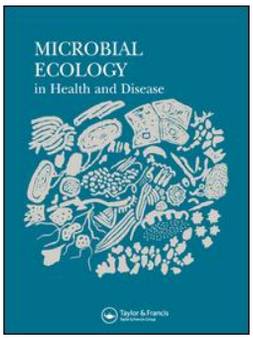

Taylor & Francis
Taylor & Francis Group

Microbial Ecology in Health and Disease

ISSN: (Print) 1651-2235 (Online) Journal homepage: informahealthcare.com/journals/zmeh20

Abstracts of the XVIII Meeting of the Society for Microbial Ecology and Disease (SOMED), Boston, Massachusetts, USA, 10–13 September 1993

To cite this article: (1994) Abstracts of the XVIII Meeting of the Society for Microbial Ecology and Disease (SOMED), Boston, Massachusetts, USA, 10–13 September 1993, Microbial Ecology in Health and Disease, 7:1, 35-55, DOI: 10.3109/08910609409141572

To link to this article: https://doi.org/10.3109/08910609409141572

© 1994 The Author(s). Published by Taylor & Francis.

Published online: 11 Jul 2009.

Submit your article to this journal

Article views: 111

View related articles

Full Terms & Conditions of access and use can be found at
https://informahealthcare.com/action/journalInformation?journalCode=zmeh20

MICROBIAL ECOLOGY IN HEALTH AND DISEASE VOL. 7: 35–55 (1994)

Abstracts of the XVIII Meeting of the Society for Microbial Ecology and Disease (SOMED), Boston, Massachusetts, USA, 10–13 September 1993

MATHEMATICAL MODELS OF VAGINAL MICROBIOTA DATA

M.-L. T. Lee, A. B. Onderdonk, R. A. Ross. Brigham & Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA

The mixed-effects model is considered for analysing results from a large data base assembled from several *in vivo* studies. This model takes into account the dependence of repeated measurements on the same set of subjects and the variability among subjects. A predictive model is constructed. Also, the method of generalised estimating equation is considered to identify abnormal bacterial counts. Unlike regular logistic regression, this model takes into account correlated repeated measurements in analysing relative risks.

USE OF CONTINUOUS CULTURE GROWTH SYSTEMS FOR MODELLING VAGINAL MICROBIOTA BEHAVIOURS

R. A. Ross, M. L. Delaney†, A. B. Onderdonk†. Channing Laboratory, Department of *Medicine and †Pathology, Brigham & Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA*

The microbiota of the vaginal environment is important for the maintenance of health and prevention of infection and disease in the host. The modelling of a host–microbe ecosystem such as the human vaginal environment may be an important method for the identification of changes that can result in disease and factors that lead to disease prevention. An *in vitro* growth system modelling microbial behaviours of the human vaginal environment has been developed and includes using bacterial isolates obtained from vaginal samples of healthy women, a culture medium simulating vaginal secretions, a continuous culture (CC) growth system, and an *in vivo* data base describing the unaltered vaginal ecosystem. To compare *in vivo* and *in vitro* results at various pH values, *in vivo* organism concentrations were expressed as ranges ($\pm$SD of mean). Various pure and mixed CC experiments produced *Lactobacillus*, *Staphylococcus*, *Prevotella*, and *Streptococcus* group D spp. concentrations within *in vivo* ranges. Only *Corynebacterium* spp. concentrations were outside the range observed *in vivo* (0.6 $\log_{10}$ units below). To clarify this result, additional vaginal *Corynebacterium* isolates and *Corynebacterium* spp. in more complex mixed CC are being examined. The levels observed in the CC *in vitro* growth system closely reflect those noted *in vivo*. The use of the CC growth system is a credible strategy for the *in vitro* modelling of the vaginal host–microbe ecosystem.

BIOTHERAPEUTIC AGENTS: BOON OR BANE?

