar-Ilan University, Ramt-Gan, ISRAEL. e-mail: henf@ariel.ac.il, tel.:97239066606

The treatment of aromatic hydrocarbons in wastewater resulting from oil spills and chemical manufactories is becoming a key concern in many modern countries. Benzene, ethylbenzene, toluene and xylene (BETX) contaminate groundwater as well as soil. These compounds have an acute effect on human health and are known to be carcinogenic. Conventional removal of these toxic materials involves separation and burning of the wastes, however, the cost of chemical treatment is very high and energy consuming. Microbial fuel cells (MFCs) have been operated successfully by using a variety of readily degradable compounds, such as glucose, acetate, monosaccharides, and complex carbohydrates. MFC is emerging as an excellent approach to treat aromatic hydrocarbons. The bacterial cells can use BETX compounds as a hydrocarbon source and transformed it directly to electricity through microbial metabolism. The overall process is economically favorable, not just as a means of water treatment, but also as an alternative energy source. Nevertheless, state of the art microbial fuel cells utilize mixed bacterial strains that are not optimized for BETX bioremediation, therefore, the total power and aromatic conversion rates are expected to be low. That is why it is essential to use specific BETX-degrading bacteria strains. In this research culture of Pseudomonas putida was grown in MFC with toluene 100 mg/l as a single carbon source under constant voltage of 125 mV, 250 mV and 500 mV. The peak current reached to 10.4 µA, 16.1 µA and 25.8 µA respectively. The culture grown in MFC reached to 0.8 OD660nm while the control culture that grown with out external voltage reached only to 0.6 OD660nm. In edition, the proteomic profile of the culture grown in MFC differ from the the proteomic profie of the control culture.

Adaptation of bacterial community during production of poly(3-hydroxybutyrate) from glycerol

P. Nipon

Division of Environmental Technology, Department of Agro-Industrial Technology, Faculty of Applied Science, King Mongkut's University of Science and Technology North Bangkok, Bangkok 10800 Thailand
e-mail: npisutpaisal@gmail.com, tel.:6686 314 7829

Glycerol byproduct from biodiesel industry has been increasingly generated since the recent rise of fossil fuel's price, and highly potential becomes waste steam required to be treated due to the over market demand and the plummet market price of pure glycerol grade. The aim of current study was to investigate the production of polyhydroxyalkanoate biopolyester from glycerol with concentration range of 0 - 70% (v/v) using an UASB bacterial consortium as a seed, under 30˚C shake flask experiment for 30 days. Result indicated that glycerol concentration of 50% appears to be a threshold level for the bacterial growth. In glycerol containing media, maximal growth and specific poly(3-hydroxybutyrate) biopolyester production of 3,518.9 mg HB g-1 cell dry weight L-1 were observed at 10% glycerol concentration after 120 h incubation time corresponding to the stationary growth phase. At the initial concentration of 10%, glycerol was consumed 34 and 85 % after 120 and 264 h incubation times respectively. Denaturing gradient gel electrophoresis (DGGE) showed the significant dynamic change in community structure based on 16S rDNA band patterns between the original seed and that cultured in glycerol containing media after 120 h incubation time. The band patterns obtained from bacterial seed grown in the glycerol containing media was identical regardless of the glycerol concentrations. Based on 16S rDNA sequence analysis, poly(3-hydroxybutyrate) producers would likely belonged to genus Citrobacter, Dysgonomonas and Klebsiella.

Real-time on-line monitoring of surface water in Athens water treatment plant by Daphnia Toximeter

G.Vassilantonopoulou [1], E.Lytras [2]

[1] Toxicological Department, Water Quality Control Division, E.YD.A.P. (Water Supply and Sewage Company of Athens), 156 Oropou str., Athens,
e-mail: ginamicro@eydap.gr.;
[2] Water Quality Control Division, E.YD.A.P. (Water Supply and Sewage Company of Athens), 156 Oropou str., Athens
e-mail: e.lytras@ontelecoms.gr., e-mail: ginamicro@eydap.gr; ginamicro@yahoo.gr
tel.:(+) 210 2144360, (+) 210 9751573

The aim of this presentation is to describe the water toxicity monitoring results via a Daphnia Toximeter that is installed for the first time in Greece, in the entrance of one of Athens Water Treatment Plants. This monitoring device is an early warning system that allows the users to respond rapidly to incidents of toxic contamination, by detecting a wide range of dissolved toxic compounds including pesticides, neurotoxins and heavy metals. The Daphnia Toximeter uses a video-camera to monitor the activity of Daphnia magna. The live images are evaluated online with an integrated PC to analyse critical changes in the behaviour of Daphnia. The determined behavioural parameters are: the average velocity, the speed class distribution, the average distance among organisms, the 'curviness' of swimming patterns as determined by two fractal dimension equations, the average altitude and the recognition rate of Daphnia. If any of mentioned parameters changes suddenly or dramatically, an alarm is triggered, when alarm conditions, as set by the operator, are met. Additional chemical analyses were performed to gain insight in the cause of the alarms. The results demonstrate that for a 6 months period of Daphnia Toximeter operation, not any real alarm situation was detected. Consequently, the input water of Athens Treatment Plant did not contain any potentially toxic contaminants. The Daphnia Toximeter proves to have a considerable potential as a tool to determine the real-time quality of drinking water sources.

Keywords : Real time early warning system, daphnia Toximeter, online test, water quality.

A study on fungal flora of tap water as a potential reservoir of fungi in hospitals from Sari city

I. M.T. Hedayati[1], S. Mayahi[1], M. Movahedi[2], T. Shokohi[1]

[1] Department of Medical Mycology and Parasitology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[2] Department of Environmental Health, School of Health, Mazandaran University of Medical Sciences, Sari, Iran
e-mail: hedayaty2001@yahoo.co.uk, tel.:00981513543088

Since the incidence of mold infections in hospitalized patients with immuno-compromised conditions continues to rise despite the widespread use of air filtration systems, suggesting that other hospital sources for molds may exist. In view of these facts, the objective of the present study was to evaluation of tap water samples of university hospitals as a probable potential reservoir of fungi from Sari city, Iran. Methods: During a one-year period, 240 water samples were collected from 4 university hospitals. All water samples were collected in sterile polystyrene bottles containing sodium thiosulfate (120 mg/L). A volume of 100 ml of the samples passed through sterile 0.45 micrometer filters. The filters were placed directly on Malt extract agar (containing gentamycin and chloramphenicol) and incubated at 27 ° C for 3-7 days. Routine mycological techniques were applied to identification of grown fungi. Results: Out of 240 plates, 77.5% were positive for fungal growth. A total of 498 fungal colonies were isolated. Twelve different genera were identified. Aspergillus (29.7%), Cladosporium (26.7%) and Penicillium (23.9%) were the most common isolated. Phoma (0.2%) had the lowest frequency. Among Aspergillus species, A. flavus (56.1%) had the highest frequency. Highest colony counts 145 (31.4%) were found in autumn. Aspergillus predominated in autumn, Cladosporium in winter and spring and Penicillium in summer. The total mean colony forming units (CFU) per 100 ml for the positive samples was 2.7. Conclusion: The results of our study showed that hospital water should be considered as a potential reservoir of fungi specially Aspergillus.

Key words: Fungi, Aspergillus, Tap water, Hospital

Effects of land use types on the activity of waterborne Escherichia coli O157:H7 within a UK catchment CE

C. Thorn[1], R.S. Quilliam[2], A.P. Williams[1], D.L. Jones[1], S. Malham[1] and B. Reynolds [1]

[1] School of the Environment, Natural Resources and Geography, Bangor University, Bangor, Gwynedd, LL57 2UW. UK;
[2] Centre for Applied Marine Sciences, School of Ocean Sciences, Bangor University, Menai Bridge, Anglesey, LL59 5AB. UK
e-mail: afpa72@bangor.ac.uk, tel.:00447833633923

Contamination of watercourses with faecal matter from sewage outlets, as well as from surface run-off and leachate originating from agricultural land, can lead to the introduction of enteric pathogens, such as Escherichia coli O157:H7, into these aquatic systems. This poses risks to public health and can also lead to re-infection of livestock and perpetuation of the cycle of infection. To understand the activity and behaviour of waterborne E. coli O157:H7, we generated microcosms using river water samples taken from areas of different land use types within the Conwy catchment, UK, such as forestry, peat moorland and agriculture. Half of these mesocosms were filtered to remove the native microbial community. Chemical properties were analysed and autochthonous populations of heterotrophic bacteria and coliforms enumerated. Filtered and unfiltered microcosms were inoculated with a chromosomally lux-marked strain of E. coli O157:H7 and kept in a constant environment. Activity was measured using a luminometer and expressed as relative light units (RLU). Results showed markedly different levels of E. coli O157:H7 activity in water from areas of different land use types. Further investigations were carried out to simulate effects of increased nutrient fluxes, e.g. from heavy rainfall events, on the activities of E. coli O157:H7. These results indicate for the first time how agricultural run-off can affect both the persistence and infectivity of this important human pathogen in watercourses downstream.