L. V. McFarland. Department of Medicinal Chemistry, University of Washington, Seattle, Washington, USA

Biotherapeutic agents have been used as alternative treatments and preventive therapies for a variety of clinical diseases. The requirements for a successful agent, possible mechanisms and types of diseases targeted will be reviewed. *Lactobacillus* spp., *Pediococcus* spp., *Streptococcus* spp. and *Bacillus cereus* have been evaluated in animal models for a diversity of diseases including antibiotic-associated diarrhoea (AAD), lactase deficiency, antibiotic-induced mortality, colon cancer and antimutagenic properties. Clinical trials of several *Lactobacillus* species have had varying effectiveness for the treatment of lactase deficiency, relapsing *Clostridium difficile* disease, traveller's diarrhoea, acute diarrhoea, AAD, cystitis and bacterial vaginosis. *Bifidobacterium longum* has been found to be effective in preventing erythromycin-induced diarrhoea. *Streptococcus faecium* has been found effective for the prevention of AAD but was not effective for cholera or enterotoxigenic *Escherichia coli* diarrhoea. *Saccharomyces boulardii* has been found effective in the treatment of *C. difficile* disease, acute diarrhoea and chronic diarrhoea in HIV-infected patients. *S. boulardii* was also effective in preventing AAD and diarrhoea associated with nasogastric

0891–060X/94/010035–21 $15.50
© 1994 by John Wiley & Sons, Ltd.

tube feeding. Biotherapeutic agents are not only effective, but research on their mechanisms of action may also provide valuable insight into the complex ecosystem of the human body.

USE OF PROBIOTICS TO PREVENT RECURRENT BLADDER INFECTIONS IN WOMEN

A. W. Bruce, G. Reid. Division of Urology, University of Toronto and Department of Microbiology and Immunology, University of Western Ontario, London, Canada

The concept of using strains of lactobacilli to prevent recurrent urinary tract infections (UTI) was established by us following clinical observations that these organisms dominated the urogenital microbial population of healthy women. After selecting properties believed to confer a competitive advantage for lactobacilli over uropathogens, and acquiring supporting data from *in vitro* and animal studies, several clinical trials were carried out. In an open study of eight women, the incidence of recurrent UTI was reduced 71 per cent over 1 year. There was also some evidence of effectiveness, from a post-antibiotic trial of 40 patients. A double-blinded, randomised trial of 55 women, mean age 34 yr with a history of four or more uncomplicated UTIs the previous year, resulted in a 73 per cent reduction in infection rate using lactobacilli and 81 per cent using a lactobacillus growth factor which stimulated the patient's own lactobacilli. Analysis of vaginal cells showed twice as many adherent lactobacilli in patients with 0–1 UTIs per year, compared to those experiencing > 2. No unusual or multidrug-resistant pathogens emerged, and no side-effects were reported. We believe there is now strong evidence to support the use of lactobacillus biotherapeutics in the female urogenital tract.

THE EFFECT OF LACTOBACILLUS GG ON HUMAN IMMUNOCOMPETENCE

B. R. Goldin, L. Gorbach*, S. Meydani†. *Tufts University School of Medicine and †Human Nutrition Research Center on Aging at Tufts University, Boston, Massachusetts, USA*

The ability of a strain of lactobacillus designated GG to modify the human immune system has been investigated. Eleven men and women were studied, between the ages of 21 and 35 yr and between the ages of 50 and 70 yr. For baseline values, blood was drawn prior to feeding the lactobacillus GG. The subjects were then fed twice daily for 30 d a 250 mg capsule of a lyophilised powder containing 2×10^{11} lactobacillus GG per gram of powder. After the 30 d period each subject again had blood drawn. As a result of the lactobacillus feeding there was a decline in the CD_8 cells and a concomitant increase in the CD_4/CD_8 ratio. The changes were greater for the older subjects. Lactobacillus GG also caused an increased peripheral blood mononuclear cell production of interleukin-2 in response to exposure to phytohaemagglutinin or concanavalin. The effect was again greatest for the older population. Mitogen-induced lymphocyte proliferation was also increased in response to lactobacillus feeding and this increase was dependent on age and type of mitogen. Therefore lactobacillus GG increased a number of quantitative parameters associated with an enhanced immunocompetent state. The bacterial supplementation appears to bolster the immune system in the elderly to the levels observed in younger subjects.