Microbial water quality and environmental conditions in a freshwater ecosystem

S.Ioannou[1], M.Tsoumani[2], T.Papadimitriou[2], &I.Kagalou[1,3]

[1] Msc, Dept. of Aquaculture –Aquatic Animal Health, TEI of Epirus-Univ. of Thessaly, Greece.
[2] Dept. of Biological Applications & Technologies, Univ. of Ioannina, 45110, Ioannina, Greece
[3] Dept. of Ichtyology& Aquatic Environment, Univ. of Thessaly, 38446,Volos, Greece
e-Mail : ikagalou@uth.gr, tel.:2421093084

Water is a renewable resource and acceptable quality is important for human health, ecological and economic reasons. Managing the water cycle in a sustainable way is the key to protect natural resources and human health. Freshwater ecosystems are frequently used as recreational, touristic and for other economic purposes. Unfortunately pollution in these systems is widespread, and areas impacted by human activity may be severely degraded. Lake eutrophication has been a major problem for decades involving a change in lake status, with detrimental effects to the ecosystem. Furthermore eutrophicated lakes ,usually, show high level of contamination posing a risk for the human health. The objective of the present study is to evaluate the abundance and the spatial distribution of indicator bacteria of Lake Ziros (N-W Greece), along with the environmental factors aiming at the assessment of the water quality. Multivariate statistical analysis was applied in order to identify the impact of abiotic factors on bacteria population. The monthly variation of the key-eutrophication parameters are presented as well as the abundances of the indicator bacteria. The trophic status, and the pollution level are discussed highlighting also implications for the Water Framework Directive.

Correlation of viral pathogens present in environmental waters with human isolates

V.Krikelis[1], A.Vontas [1], E.Plakokefalos[1], E.Gramoustianou[1], Minas A.[1], A.Noulas [1], C.Hadjichristodoulou [2]

[1] Medical Laboratories Department, SEYP, TEI Larissas, 41110, Larissa
[2] Epidemiology Laboratory, Department of Medicine, School of Health Sciences, University of Thessaly, 41221 Larissa, Greece
e-mail: vkrikelis@teilar.gr, tel.:+30 6976568648

Human enteric viruses belonging to different families and genera are being detected for the past 25 years from environmental water samples, such as costal sea waters, river waters, urban effluents and lake waters in Greece. The most often detected and identified viral pathogens belong to: Adenovirus serotypes 1 to 7, 15 and 40/41, with the most often found in cell cultures serotype 7. Enteroviruses (Polio all three serotypes , Coxsackie B serotypes 1 to 5, and Echo serotypes 1, 7, 14, and 30). Noroviruses , both genotypes and Rotaviruses. All the above viruses have also been found in various clinical samples from humans such as throat, stool, cerebrospinal fluid, lung necropsy tissue and eye swabs. The presence of the these viruses in water ecosystems chronologically anticipates epidemic outbreaks and their study is possibly a good indicator of viral disease outbreaks in the community. Therefore the study of viral circulation in water ecosystems and the community gives important information to health authorities in planning and implementing health directives and measures.

Diversity of marine actinomycetes from polluted waters of ennore and pulicat and their response to environmental hazards

Periyanayagam Sagaya Victor

Matha kovil street, akkarayapalayam, kallakurchi(tk), villupurum(dt).tamil nadu.606207, India e-mail: marynaveena1993@gmail.com

Water and soil pollution has become a major concern in the world, as much of the population relies on Marine resources. Heavy-metal contamination brings a potential health hazard that can cause metal toxicoses in animals and humans. Trace elements such as cadmium, copper, and mercury are very toxic heavy metals and have been found in the human environment at increased concentrations, because a wide variety of industrial activities have accelerated the release of these metals at higher rates than natural geochemical cycling processes. Diversity of marine actinomycetes in the polluted waters and sediments of Ennore and Pulicat ecosystem was studied. Actinomycetes in marine environments are often under extreme conditions of e.g., pressure, temperature, salinity, and depletion of micronutrients, with survival and proliferation often depending on the ability to produce biologically active compounds. Actinomycetes highly resistant to environmental hazards has been isolated from seawater and sediment samples were tested for growth in the presence of different heavy metals, pesticides, and plastics to investigate their potential for growth in the presence of a variety of toxic xenobiotics. We hypothesized those actinomycetes resistant to high concentrations of heavy metals would have potential capacities to tolerate or possibly degrade a variety of toxic materials and thus would be important in environmental pollution bioremediation.

Meta-analysis of *Cryptosporidium* and *Giardia* concentrations in sewage for public health risk assessment

Hamilton Kerry

1862 Mintwood Pl NW Apt 404 United States, tel.:516-557-3772
e-mail: kahamilton17@gmail.com

World Bank estimated that 15-20% of the recorded diagnoses recorded in Dar es Salaam from 1996 through 2003 were due to waterborne diseases, with 15% total cases (16.5% deaths) residing within the Ilala district. Water rationing, overflowing septic tanks, infrastructural degradation, and unserviced and unplanned squatter communities contribute to this disease burden. Drinking water in the Ilala district is provided primarily through shallow and deep wells. Shallow wells are usually 1-5 m deep, are uncovered or semi-covered, and are publicly owned and maintained to provide water free of charge to residents. Deep wells are typically privately owned for profit (89% studied). They are perceived to contain higher quality water since they are typically greater than 5 m deep and are closed and capped with access via a tap. The goal of this study was to evaluate public health risks from exposure to waterborne contaminants in shallow and deep well types, and make risk management recommendations to prevent and mitigate potential exposures. This study evaluated potential public health risks from open and “semi-closed” shallow wells, and deep wells in the Ilala district by assessing well water quality for concentrations of nutrients and indicator bacteria, and surveying for access, construction, and maintenance factors. Although “semi-closed” wells were partially covered by wooden boards or other means and closed wells were completely covered, the indicator bacteria total and fecal coliforms exceeded U.S.EPA and WHO guidelines 1000-fold across all well types. Concentrations of nitrates also exceeded guideline values ten-fold in all well types. Nitrate concentrations are of particular concern because of acute risk of infant methemoglobemia and chronic cancer risks from ingestion. Only 30% well owners treated their wells with some form, and only one well-owner could produce the means of treatment when requested, indicating that potential exposures are not being mitigated by on-site treatment. Despite the willingness to pay for cleaner water from closed wells, closed well water quality exceeded water quality guidelines as frequently as open and semi-closed wells for all parameters. Although semi-closed wells were expected to have better water quality than completely open wells, this well type had the highest concentrations of all contaminants. Based on the water quality data observed, well water in the Ilala District needs to be further monitored for microbial, organic, and inorganic contaminants that may pose a threat to human health. High nitrate concentrations observed indicate a potential acute risk of methemoglobemia and warrant further sampling. Although health records could not be obtained, these data must be compared to health statistics to fully evaluate public health risk. Surveys must be administered to residents in addition to well-owners to more accurately evaluate water use practices and well access. Development and education interventions should target public perceptions of drinking water, and encourage on-site filtration practices until human health risks can be elucidated.

Drinking water risks associated with open and closed wells in Dar es Salaam, Tanzania

Hamilton Kerry
1862 Mintwood Pl NW Apt 404 ,United States, tel.:5165573772
e-mail: kahamilton17@gmail.com

Leaking sewer mains are a potential source of contamination if they are located near leaking drinking water mains. Under low or negative pressures in the drinking water system, sewage contaminated water could be introduced and eventually reach customer's taps. In the process of evaluating the potential public health risks associated with this type of intrusion event, the concentrations of pathogenic microorganisms in wastewater must be evaluated. To capture variability and uncertainty of microbial occurrence data, probability density functions (PDF) are frequently used as risk model inputs.Because of the heavy reliance on occurrence data for policy decisions, a literature review was performed for waterborne pathogens to highlight research gaps in microbial data collection. Cryptosporidium and Giardia were selected for this case study because of their resistance to disinfection, association with disease outbreaks, and typically high occurrence in wastewater influent (especially for Giardia). Thirty-two studies were reviewed for Cryptosporidium and Giardia concentrations in sewage and their corresponding parameters. Cryptosporidium concentrations in untreated wastewater ranged from zero to 13,700 oocysts per litre, while Giardia concentrations ranged from zero to 88,000 cysts per litre. Arithmetic mean concentrations were up to 1,500 Cryptosporidium oocysts per litre and 13,000 Giardia cysts per litre. Only a small portion of studies assessed parameters relevant for giving context to these values: 7 studies assessed viability; 4 assessed infectivity; 9 assessed genotype (all found human infectious genotypes). The reliability of these values cannot be fully assessed because of the inadequate reporting of sampling characteristics such as time of day, weather, and plant specifics. Lack of information regarding percent recovery, method detection limits, and non-detect interpretation is problematic when comparing data across studies and limit the use of these data as inputs into risk models. The main hurdles for the consideration of available Cryptosporidium and Giardia datasets is lack of contextual data associated with pathogen concentrations in sewage. Although standard methods such as USEPA 1623 are available, formal reporting guidelines for pathogen occurrence data are necessary for complex environmental matrices such as sewage. Better quality control and information regarding non-detect samples will be vital to achieving more accurate public health risk assessment and policy decisions based on robust occurrence data.

Isolation and Identification of Human Pathogenic Aerobic Actinomycetes And Fungi From Soil In Greece

V.Papaemmanouil, D. Lidakis, M. Plega

Microbiological laboratory of Anticancer Hospital of Piraeus METAXA, ELIKONOS 18, Greece, e-mail: vpapaemmanouil@yahoo.gr, tel.: 2104818333

The aim of this study was to identify the prevalence and geographic distribution of human pathogenic aerobic actinomycetes and fungi in the soil from differrent parts of Greece. 26 soil samples were collected from different and geographically significative areas of the country. The speciments were collected over a period of eight months, from surface soil, were numbered and put in a sterilized plastic tube and transferred to the Laboratory. Each soil sample was examined by three different techniques: a) the hair bait technique b) the cultured methods and c) the mouse procedure, to detect the possible spectrum of pathogenic actinomycetes and fungi. 21 species had been isolated belonging to 12 genera. The isolated microorganisms belonging to the genus : Nocardia, Streptomyces, Aspergillus, Candida, Cryptococcus, zygomycetes, Acremonium , Fusarium, Microsporum and Chrysosporium have been the genera most represented in the keratinophilic fungi, also were isolated four Nocardia asteroides and one Streptomyces. The successful isolation of these important etiologic agents of mycoses in man, encourages for further investigations in the soil of Greece, because of climate conditions and geographical position, could represent an ideal reservoir for other significant pathogenic fungi and aerobic actinomycetes. The recovery of nocardia in the soil of Greece, even in this small proportion, acquires importance, because potentially it is able to explain individual cases of human nocardiosis,which are observed at certain times in our country.

Water Microbial Ecology – Faecal and Waste Bacteria in Marine Environments

Poster Presentations

Annual variation of the pathogens *Salmonella sp.* and *Pseudomonas aeruginosa* in laganas bay

A. Pournara, D. S. Lymperopoulou, E. A. Katsifas and A. D. Karagouni

National and Kapodistrian University of Athens, Faculty of Biology, Department of Botany, Microbiology Group, 157 73, Athens, Greece
e-mail: apurnara@yahoo.com, tel.: 00302107274704

The detection of pathogenic microorganisms in bathing waters is quite common and should be seriously considered according to bathers' health. Since 1976 a series of parameters has been suggested to assess water's quality and thus the infectious diseases risk from bathing within. The World Health Organization, European Union and many countries all over the world use specific microorganisms as indicators, the concentration of which can predict the presence of pathogens in the water. According to the relative European Union's directive, Escherichia coli and Enterococcus sp. are used as microbial indicators. The estimation of their population leads to the characterization of waters' quality as excellent, good and sufficient. The aim of the project was to detect the presence of two important human pathogens, Salmonella sp. and Pseudomonas aeruginosa, and to ascertain that the microbial indicators used can successfully predict it. The research was conducted in Laganas Bay region and both pure culture and molecular techniques were used. Three samplings were performed within a year's period in 9 regions at Laganas bay. To detect both microbial indicators and pathogens selective culture media were used together with biochemical tests. Isolated microorganisms were identified by using molecular techniques

Effects of rain on coastal bathing waters in laganas bay

A. Pournara, D.S. Lymperopoulou, E.A. Katsifas and A. D. Karagouni

National and Kapodistrian University of Athens, Faculty of Biology, Department of Botany, Microbiology Group, Panepistimioupoli, 157 73, Athens, Greece
e-mail: apurnara@yahoo.com, tel: 00302107274704

Coastal waters' quality is influenced by a series of factors, such as the weather, the number of bathers, the activities of nearby urban areas etc. Sewage or storm effluents may enter the water, leading in deterioration of its quality and thus affecting both the equilibrium of the marine ecosystem and bathers' health. The World Health Organization (WHO) has suggested a series of microbial indicators and limits, so as to evaluate both fresh and marine water's quality. In addition, many countries have relative legislation. The aim of the project was to assess the microbiological quality of coastal bathing waters in correlation with the effect of a storm in Laganas Bay in Zakynthos, which is a protected area since 1999. Assessment of the quality was accomplished by estimating through pure culture techniques the concentration of Escherichia coli and Enterococcus sp., both microbial indicators suggested by the European Union's directive. Three samplings were performed within a six months' period in the following areas: Marathonisi, Laganas and Kalamaki. To detect microbial indicators selective culture media were used followed by biochemical tests. The results showed that the rain had a serious effect on coastal waters' quality, while the deterioration was directly related to the number of bathers.

Effect of antimicrobial agents on indigenous population of the chemolithotrophic bacteria in marbles

P.Tsakiroglou[1], G.Kanini[1], A. Savvides[1], E. Papakonstantinou[2], E. A. Katsifas[1] and A. D. Karagouni[1]

[1] National and Kapodistrian University of Athens, Faculty of Biology, Department of Botany, Microbiology Group 157 73, Athens, Greece
[2] Acropolis Restoration Service, 10 Polygnotou St., 105 55, Athens, Greece
e-mail: panost04@gmail.com,tel.: 00302107274704

The main building material of the Acropolis Monuments is the pentelic marble. The damage observed on the marbles is a biological one along with seven other categories. The aim of this work was to study the effect in situ of three chemicals (Algophase, D / 2 and Preventol R80), selected in collaboration with the Acropolis Restoration Service (YSMA) and which had the strongest effect on the indigenous population of the chemolithotrophic bacteria in in vitro experiments. The in situ experiments were conducted on two identical marble beams. One beam was treated with the method used by YSMA prior to the application of biocides, while the other was not. The application of all three biocides was applied to each one of the two beams at a specific area. The four samples taken in a year were followed by quantitative and qualitative analysis of the chemolithotrophic bacteria. The experimental procedure included the isolation of bacteria in pure cultures, the extraction of bacterium DNA, BOX PCR for the grouping of strains and sequence analysis of 16S rRNA gene for the final identification of the bacteria. The results showed that among the three biocides Algophase had the greatest long-term impact on the colonization especially when combined with the previous preservation applied by YSMA. The results were discussed in relation to the colonization of beams by the group of the chemolithotrophic bacteria.

Isolation of endophytic microorganisms from the red alga laurencia glandulifera

G.E. Malliarakis[1], E. Ioannou[2], E. A. Katsifas[1] C. Vagias[2], V. Roussis[2] and A. D. Karagouni[1]

[1] National and Kapodistrian University of Athens, Faculty of Biology, Department of Botany, Microbiology Group, Panepistimiopolis, Athens, 157 73
[2] National and Kapodistrian University of Athens, School of Pharmacy, Department of Pharmacognosy and Chemistry of Natural Products, Panepistimiopolis, Athens, 157 71, Greece e-mail : bioscientist84@gmail.com, tel: 00302107274704

Species of the genus Laurencia (Ceramiales, Rhodomelaceae) have been the subject of intensive research and proved to be a rich source of halogenated sesquiterpenes, diterpenes, non-terpenoid C15 acetogenins and a number of unique chemical structures. An important number of these metabolites, exhibit a wide range of biological properties and have been valuable in chemosystematic studies of the genus. The aim of the project was to isolate new bioactive compounds from the red alga L. glandulifera and to estimate their origin (products of microbial metabolism or products of the algal metabolism). The samples were collected from the rocks of Zoumberi, near Athens. The chemical profile of the alga was examined by means of NMR and TLC, of the organic extract. The samples were homogenized in sterilized Ringer solution and the microorganisms (bacteria and fungi) were isolated using typical, selective and diagnostic culture media. This procedure was followed by total DNA isolation from each microorganism, BOX-PCR (bacteria) and ISS-PCR (fungi) for the grouping of the isolates and the subsequent sequencing analysis of the genes 16s rRNA and 18s rRNA respectively, for their final identification.

Assessment of the microbial biodiversity at the nests of the sea turtle *Caretta caretta*

E.N.A. Prifti, G.Kanini, E. A. Katsifas and A. D. Karagouni

National and Kapodistrian University of Athens, Faculty of Biology, Department of Botany, Microbiology group 15773, Athens, Greece
e-mail: elektra-niki@hotmail.com, tel.: 00302107274704

Laganas bay is one of the most important places of reproduction of Caretta caretta in the Mediterenean area. Within a research project concerning the biology of the loggerhead turtle nesting we studied the microbial biodiversity within the nests. Samples of the eggs, coming from the inner part of the nests being in contact with roots, were taken right after the hatching and the removal of the hatchlings. The sampling was done in August and September 2008 and the samples collected were categorized to these being in contact with roots (group A) and those not surrounded by roots (group B). The experimental procedure included the isolation of bacteria using typical, selective and diagnostic media and grouping them by biochemical, physiological and morphological traits. The extracted DNA was followed by BOX PCR and analysis of the 16s rRNA gene in order to identify each bacterial strain. Totally 276 bacterial isolates were categorized to 217 different strains, of which the 69% belonged to the group A and the 31% to the group B. 63% of the bacteria isolated from the group A were Gram positive and 37% were negative, while 23 strains were isolated from a selective media for enterobacteria. Equivalently 43% from the group B were Gram positive and the 57% negative, while 12 strains were estimated as enterobacteria. The results were discussed in relation to the mortality or not of the hatchlings.