BINDING OF FOOD MUTAGENS BY INTESTINAL AND LACTIC ACID BACTERIA

K. Orrhage, J. Rafter†, C. E. Nord*. Departments of *Microbiology and †Medical Nutrition, Karolinska Institute, Stockholm, Sweden*

The purpose of the study was to investigate the binding capacity of intestinal and lactic acid bacteria for mutagenic heterocyclic amines created during cooking of protein-rich food such as meat. Five strains, two lactobacilli, two lactococci and one strain of *Bifidobacterium* sp., used in fermented milk products and one strain of *Escherichia coli*, *Clostridium perfringens* and *Bacteroides fragilis* were lyophilised and incubated separately with the heterocyclic amines 3-amino-1-methyl5H-pyrido[2,3-b]indole (Trp-P-2), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) or 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx). The mixture was centrifuged and ultrafiltered and the amount of unbound mutagen in the supernatant was quantitated by HPLC. The mutagenic activity of Trp-P-2 in the presence of bacteria was also determined by the Ames' salmonella mutagenicity test. All bacterial strains effectively bound Trp-P-2 (more than 90 per cent). The binding was most effective in an incubation medium without metal ions and occurred very rapidly. A dramatic decrease in mutagenic activity correlated with the

amount of bound Trp-P-2. Dose-dependent binding of IQ and MeIQx was also observed with all strains tested, but the binding was much less efficient than with Trp-P-2. These results indicate that lactic acid bacteria in fermented milk products and normal intestinal bacteria can bind mutagenic heterocyclic amines formed in cooked food and may decrease their genotoxic potential.

EFFECTS OF PROLONGED INGESTION OF FRUCTO-OLIGOSACCHARIDES (ACTILIGHT®) ON FAECAL INDIGENOUS BIFIDOBACTERIA AND BACTERIAL ENZYMES IN HUMANS

Y. Bouhnik, B. Flourié*, F. Ouarne†, N. Bisetti†, P. Marteau*, P. Pochart*, F. Bornet†, J. C. Rambaud*. *INSERM U 290, Hôpital Saint-Lazare, Paris; †Eridania Béghin-Say, Service Nutrition & Santé, Paris, France*

Actilight's fructo-oligosaccharides (FOS) are enzymatically obtained from sucrose (GF_n, $n \leqslant 4$). They are non-digestible in the human small intestine and reach the colon where they may promote the growth of the indigenous microflora. The *aim* of this study was to assess the effects of prolonged ingestion of FOS on faecal indigenous bifidobacteria and bacterial enzymes in humans. Fourteen healthy volunteers randomly divided into two groups were studied for three consecutive 12 d periods. In period 1, they consumed their usual diet; in period 2, they received in addition either 12·5 g/day FOS or placebo in three oral doses; in period 3, they only received their usual diet. Stools were regularly recovered throughout the study. They were analysed for total anaerobes and *Bifidobacterium* spp. enumeration and the following bacterial enzymes: β-galactosidase, β-glucosidase, nitroreductase, azoreductase and β-glucuronidase. In the placebo group, there were no changes in faecal bacterial levels and enzymes during the three periods. In the FOS group, some significant effects were evident:

	Period 1	*Period 2*	*Period 3*
Bifidobacterium spp.*	$8{\cdot}2 \pm 0{\cdot}6^{a}$	$9{\cdot}3 \pm 0{\cdot}3^{b}$	$8{\cdot}3 \pm 0{\cdot}4$
β-galactosidase†	38 ± 6^{c}	48 ± 6^{d}	41 ± 4
β-glucosidase†	$7{\cdot}3 \pm 1{\cdot}2^{a}$	$9{\cdot}8 \pm 1{\cdot}4^{b}$	$6{\cdot}4 \pm 0{\cdot}8$
	$a \neq b$: $P < 0{\cdot}01$	$c \neq d$: $P < 0{\cdot}05$	

*bacterial units = mean ± SD log colony-forming unit per gram of fresh stool.
†enzymes units = mean ± SD mUI per gram of dry weight.

All these parameters returned to their initial level during the last period. In contrast, FOS supplements had no effect on faecal total anaerobes, nitroreductase, azoreductase or β-glucuronidase activities. Ingestion of FOS significantly increases the level of indigenous bifidobacteria, β-galactosidase and β-glucosidase activities in stools. These effects are transient, occurring only at times when FOS is consumed.