Comparison of some microbial and physico-chemical parameters in two streams from Turkey

N. Hacioglu, B.Dulger

Department of Biology, Faculty of Atrs and Sciences, Canakkale Onsekiz Mart University, 17100, Canakkale, Turkey
e-mail : nurcihan.n@gmail.com, tel.:+902862180018/1950

Water samples were collected from three different sites of Saricay and Biga Streams (Canakkale, Turkey) in the months of October 2007 – September 2008 for the analyzing of some physico-chemical and microbiological parameters of two streams. In the present investigation, the mean average value (mean ± SD) of the Saricay stream temperature, dissolved oxygen (DO), biochemical oxygen demand (BOD5), pH, electrical conductivity (EC), total coliform (TC), faecal coliform (FC) were 17.737 ± 0.199 oC, 7.117 ± 0650 mg/L, 170.4 ± 50.3 mg/L, 7.7018 ± 0.0325, 18.50 ± 2.33 µs/cm, 46461 ± 10311 MPN/mL and 33103 ± 5863 MPN/mL, respectively. And the mean average value (mean ± SD) of the Biga stream temperature, dissolved oxygen (DO), biochemical oxygen demand (BOD5), pH, electrical conductivity (EC), total coliform (TC), faecal coliform (FC) were noted as 15.533 ± 0.199 oC, 8.332 ± 0.253 mg/L, 136.60 ± 2.51 mg/L, 7.5078 ± 0.0427, 869.93 ±3.72 µs/cm, 39381 ± 7952 MPN/mL, 42500 ± 7072 MPN/mL, respectively. We conclude that there is a great potential risk infection of waters from the Saricay and Biga Streams.

Key words: Saricay, Biga Stream, Total coliform, Faecal coliform

Isolation and monthly variation of nitrogen cycle bacteria in saricay stream (Turkey)

N.Hacioglu, B. Dulger

Department of Biology, Faculty of Atrs and Sciences, Canakkale Onsekiz Mart University, 17100, Canakkale, Turkey
e-mail: nurcihan.n@gmail.com, tel.:+902862180018/1950

Saricay stream containing pollutions from various sources is a great role in pollution this fresh water resource. Saricay stream was particularly studied periodically for every month during October 2007 – September 2008. These pollutants were industrial, agricultural and domestic sources. In studying of Saricay stream, various parameters indicating pollution has been measured in the sample taking from definite stations. These parameters temperature, dissolved oxygen (DO), biochemical oxygen demand (BOD5), pH, electrical conductivity (EC). And most probable number of denitrificating and ammonifying bacteria by using the multiple tube fermentation technique were determined. The number of these bacteria show what kind of pollutants are found and level of pollution Saricay Stream. On the other hand, some nitrite and nitrate bacteria were also isolated in Saricay stream.

Key word: Saricay stream, nitrite bacteria, nitrate bacteria.

Molecular Identification of Polyomaviruses isolated in raw sewage

Kolla S[1]., Galanis A.[1], Kokkinos P.[2], Vantarakis A.[2]*

[1]Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupolis, GR 68100, Greece
[2]Environmental Microbiology Unit, Department of Public Health, University of Patras, Patras, GR 26504, Greece

*Correspondence to:
Dr Apostolos Vantarakis
Tel/Fax: (+30)2610 969875 Mob: (+30) 6945336243
e-mail: avantar@med.upatras.gr

Infection with polyomaviruses causes a wide range of clinical outcomes and serious illnesses, especially in infants, adolescents and immunocompromised patients. These viruses are highly spread among adults. Significant antibody titres are encountered in 70–80% of adult populations throughout the world. The aim of the study concerns with the isolation and molecular identification of polyomaviruses in raw sewage. The samples were collected from the inlet of the wastewater treatment plant of the municipality of Patras, from November 2007 to December 2008. Thirty-seven (37) samples (100 ml) were collected and concentrated; the viral genome was extracted, and amplified by Polymerase Chain Reaction (PCR) by using a pair of specific primers. The amplification products were electrophoresed on an agarose gel, isolated and typified by sequencing, in order to verify the isolated strains and to type the existing polyomaviruses. In total, (33) thirty-three positive samples were detected, while three (3) samples were non-confirmed and one (1) was negative. To determine the relatedness between the different sequences a phylogenetic tree was constructed according to the neighbor-joining (NJ) method. Sewage viral load is discharged to the environment and constitutes a serious hazard of public health. The application of advanced sewage treatment processes and of virological monitoring as a critical component of the evaluation of sewage quality should be seriously considered to be added to the routine testing performed for wastewater plant management, as the discharged sewage can be a severe source of environmental viral contamination and constitute a major public health problem.

Effect of charcoal-sand filtration and solar disinfection on drinking qualities of raw water sourced from omisanjana river in ADO-EKITI, NIGERIA

J.O. Oluyege, A.O Omolayo, and A.O. Oluyege

Department of Microbiology, Faculty of Science University of ado ekiti pmb 5363 ado ekiti, Nigeria, e-mail: jacoboluyege@yahoo.co.uk, tel.:+2348033883194

The provision of portable water to rural and urban population is necessary to prevent health hazard. Before water can be described as portable, it has to comply with certain physical, chemical and microbiological standards which were designed to ensure that the water is portable and safe for drinking. In developing countries, 90% of the industrial waste water discharged into rivers and steams which serve as sources of drinking water for rural communities. In our effort to contribute to availability of safe drinking water, we designed a laboratory scale and prototype charcoal- sand filtration (CSF) reactor (31.5 by 35.0 cm) for treatment of surface water for rural dwellers. The qualities of charcoal-sand filtered solar treated water samples were monitored using standard plate count technique for total bacteria and total coliform counts. Bacterial isolates from CSF water were evaluated for antibiotic resistance pattern. Sensory evaluation of the treated surface water was done using a 20- men and women panel and statistically analyzed. Charcoal-sand filtration of the raw water produced reduction of total viable bacteria counts (TVB) of 91.3% and 77.0 % of TCC. The CSF in combination with solar disinfection (SODIS) resulted to reduction by 92.7% and 97.4% of TVB and TCC respectively. The CSF-chlorinated water and CSF-citrated water were free of bacteria. Thirty-Six Gram-negative bacterial isolates were recovered from CSF water. They comprised Escherichia coli (33.3%), Enterobacter aerogenes (19.4%), Klebsiella pneumoniea (11.0%), Salmonella paratyphi (13.8%), Shigella dysenterea (8.3%) and Pseudomonas aeruginosa (13.8%). The overall resistance patterns of the bacterial isolates to six antibiotics were: ofloxacin (2.8%), gentamicin (25.0%), nitrofurantoin (63.8%), nalixidic acid (69.4%) cotrimoxazone (80.6%), augmentin (86.1%), tetracycline (88.8%) and amoxicillin (94.4%). No significant difference was noticed between the overall acceptability and organoleptic properties in terms of taste, odor as well as clarity of CSF-SODIS water and CSF-chlorinated or CSF-citrated water (Probability=95%). The physic-chemical quality of CSF-SODIS water met the standard limit for drinking water. Hence, CFS-SODIS may be recommended for surface water treatment in rural communities to prevent outbreak of waterborne diseases.

Key words: charcoal-sand filtration, solar disinfection, drinking water, antibiotic resistance, coliform bacteria, waterborne disease.

Effect of light intensity on α-Tocopherol production by the marine microalgae *Dunaliella tertiolecta* DCCBC26

N.Samadi, M.R.Fazeli and H. Tofighi

Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran; e-mail: samadin@tums.ac.ir**,** tel.:+98 21 66482608

Abstract Production of α-Tocopherol (α-T) by Dunaliella tertiolecta DCCBC26 isolated from the hypersaline lake of Urmia (north of Iran) investigated under fluorescent and halogen light sources. The isolate was grown in artificial seawater containing different concentrations of NaCl ranging from 0.05M to 3M. Under illuminance of gμfluorescent light, the maximum α-T accumulated by the microalgae was 135 g-1dw which was achieved at salinity of 0.5M after 28 days incubation at 25oC. α-Tocopherol content of the cells decreased with increasing light intensity from g/g dw at 600μg/g dw at light intensity of 150 μmol photons/m2 s to 80 μ217 μmol photons/m2 s when halogen lamps were used as light source. Although the level of dry weight obtained (0.965 g/l) after 28 days of cultivation using halogen lamp source was not much different from that achieved under illuminance of fluorescent lamp (1.045 g/l) but cell counts were higher with fluorescent lamp compared to those of halogen light.