IN VITRO INHIBITION OF GROWTH AND ADHESION OF *CANDIDA ALBICANS* BY VAGINAL ISOLATES OF LACTOBACILLI

J. A. McGroarty. Glasgow Caledonian University, Glasgow, Scotland, UK

Vulvovaginal candidiasis is a common infection of women of child-bearing age, caused mainly by *C. albicans*. Adhesion of the yeast is a prerequisite for establishing infection and can be inhibited *in vitro* by the addition of L-fucose. A range of lactobacilli isolated from healthy females was tested for inhibitory activity against *C. albicans*. 13/80 isolates tested were capable of inhibiting the growth of a range of *C. albicans* strains. The inhibition was consistent with organic acids and hydrogen peroxide production by the lactobacilli. Rough-smooth colony phase variation was noted among a number of the lactobacilli examined, which was influenced by culture conditions and correlated with the production of an adhesin involved in attachment of the lactobacilli to vaginal epithelial cells. Preliminary characterisation of the adhesin revealed it to be proteinaceous in nature and that adhesion to the corresponding receptor on the epithelial cells could be inhibited by the addition of L-fucose to the adhesion assay. A comparison was made of the ability of phase variants of lactobacilli to inhibit the adhesion of *C. albicans in vitro*. Exfoliated vaginal epithelial cells were pretreated with either buffer or *Lactobacillus acidophilus*. The vaginal cells were washed to remove unattached lactobacilli before the addition of *C. albicans*. The number of yeasts adherent to control and lactobacillus-treated cells were enumerated. The lactobacilli differed significantly in their ability to inhibit candida adhesion, with the smooth variants inhibiting adhesion by between 50 and 85 per cent and the rough variants producing between 0 and 25 per cent inhibition. We suggest that certain lactobacilli produce a proteinaceous adhesin which could be used to prevent vulvovaginal candidiasis by competing with candida for specific receptors on the vaginal epithelium.

A HUMAN LACTOBACILLUS STRAIN ENHANCES GUT IMMUNE RESPONSE IN CROHN'S DISEASE

M. Malin, H. Suomalainen, E. Isolauri. Department of Clinical Medicine, University of Tampere, Finland

Gut mucosa provides selective exclusion and immune elimination of potentially harmful substances. Lactobacilli have been shown to inhibit pathogenic microorganisms and to afford protection against increased intestinal permeability. They therefore appear promising candidates for the treatment of clinical conditions with altered gut mucosal barrier functions. The aim was to investigate the effect of human *Lactobacillus casei* strain GG on gut immune response in patients with Crohn's disease ($n=15$) and juvenile chronic arthritis ($n=9$), and controls ($n=8$). The patients (2–17 yr) were given *Lactobacillus* GG (10^{10} c.f.u.) twice daily for 10 d. Gut immune response was indirectly assessed by the ELISPOT method of specific antibody secreting cells (sASC) to dietary β-lactoglobulin, casein and gliadin, before and a week after commencing the treatment. The patients showed significantly higher numbers of sASC in the IgG class compared to controls. Oral bacteriotherapy was associated with a rise in IgA sASC to cow's milk antigens in patients with Crohn's disease, but not in those with juvenile chronic arthritis and controls. The mean (95 per cent CI) numbers of IgA sASC to β-lactoglobulin and casein increased from 0.4 (0.1, 2.4) to 3.2 (0.7, 13.9)/10^6 cells; $P=0{\cdot}01$. The results indicate that in Crohn's disease and juvenile chronic arthritis the local immune system is activated against dietary antigens with disproportional increase in IgG-producing cells. They further suggest that *Lactobacillus* GG may aid in host defence by intensifying the IgA response in patients who have Crohn's disease.

NEWLY DESCRIBED CLOSTRIDIAL DISEASES OF THE GUT

S. P. Borriello. Microbial Pathogenicity Research Group, Department of Microbiology, Queens Medical Centre, Nottingham, UK

As in most bacterial diseases, the diseases themselves have been known for some time, but the bacterial pathogen has only recently been identified. Within clostridia three pathogens have been described over the last decade. In two cases it was the first time that the particular species had been associated with disease, i.e. *Clostridium difficile* causing pseudomembranous colitis and some cases of antibiotic-associated diarrhoea, and *C. spiroforme* causing enterotoxaemia in rabbits. In the third example, *C. perfringens*, it was already known that the organism could cause diarrhoea (food poisoning), but it has now been firmly implicated in nosocomial diarrhoea unrelated to food poisoning. For all three pathogens the critical observation that led to identification of their role in disease was the observation of a cytopathic effect in tissue culture exhibited by faecal filtrates. In two cases this led to misidentification in the first instance of the potential pathogen due to serological cross-reactivity of the toxins. The cytopathic effect of the *C. difficile* toxins being neutralised by antisera to *C. sordellii*, and the *C. spiroforme* cytotoxic effect being neutralised by *C. perfringens* iota antitoxin.

CLOSTRIDIUM DIFFICILE AND ITS SURFACE PROTEINS

P. Mastrantonio, A. Pantosti, M. Cerquetti, A. Molinari, A. Gelosia, G. Donelli. Istituto Superiore di Sanità, Rome, Italy

The pathogenicity of *C. difficile* has been mainly related to the production of toxins A and B. However, some pathophysiological and epidemiological aspects of *C. difficile*-induced disease such as the early phases of mucosal colonisation or the asymptomatic carriage of toxigenic strains, are still far from being understood. This suggests that microbial factors other than toxins may play an important role, especially in promoting the establishment of the disease. One of these factors may be surface-expressed antigens which could mediate essential functions such as adhesion to the epithelial layer of the gut or interaction with mediators of local immunity. In recent years, several works have demonstrated that only strains of *C. difficile* with certain cell wall-associated protein patterns are commonly isolated from patients and these proteins may thus represent as yet undefined virulence factors of the organism.

Our study in this field started with the observation that an electrophoretic pattern (group 2) which was characterised by a major protein of 36 kDa was characteristic of strains isolated from pseudomembraneous colitis and antibiotic-associated diarrhoea (AAD). We found antibodies to this 36 kDa antigen in the majority of patients with AAD. We purified and characterised the immunodominant protein from a representative strain, demonstrating that it is expressed on the surface of the microorganism and that it is a common cross-reactive antigen of most group 2 *C.*

difficile strains. Experiments were performed to verify its surface localisation, involvement in possible S-layer structures and interaction with phagocytosis. Moreover, by electron microscopy, we examined strains belonging to the electrophoretic group 2 for surface structures such as fimbriae and capsule in comparison with strains isolated from asymptomatic carriers.

INTESTINAL RECEPTORS FOR ENTEROTOXIGENIC *ESCHERICHIA COLI* THAT CAUSE DIARRHOEA IN SWINE

E. A. Dean-Nystrom, B. T. Bosworth. National Animal Disease Center, ARS-USDA Ames, Iowa, USA

Enterotoxigenic *E. coli* (ETEC) cause diarrhoea in pigs during the neonatal period and immediately after weaning. An important virulence attribute of ETEC is their ability to colonise the small intestine. Fimbriae (pili) facilitate intestinal colonisation by promoting adherence of ETEC to fimbriae-specific receptors on villous epithelium. We are examining the molecular basis of bacteria–host interactions by studying mechanisms of age-related and genetic resistance to ETEC adherence. Neonatal pigs are susceptible to 987P-mediated ETEC diarrhoea, but weaned pigs are resistant. We compared 987P binding to brush borders from susceptible (< 3 d old) and resistant (3–6 wk old) pigs. Multiple 987P-binding glycoproteins (987R, 33–40 kDa) in brush borders from both susceptible and resistant pigs did not correlate with susceptibility. A glycolipid 987P receptor (987M, < 17 kDa) was present in intestinal mucus of resistant pigs, but was absent or present only in trace amounts in susceptible pigs. We are testing the hypothesis that 987M inhibits 987P-mediated colonisation of weaned (resistant) pigs by preventing attachment of $987P^+$ ETEC to 987R. Unlike 987P, K88 mediates ETEC diarrhoea in both neonatal and weaned pigs. However, some pigs lack K88 receptors and are genetically resistant to diarrhoea caused by $K88^+$ ETEC. Erickson *et al.* (*Infection and Immunology* 60: 983–988, 1992) identified two K88-binding glycoproteins (> 200 kDa) in brush borders from K88-susceptible pigs that are not found in brush borders from genetically resistant pigs. We found that intestinal mucus from K88-susceptible pigs, but not K88-resistant pigs, contained K88 receptors like those in brush borders. Thus, unlike 987 receptors in mucus which may be protective, K88 receptors in mucus may promote adherence of $K88^+$ ETEC. These observations increase our understanding of host–pathogen interactions. They may also lead to novel intervention measures to protect pigs from ETEC diarrhoea, including the use of receptor analogues to inhibit ETEC adherence in susceptible pigs and development of genetically resistant (receptor-negative) breeding stock.