Distribution of *Borrelia Spirochetes* by passerine birds: data from ticks collected from birds during breeding and postbreeding migration period in central Europe

L. Dubska[1] , I. Literak[1] ,E. Kocianova[2] , V. Taragelova[3] and O. Sychra[1]

[1] Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Palackeho 1-3, 612 42 Brno, Czech Republic
[2] Institute of Virology, Slovak Academy of Sciences, Dubravska cesta 9, 842 45 Bratislava, Slovakia
[3] Institute of Zoology, Slovak Academy of Sciences, Dubravska cesta 9, 845 06 Bratislava, Slovakia3
e-mail: dubska@mou.cz, tel.: +420 543 136 730

Borrelia spirochetes in bird-feeding ticks were studied in the Czech Republic. During the postbreeding period (July to September 2005) and breeding period (April to June 2007), respectively, 1080 and 835, respectively, passerine birds infested by 2240 and 1016, respectively, Ixodes ricinus subadult ticks were examined. Borrelia garinii was detected in 22.2% of the ticks, B. valaisiana was detected in 12.8% of the ticks, B. afzelii was detected in 1.6% of the ticks, and B. burgdorferi sensu stricto was detected in 0.3% of the ticks in postbreeding period. B. garinii was detected in 12.1% of the ticks, B. valaisiana was detected in 13.5% of the ticks, B. afzelii was detected in 3.7% of the ticks, B. burgdorferi sensu stricto was detected in 0.1% of the ticks, and B. lusitaniae was detected in 0.4% of the ticks in breeding period. After analysis of infections in which the blood meal volume and the stage of the ticks were considered, we concluded that Eurasian blackbirds (Turdus merula), song thrushes (Turdus philomelos), and great tits (Parus major) are capable of transmitting B. garinii; that juvenile blackbirds and song thrushes are prominent reservoirs for B. garinii spirochetes; that some other passerine birds investigated play minor roles in transmitting B. garinii; that during postbreeding period the transmission of B. garinii by competent reservoir birds is higher; and that the presence B. afzelii in ticks results from infection in a former stage.

This study was funded by the Czech Science Foundation (grant 524-08-P139) and by the Ministry of Education, Youth and Sports of the Czech Republic (grant MSM6215712402).

Fertility and viability rate of hydatid cyst in slaughtered sheep and cattle in Sari, Iran

A.Daryani , M. Sharif, H. Ziaei, Sh. Gohardehi, R. Bastani

Department of Parasitology and Mycology, School of Medicine, Mazandaran University of Medical Sciences, PC 48168-95475, Sari, Iran.
e-mail : daryanii@yahoo.com, tel.: 0098-9111587234

The aim of this study was to determine the fertility and viability of hydatid cysts in slaughtered sheep and cattle. Cysts were collected from the liver and lungs of 169 sheep and 171 cattle infected with Echinococcus granulosus when slaughtered in industrial abattoir in Sari, Iran 2007. Fertility was determined by the examination of cyst fluid for the presence of protoscolices. The viability of the protoscolices was assessed by staining with 0.1% aqueous eosin solution. The fertility rates of hepatic cyst of sheep and cattle were 47.1% and 1.4%, respectively and the fertility rates of pulmonary cyst of sheep and cattle were 39.4% and 8.1%. In the sheep, the fertility of cysts in the liver was higher than that in lungs, but in the cattle the fertility of cysts in lungs was higher than liver. The viability of protoscolices of fertile cysts for sheep and cattle were about 76.9% and 82.5%, respectively. Based on the finding in the present study, effort should be made to control transmission of cystic echinococcosis by safe disposal of Echinococcus cysts such that dogs cannot have access to the cysts.

Studies on the detection of Tubercuolsis in cattle and buffaloes in Egypt using different antigens

A. Elaziz, A. Mosaad[1]; S. I. Fathalla[2] and N. A. Badr [1]

[1] Dept of Bacteriol., Immunol. and Mycol.,Fac. of Vet. Med., Sadat City, Minufiya Univercity
[2] Dept of Physiol..,Fac. of Vet. Med., Sadat City, Minufiya Univ
e-mail: abdelazizmosaad@yahoo.com, tel.: (+20)12260-4371

Abstract The early diagnosis of bovine tuberculosis is very important due to its wide spread in the world. It is considered highly zoonotic diseases between man and animal, due to the highly economic losses in the world. In addition to the highly spreading of these diseases in the development country. This study was done on different localities at Minufiya governorate within the period from 2005-2008 years, by application of tuberculin test. The number of positive tuberculin cases 23 were slaughtered and subjected for post mortem examinations and noted a different lesions in 14 cases (60.86%) with visible lesion and 9 cases (39.13%) non visible lesion we observed that most lesion in the lung 6/23 (26.8%) followed by digestive 4/23 (17.39%). Also, the bacteriological examination revealed that 15/23 (65.21%) isolated M.bovis, while 8/23 (37.61%) isolated as Mycobacterium Other Than Tuberculosis (MOTT). An ELISA assay were applied onto collected 23 serum sample of tuberculin positive cattle using ESAT-6 CFB10 12/23 (52.17%) MPB70 11/23 (47.82%), MPB83 19/23 (82.6%). Also, used a rapid test kits (immunchromatograbhic assay containing a specific antigen in comparing with the positive tuberculin animals , the result of examined cases using culture filtrate of MAP (sonicated cell), the positive result were 15/23 (65.21%) the negative result 8/23(34.78%. The result cocktail antigen (ESAT-6, MPB 70 and MPB 83) by using immunochromatographic (rapid test kit) were 15/23 (65.21%) the negative result 8/23(34.78%). The result of human cases infected using TB antigen 38 KD gave a positive result were 19/23 (82.6%) the negative result 4/23 (17.39%). our studies it can be concluded that, using of tuberculin and ELISA serum assays depending upon a specific antigens which have the ability to discovering the infection in early stages before shedding the microorganisms into the environment specially if we used these antigens as cocktails, which showed high sensitivity and specificity to help us in control the Tuberculosis infection, differentiate between the typical Mycobacteria and Mycobacteria other than Tuberculosis and for discovering the tuberculosis infected animals in parallel with other assays.

Antimicrobial suseptibility of thermophilic C*ampylobacter* spp. isolated from environmental samples in north of IRAN

M. Ghane and M. Eghbali

Department of Microbiology, Islamic Azad University, Tonekabon Branch, Iran
e-mail: masoodghane@gmail.com

ABSTRACT: The major purpose of this study was isolation, identification and antimicrobial susceptibility of thermophilic Campylobacter spp. from different sources including domestic animals (cow, sheep, horses) , poultry, river water and sewage in north of Iran. Campylobacter spp. was isolated using pret-KB method and identified by phenotyping tests. Antimicrobial susceptibility of the isolates against different antibiotics and Minimal inhibitory concentration (MIC) values were determined by disc diffusion and double dilution methods respectively. In general, 44 strains of thermophilic Campylobacter were isolated from all of the sources. The results obtained indicated that frequency of occurrence of Campylobacter in poultry was high and in sewage was low. In addition, thermophilic Campylobacter isolates were sensitive to Ciprofloxacin, Gentamicin, Amikacin and Streptomycin and resistant to Ampicillin, Amoxicillin, Penicillin, Amoxiclave and Vancomycin. The lowest values of MIC were found for Ciprofloxacin and Gentamicin, while the highest value was found for Streptomycin. Overall, our observations, illustrated that Campylobacter exist in approximately all of the sources (domestic animals, poultry, river water and sewage) in north of Iran. Furthermore, they were mostly resistant to β lactam antibiotic (penicillin).

Key word: thermophilic Campylobacter, Antimicrobial sensitivity, Environment samples

Screening of bovine leukemia virus (BLV) infection in bulk tank milk of dairy cattle herds of Mashhad area of Iran

A. Haghparast[1,3], G. Mohammadi[2], N. Kord[1]

[1]Immunology Section, Department of Pathobiology and
[2]Department of Clinical Siences, Faculty of Veterinary Medicine,
[3]Institute of Biotechnology, Ferdowsi University of Mashhad, P.O.Box: 91775-1793, Mashhad-Iran, e-mail: alireza.haghparast@gmail.com

Bovine leukemia virus (BLV), a member of retroviridae, is an oncovirus that causes a chronic infection in cattle. Enzootic Bovine Leukosis (EBL) causes significant economic loss associated with the costs of control and eradication, loss in milk production and difficulties in exports. There are a few serolgical tests for detection of BLV infection such as AGID and ELISA. ELISA has advantages over AGID and has been approved by OIE. This study was performed by an indirect ELISA technique to determine the presence of antibody against BLV in bulk tank milk of dairy cattle herds of Mashhad and suburb. Totally, 92 bulk milk samples, which were taken from dairy herds of Mashhad area in summer 2009, were used in this study. After ELISA test on milk samples, 41.3% of the examined samples showed positive reaction. The positive results in East and Western region of Mashhad were 40.57% and 43.4%, respectively. Moreover, the prevalence of BLV in bulk tank milk of the dairy herds with less and more than 100 cattle per herds were 32.8% and 73.6%, respectively. Statistical analysis revealed a direct correlation between herd size and BLV infection, but did not indicate a direct correlation between geographical location of herds and BLV infection. However, industrial dairy herds showed significant difference with non-industrial herds in terms of BLV infection. In other words, the BLV infection was higher in industrial dairy herds as compared to non-industrial herds. The results presented in this study indicate a high prevalence of BLV infection among dairy cattle herds in Mashhad area. Therefore, control strategies based on test and implementation of improved management should be taken into consideration in order to control and eventually eliminate this economically important disease.

Keywords: Bovine leukemia virus, Bulk tank milk, Mashhad, Iran

Microbial ecology of the watery ecosystems of Evros river in North Eastern Greece

S. Vavias*, A. Alexopoulos, S. Plessas, I. Mantzourani, C. Stefanis, C. Voidarou, E. Stavropoulou and E. Bezirtzoglou.

Democritus University of Thrace. Faculty of Agricultural Development. Laboratory of Microbiology, Biotechnology and Hygiene. Pandazidou 193, Orestiada, GR68200, Greece. (Email address: vavias@gmail.com).

The aim of the present study was to evaluate the microbial ecosystem of cultivated soils along the Evros river in NE Greece. Evros river together with its derivative rivers constitute the capital source of life and sustainable development of the area. Along this riverside watery ecosystem systematic agro-cultures were developed such as wheat, corn and vegetable cultures. The evaluation of the ecosystem microbial charge was conducted in both axes which are the watery ecosystem and the riverside cultivated soil area. Considerable discrimination of water quality was observed when considering chemical and microbiological parameters of the Evros river ecosystem. Ardas river possesses a better water quality than Evros an Erythropotamos, which is mainly due to the higher quantities that these two rivers accumulate from industrial, farming and urban residues leading to higher degree of pollution. Samples were collected from environment with slightly alkaline pH, suggesting relative high ammonia content. Staphylococccus was identified in all water samples (100%) mostly in concentrations of 2,59 log per 100 ml. Enterococcus was also identified in all water samples (100%) but in lower levels. The respective concentration reached 2,55 log per 100 ml. Nitrates, nitrites and phosphate ions level increased after the junction of Erythropotamos and Ardas river to the main body of Evros river suggesting a charge with non oxidised nitrogen forms originating from fertilizers or urban type residues. The samples were collected from environments which showed a slightly alkaline pH, suggesting high ammonia charge. Escherichia coli was identified in all samples, in mean concentrations of 2,2 log cfu/100 ml. Enterococcus was also identified in all water samples reaching concentrations of 3.07 log cfu/100 ml. The organic material ranged from 0,57 to 1,69%. The levels of nitrates ranged from 7,82 mgNO_3-N/kg to 14,21 mgNO_3-N/kg and phosphorous ranged from 42,95 mg/kg to 115,75 mg/kg. In addition slightly alkaline pH was recorded in most of the areas.In conclusion, increased pollution and contamination was observed following a small area of the downstream of the river (30km) was observed. It should be highlighted, that there is a direct relation in microbial and chemical charging between water and cultivated-soil ecosystems. The protection of these ecosystems with appropriate cultivated methods, control of human and animal activities will define the homeostasis of the environmental area.

Isolation and typing of environmental mycobacteria in the tap water of hospitals, schools, kindergartens and swimming pools

G.Kelnar[1], A.Skouroglou[2], V.Krikelis[3], A.Minas[3], C.Chatzichristodoulou[4], A.Mavridou[5]

[1] Medical Laboratory Technologist, M.Sc. "Applied Public Health and Environmental Hygiene",
[2] Medical Laboratory Technologist, "Sotiria" Chest Diseases Hospital,
[3]Technological Educational Institution of Larissa, Department of Medical Laboratories,
[4]Department of Hygiene and Epidemiology, Faculty of Medicine, University of Thessaly,
[5]Technological Educational Institution of Athens, Department of Medical Laboratories
e-mail : kelnar@med.uth.gr

The role of Non-Tuberculosis Mycobacteria (NTM) in Public Health hygiene is increasing in importance. Their presence in water distribution systems recently gained attention as they can provoke health problems in immunocompromised people while they have been isolated from water distribution systems in hospitals as a result of poor hygiene. NTM are able to survive in water distribution systems because of their relative resistance to chlorine. Biofilms and protozoal interactions have been suggested as favorable to their growth in the water supply. The growth of NTM is increasingly implicated as causing lung and extrapulmonary infections. Patients with a history of chronic lung disease, with skin lesions, or immunocompromised, as well as children with poor immune system are highly susceptible to infection. In particular, AIDS has contributed significantly to increasing the frequency of NTM infections. In Greece, the use of lakes and rivers as public water supplies increases the health risks for the population as a result of NTM. Purpose: The purpose of this study was to isolate atypical mycobacteria in water distribution systems of selected buildings and identify species using molecular techniques. Materials and methods: 112 water samples were collected from various sampling points from the distribution system of hospitals, schools, kindergartens and swimming pools in the Greek cities of Athens, Corinth, Patras, Larissa, Karditsa, during a six-month period (September 2009–February 2010). One litre of sample was collected in dark sterile bottles containing 1 ml of 10% (w/v) Na2S2O3 solution. The samples were placed in a portable cooler and transported to the laboratory. They were refrigerated and analyzed within 7 hours of sampling. Each sample was decontaminated with 0.005% of CetylPyridinum Chloride (CPC). After an exposure time of 30 min the samples were filtered through membrane. The filter membrane was incubated on Middlebrook 7H10 OADC agar (Difco, Laboratories, USA) at 37oC/24days. Typical colonies were identified by PCR and Reverse Hybridization (Hain Lifescience GenoType Mycobacterium CM / AS kit, Hain Lifescience, Germany) (Torvinen Eila, 2009). Results: NTM were isolated and identified in 14 (12.5%) out of 112 examined samples 13 of the positive samples were collected from hospitals and one from a school. The isolated species were M. gordonae (6 strains) M. chelonae (4 strains), M. lentiflavum (3 strains) and M. fortuitum (1 strain). Conclusions: Continuous monitoring of NTM throughout the entire water supply network is not recommended, as the isolation and identification is a costly and time-consuming process. Periodic examination of water, especially in areas of hospitals and clinics that host

immunocompromised patients is necessary. Acknowlegements: * The study was carried out as a dissertation for the “Applied Public Health and Environmental Hygiene” M.Sc. course in the Faculty of Medicine, University of Thessaly, and conducted in the Research Laboratory of the Department of Medical Laboratories, Technological Education Institution, Athens.

Importance of echinococcus genetics in ecosystem

N. Altintas[1], N. Altintas[2]

[1]Celal Bayar University, School of Medicine, Medical Biology and Genetics, Manisa-TURKEY
[2]Ege Üniversity, School of Medicine, Deparment of Parasitology, Izmir-TURKEY
e-mail: naltintas35@gmail.com

Echinococcosis is a chronic disease of humans which has a serious prognosis and is caused by metacestodes of the genus *Echinococcus* (Taeniidae) which include *Echinococcus granulosus*, *E.multilocularis*, *E.vogeli* or *E.oligarthrus* species. *E.granulosus* has a world wide distribution and occurs in all continents. *E.multilocularis* is distributed in the northern hemisphere. *E.vogeli* and *E.oligarthrus* are endemic in Central and South American countries Larval infection (hydatid disease; hydatidosis) is characterized by long-term growth of metacestode (hydatid) cysts in the intermediate host. *Echinococcus granulosus* and *E. multilocularis* - the two major species of medical and public health importance-cause cystic echinococcosis (CE) and alveolar echinococcosis (AE), respectively. Recently, the application of molecular biological tools has provided a wealth of knowledge regarding gene structure, organization and expression in *Echinococcus* parasitic organisms. Molecular techniques provide of value in the study of *Echinococcus*, in particular for investigating genetic variation, phylogenetic relationships and molecular epidemiology, for the detection of parasite nucleic acids in clinical samples and in the identification of eggs. Molecular epidemiology for the study of echinococcosis disease includes to evaluate host/environmental interactions in disease and the development of strategies for the control of echinococcosis. It has recently been defined as a tool for contribution of potential genetic and environmental risk factors, identification at the molecular level on distribution and prevention of disease. It is now generally recognized that *Echinococcus granulosus* exhibits substantial genetic diversity. At least ten genotypically defined strains (G1–G10) were described within the *E. granulosus* complex, some of which exhibit marked biological and morphological differences. Even though, there has been some progress in the control of echinococcosis, this disease continues to be a major public health problem in most of the countries and it constitutes an emerging and re-emerging disease.

Last minute submissions (Invited/oral/poster)

Application of novel starter cultures for sourdough bread production.

S. Plessas[1], A. Alexopoulos[1], I. Mantzourani[1], C. Voidarou[1], E. Stavropoulou[1], A. Koutinas[2] and E.Bezirtzoglou[1]

[1]Democritus University of Thrace, Faculty of Agriculture Development, Laboratory of Microbiology, Biotechnology and Hygiene, 193 Pantazidou str. GR-68200, Orestiada, Greece, e-mail: splessas@agro.duth.gr

[2]Food Biotechnology Group, Department of Chemistry, University of Patras, Greece

Sourdough application has been extensively increased the last years due to the consumers demand for food consumption without the addition chemical preservatives. Several starter cultures have been applied in sourdough bread making targeting to the increased of bread self life and the improvement of sensorial character. More specific, Lactobacillus acidophilus and Lactobacillus sakei as single and mixed cultures were used for sourdough bread making. Various sourdough breads were produced with the addition of sourdough pervious prepared with 10% w/w Lactobacillus acidophilus, 10% w/w Lactobacillus sakei and 5% w/w Lactobacillus acidophilus and 5% w/w Lactobacillus sakei at the same time. Various chemical parameters were determines such as lactic acid, total titratable acidity and pH. The results revealed that the produced sourdough bread made with sourdough containing the mixed culture was preserved for more days (12 days) than all the other breads produced in the frame of this study, since it contained lactic acid in higher concentrations. The respective total titritable acidity varied between (10.5 and 11ml NaOH N/10. The same sourdough bread had a firmer texture, better aroma, flavor and overall quality compared to other sourdough breads examined in this study, as shown by sensory evaluation tests and results obtained through SPME GC-MS analysis, which revealed significant differences among the different bread types.

Keywords: Lactobacillus acidophilus, Lactobacillus sakei, sourdough, bread

Occurrence of *Clostridium perfringens* from Different Cultivated Soils.

C.Voidarou[1], E.Bezirtzoglou[2], A.Alexopoulos[2], S. Plessas[2], C.Stefanis[2], I. Papadopoulos[2], S. Vavias[2] E. Stavropoulou[2], K.Fotou[1], A. Tzora[1] and I. Skoufos[1]

[1]Technological Educational Institute of Epirus, Arta, GR47100, Greece
[2]Democritus University of Thrace, Faculty of Agricultural Development, Orestiada, GR68200, Greece

The occurrence of Clostridium perfringens was estimated in 750 samples originating from a variety of soils bearing various bulb crops: Brawnica oderacea (vegetable), Olea europaea, Daucus carota (carote), Solanum tuberosum (potato), Phaseolus vulgaris (green haricot), Beta vulgaris var. rapaceum (beetroot), Cucurbita pepo (squash), Allium cepa (onion), Cucumis sativus (cucumber) and Capsicum annum (pepper). All isolated strains were tested for their antimicrobial activities to amoxicillin, penicillin G, kanamycin, tetracycline, streptomycin, erythromycin, chloramphenicol and metronidazole.

When considering the type of the bulb production, increased C.perfringens spore densities in most subterranean bulb soils were observed. Moreover, C.perfringens spores are likely to occur in particularly large numbers in soils contaminated by fecal matter. Additionally, there is a close relationship between the spore amount and the nature of soil's organic content. The presence of C.perfringens was associated with acidic soil. Most of our strains showed resistance to the studied antibiotics applied usually for human and veterinary care. A systematic monitoring of the cultivated soil ecosystems must include bacteriological parameters together with chemical indicators of organic pollution in order to obtain information adequate for assessing their overall quality.

Keywords: Soil; microbial ecology; environment; agriculture; C. perfringens

Hygienic quality and antibiotic resistance profile of sliced butchery.

C.Voidarou[1], A.Alexopoulos[2], S. Plessas[2], E. Stavropoulou[2], K.Fotou[1], A. Tzora[1], I. Skoufos[1] and E.Bezirtzoglou[2]

[1]Technological Educational Institute of Epirus, Arta, GR47100, Greece
[2]Democritus University of Thrace, Faculty of Agricultural Development, Orestiada, GR68200, Greece

E-mail:empezirt@agro.duth.gr

In order to investigate the microbiological quality of different ready –to-eat meat products, 200 samples were collected from the following preparations: boiled turkey (n=50), boiled pork ham (n=50), smoked turkey (n=50) and smoked pork ham (n=50). All cold ham products were collected from commercial areas and were homogenized in PBS by the aid of a Stomacher. An aliquot of Ringer's solution was heated for 10 min at 800 C and from each dilution a second plate of non selective medium was seeded for detection of the germinated spore forms. Our media were incubated aerobically and anaerobicaly for 48 h at 370 C. To confirm the presence of C. perfringens, L.S. (Lactose-Sulfite) medium was used. Identification of the bacteria was carried out according to Bergey's manual. Microscopic examination of Gram-stained cells, catalase, oxidase and biochemical tests were performed when necessary. In boiled turkey and pork ham preparations C. perfringens vegetative (16% and 20%) and spore (48% and 44%) forms, S. aureus (40% and 32%) , E. coli (20% and 16%), other Clostridium sp lec(-) (36% and 24%), Lactobacillus (24% and 48%), Bacillus sp (20% and 32%) and Salmonella (24% and 20%) sp were present respectively. B. cereus (4%) was rarely present in boiled butchery. In smoked turkey and pork ham butchery C. perfringens vegetative (16% and 20%) and spore (72% and 88%) forms, S. aureus (28% and 20%), E. coli (12% and 8%), other Clostridium sp lec(-)(36% and 20%), Lactobacillus (36% and 56%), Bacillus sp (20% and 28%) and Salmonella (16% and 12%) sp were found respectively, together with low levels of B. cereus (8%). The persistent occurrence of S. aureus in these products is associated with the salted conditions of these preparations, especially in boiled butchery (32-40%). C. perfringens spore forms seems to be present in higher numbers in the pork ham preparations. Moreover, smoking processing of ham and turkey preparations compared to boiling processing is associated to a higher microbial charge by C. perfringens spore forms.

Systematically monitoring of the microbiological quality of cold butchery preparations must be done, in order to preserve food quality and optimizing the processing and elaboration methods of the product.

Keywords: Ham, cold butchery, microbe, antibiotic resistance, E. coli, S. aureus , C.perfringens, Salmonella, B. cereus

Microbiological quality of grey-mullet roe.

C.Voidarou[1], A.Alexopoulos[2], S. Plessas[2], H. Noussias[2], E. Stavropoulou[2], K.Fotou[1], A. Tzora[1], I. Skoufos[1] and E.Bezirtzoglou[2]

[1]Technological Educational Institute of Epirus, Arta, GR47100, Greece
[2]Democritus University of Thrace, Faculty of Agricultural Development, Orestiada, GR68200, Greece

E-mail:empezirt@agro.duth.gr

The Greek grey mullet roe is produced from the fully developed gonads of the female mullet (Mugil cephalus) couth in lagoons during their reproductive migration. The traditional processing method of the roe includes, air drying, salting, shape formation and covering with multiple layers of natural beeswax for preservation and distribution. Fish Roe brands have been a staple in local diet and is increasingly becoming popular in the international market. As a ready-to-eat food it's microbial quality should be of concern for the protection of consumers health. 48 samples of fish roe, just before waxing, were collected from various local processors during August and September. For microbiological examination, the samples were homogenized in peptone water by the aid of a Stomacher and a series of dilutions were prepared. Aliquots of 100µl were plated on the surface of selective media and incubated under aerobic and anaerobic conditions. The identification of the bacteria was carried out according to the Bergey's manual. Microscopic examination of Gram stained cells, catalase, oxidase and biochemical tests were performed when necessary to further identify. *V. parahaemolyticus, Vibrio spp., Salmonella spp.*, and *Aeromonas hydrophila* were detected in one sample (2%). Shigella spp., and Flavobacterium spp. in two samples (4%), C. perfringens (vegetative forms), E. coli, and spores of Bacillus spp., were detected in three samples (6%), Staphylococcus aureus in four samples (8%). Various Micrococcus spp., and spores of C. perfringens in 16% and 35% of the samples respectively. From the Listeria genus, only the species L. innocua, L. welshimeri, L. seeligeri L. ivanovii and L. grayi were recovered from 2-10% of the samples.

Microbiological analyses revealed the presence of a small number of pathogens in grey mullet roe samples which are in accordance with the findings of similar studies. Traditional processing of the fish roe, seems inadequate to ensure the food safety and even waxing isn't expected to fully protect them against facultative anaerobes with salt tolerance. Therefore, additional measures should be taken during processing and marketing of fish roe to minimize potential health risks for the consumers.

Keywords: Microbiology,roe grey-mullet,fish

Microbial ecology of fish species ongrowing in Greek sea farms and their watery environment.

A.Alexopoulos[1], S. Plessas[1], C.Voidarou[2], H. Noussias[1], E. Stavropoulou[1], A. Tzora[2], I. Skoufos[2] and E.Bezirtzoglou[1]

[1]Democritus University of Thrace, Faculty of Agricultural Development, Orestiada, GR68200, Greece

[2]Technological Educational Institute of Epirus, Arta, GR47100, Greece

Aquaculture is a dynamic branch of Greek economy having a great expansion the last years. The present study focuses on the bacteriological profile of both watery ecosystem and fishes from different North and Central West Greek fish-farms ongrowing euryhaline fish species. The natural microflora of the fish and the water of their ongrowing units in selected farms were studied for a period of 20 months. The analysed samples were mainly sea bream (Sparus aurata) 61.3% and sea bass (Dicentrarchus labrax) 24%. In most of the watery ecosystems coming from the different sampling areas, total and fecal coliforms as well as total and fecal streptococci were abundant in all water samples. Enterococcus, E.coli and Pseudomonas were present at a level of 3 logs cfu/100 ml. The anaerobic C. perfringens was found in vegetative (21.3%) and spore forms (13.3%), when was diluted 1/10. It is of interest to note that P. piscicida and V.anguillarum were isolated only from fishes ongrowing in waters with pH values below 8.2. Staphylococcus aureus was detected in 4% of the samples, other Staphylococcus sp. in 29.3%, E. coli in 30.7%, Salmonella sp. in 1.3%, Pseudomonas sp. in 13.3%, Clostridia lec(-) in 49.3%, Bacillus sp. in 38.7%, Vibrio sp. in 18.7%, Lactobacillus and Lactococcus sp in 36% και 29.3% respectively.Vegetative forms of C. perfringens were detected in 22.7%. Microorganisms in aquatic environment are constantly exposed to microorganisms coming from the watery ecosystem which is relatively rich in microorganisms. It is then conceivable that the relationship between normal watery environmental and fish microflora is capital to the monitoring of changes in fish farms. Increasingly, more focus on this bipolar interacting system should be necessary in order to avoid any possible disturbance in the balance of the healthy farming ecosystem with the host organisms.

Keywords: euryhaline fish, aquatic environment, Vibrio, P. piscicida

Antibacterial activity of different honeys against pathogenic bacteria.

C.Voidarou[1], A.Alexopoulos[2], S. Plessas[2], A. Karapanou[3], E. Stavropoulou[2], K.Fotou[1], A. Tzora[1], I. Skoufos[1] and E.Bezirtzoglou[2]

[1]Technological Educational Institute of Epirus, Arta, GR47100, Greece
[2]Democritus University of Thrace, Faculty of Agricultural Development, Orestiada, GR68200, Greece
[3]General Dental Clinic, GR47100, Arta, Greece

E-mail:empezirt@agro.duth.gr

To study the antimicrobial activity of honey, 60 samples of various botanical origins were evaluated for their antimicrobial activities against 16 clinical pathogens and their respective reference strains. The microbiological quality of honeys and the antibiotic susceptibly of the various isolates were also examined. The bioassay applied for determining the antimicrobial effect employs the well-agar diffusion method and the estimation of minimum active dilution which producing a 1 mm diameter inhibition zone. All honey samples, despite their origin (coniferous, citrus, thyme or polyfloral), showed antibacterial activity against the pathogenic and their respective reference strains at variable levels. Coniferous and thyme honeys showed the highest activity with an average minimum dilution of 17.4 and 19. 2% (w/v) followed by citrus and polyfloral honeys with 20.8 and 23,8% respectively. Clinical isolates of Staphylococcus aureus subsp. aureus, Escherichia coli, Salmonella enterica subsp. enterica and Bacillus cereus were proven to be up to 60% more resistant than their equal reference strains thus emphasizing the variability in the antibacterial effect of honey and the need for further research.

Keywords: Honey, antibacterial activity, antibiotics, sensitivity

Biodiversity and antimicrobial susceptibility of mastitis microflora pre and post treatment by antimicrobials agents, in sheep.

K. Fotou[1], K.Demertzi-Akrida[2], G.Rozos[3], A. Alexopoulos[3], S.Plessas[3], M.Skoufou[4], P.G. Demertzis[2], C. Voidarou[1], I. Skoufos[1], E. Bezirtzoglou[3] and A. Tzora[1]

[1]TEI of Epirus, Laboratory of Animal Health and Infectious Diseases, Department of Animal Production, Arta (Greece)

[2]Laboratory of Food Chemistry, Department of Chemistry, University of Ioannina, GR-45110 Ioannina (Greece)

[3]Democritus University of Thrace, Faculty of Agricultural Development, Dept of Food Science and Technology, Lab of Microbiology, Biotechnology and Hygiene (Greece)

[4]Aristotle University Of Thessaloniki, Medical School (Greece)

Despite technical advances in milk processing, the quality of milk is still determined at the dairy farm. Mastitis, milk quality and dairy products safety are all very much interrelated. Mastitis, an inflammation of the mammary gland caused by bacterial infection, trauma, or injury to the udder, remains the most common and most expensive disease affecting dairy livestock and hence dairy products' quality, throughout the world. Several different bacteria genii can invade the udder, multiply there and produce harmful substances that result in inflammation. Various therapeutic protocols exist for the treatment of mastitis and all of them involve the usage of antibiotics. The aim of this research is to study the biodiversity and antimicrobial susceptibility of the involved microorganisms in mastitis before and after the treatment. 300 samples were collected from ewes in farms situated in the mountainous rural area of Epirus, Greece: Group A: 100 samples of milk collected from 25 farms, from 4 clinically healthy ewes from each farm and were used as martyrs. Group B: 100 samples of milk collected from 25 farms with high incidence of mastitis from 4 ewes with clinical signs of mastitis, from each farm. These animals were marked with a numbered earring and after sampling were treated with various combinations of antibiotics as prescribed by the clinician veterinarian. (Streptomycin/Penicillin , Oxytetracycline, Quinolones). Group C: 100 samples of milk collected from the same animals as in Group B, after the treatment was completed. From the animals treated with Penicillin/Streptomycin and with Oxytetracycline, the samples were collected 8 days after the last administration if the drugs. From the animals treated with Quinolones/Vetimast the samples were collected 13 days after the last administration. These time intervals from the last administration to the sampling are exact to the ones imposed by the EE regulations. All samples were analyzed for Total Plate Count (TPC), Staphylococcus aureus, Escherichia coli as well as the prevalence of selected pathogens such as Listeria monocytogenes, Salmonella spp., Bacillus cereus and Clostridium perfringens. The susceptibility of all isolates originating from milk (E.coli, Salmonella, S.aureus, B.cereus, C.perfringens) to selected antimicrobial drugs (amikacin, ampicillin,

cephalothin, cefotaxime, gentamicin, neomycin, streptomycin, tetracycline, norfloxacin, chloramphenicol, metronidazole and erythromycin) was analyzed. The above mentioned bacteria presented various degrees of resistance to the antimicrobial agents used, pre and post treatment by antimicrobials agents. This finding leads to a serious concern for the quality and the safety of the milk and the dairy products. Furthermore a concern rises regarding the transmission of the resistance genes to the consumer. In such a case the implications in human therapeutics could be disastrous, given that antibiotics such as quinolones, oxytetracycline and penicillin/streptomycin are widely used in the clinical practice as frontline medicines for the treatment of various infections. Strains of indicator bacteria such Escherichia coli (E. coli) contaminating raw milk can, under specific conditions, become vectors of genes encoding resistance to antimicrobial drugs.

Probiotics, prebiotics and infection in infants and children.

C. Edwards

University of Glasgow, Division of Developmental Medicine, Human Nutrition Department, Edinburg, UK

The intestinal microbiota of the newborn is heavily influenced by mode of birth, perinatal antibiotics, breast feeding and geography. The development in the first year can be influenced by dietary carbohydrates and there are several studies showing the impact of prebiotics on the development of the infant microbiota and bacterial metabolism. Probiotics have also been shown to alter the bacterial populations and reduce some allergy symptoms. In addition in preterm neonates there have been studies looking at use of probiotics to reduce the incidence of conditions such as necrotising enterocolitis (NEC). However, much more research is needed to establish the normal colonisation process in preterm infants, who have interrupted feeding and also often have antibiotics. The impact of particular strains of probiotics also needs to be evaluated in sufficient numbers of infants to provide convincing evidence for their protective effects against NEC and other conditions.

Microbiology of some fresh fishes and seafoods in the Mediterranean basin.

Voidarou C[1], Tzora A[1], Skoufos I[1], Fotou K[1], Noussias H[2], Alexopoulos A[2] , Plessas S[2], Stavropoulou E[2] and Bezirtzoglou E[2]

[1]T.E.I. of Epirus, Laboratory of Animal Health and Infections Diseases, Department of Animal Production, Arta, Greece.

[2]Democritus University of Thrace, Faculty of Agriculture Development, Laboratory of Food Microbiology ,Biotechnology and Hygiene, Orestiada, Greece.

In order to investigate the hygienic quality of fresh fish and other fresh marine products, 100 samples of mussels, salmon fish, shrimps, tuna fish collected directly from aquaculture pools and 20 samples and eels collected from hermetically closed aquaculture pools, ready to be exported, were investigated for their microbiological quality. Variations in marine environment influence the bacterial microflora. As our fresh fishes are coming from the open sea, they seem not be exposed to a high fecal human or animal contamination showed by the classic fecal bacterial indicator which is E. coli. The prevalence of C. perfringens (spores and vegetative forms) could be due to the aerobic conditions in deep waters. Additionally, fresh shrimps seems to be less contaminated by all studied bacterial species, but this must be explained by the fact that our shrimps are coming from controlled cultured areas and not from naturally muddy sea areas.

Keywords: Fish, Seafood, Quality, Microbiology, Clostridium.

Index of Authors

The XXXIII International SOMED Congress was sponsored and supported by:

Hellenic Ministry for Health and Social Solidarity

Hellenic Ministry of Culture and Tourism

Municipality of Mykonos

Municipality of Patmos

Municipality of Thira

Prefecture of Heraklion

Municipality of Heraklion

The congress has been officially approved by the National Organization for Medicines

Registration Number: 39908