ESCHERICHIA COLI HEAT-STABLE ENTEROTOXINS: MODES OF ACTION AND MOLECULAR BIOLOGY

L. A. Dreyfus, B. Harville, S. Morris. School of Biological Sciences, University of Missouri-Kansas City, Kansas City, Missouri, USA

Enterotoxigenic *Escherichia coli* (ETEC) cause serious and sometimes life-threatening diarrhoeal disease by the elaboration of protein enterotoxins. Two classes of enterotoxins exist and are differentiated on the basis of thermal stability. The heat-labile enterotoxins (LTI and LTII) are structurally and functionally related to cholera toxin and appear to mediate secretion by similar mechanisms. The heat-stable enterotoxins (STa and STb) are peptides which share no structural or functional properties with LT. STa is an 18 amino acid cysteine-rich peptide which, following binding to the intestinal cell membrane, activates a membrane-bound guanylate cyclase. The rise in mucosal cGMP following STa action is thought to be responsible for the secretion of chloride and water which follows. The second heat-stable toxin is a 48 amino acid basic peptide containing four cysteine residues and two disulphide bonds which are required for biological activity. Unlike other cytotoxic enterotoxins of *E. coli*, STb causes intestinal secretion without apparent elevation of cyclic nucleotides. In an attempt to define a second messenger response for STb action, we investigated calcium ion fluxes in STb-treated cells by real-time multiwavelength fluorescence imaging. Cultured Madin Darby Canine Kidney (MDCK) and HT29/C1 cells were loaded with calcium- and pH-sensitive fluorescent dyes (indo-1 and SNARF-1, respectively) then placed in a video fluorescent microscope thermostastic stage bath for image analysis. Control and STb-treated cells were excited at 340 (indo-1) and 540 nm (SNARF-1) and imaged at 405 and 475 nm for indo-1 and 575 and 640 nm for SNARF-1. Each fluorescent emission was simultaneously captured by video tape for off-line analysis. Our data indicate that STb opens a ligand-gated calcium ion channel in the plasma membrane of treated cells. The rise in intracellular calcium ($[Ca^{2+}]_i$) due to

STb was not affected by agents which block voltage gated calcium ion channels. Calcium influx was completely inhibited however, by pertussis toxin and somatostatin, agents which block G protein coupled function. In addition, the elevation of $[Ca^{2+}]_i$ due to STb treatment was blocked by guanosine 5'-(β-thio)-diphosphate (GDP-βS), a specific inhibitor of the α subunit of GTP-binding regulatory proteins. The role of calcium influx in STb-mediated intestinal secretion is currently being investigated.

OXYGEN METABOLISM BY AN ANAEROBIC, MUCOSAL PATHOGEN, *SERPULINA HYODYSENTERIAE*

T. B. Stanton, B. L. Hanzelka, N. S. Jensen. National Animal Disease Center, USDA-ARS, Ames, Iowa, USA

In the early stages of swine dysentery, *S. hyodysenteriae* cells colonise oxygen-respiring mucosal tissues of the swine caecum and colon. During colonisation, this anerobic spirochaete is likely to be exposed to oxygen. Our research focuses on mechanisms by which *S. hyodysenteriae* cells metabolise oxygen and protect themselves from oxidative stress. *S. hyodysenteriae* cells growing beneath an atmosphere of N_2:O_2 (99:1) consume oxygen (1·6–2·0 μmol/ml culture). Oxygen consumption by this bacterium is accompanied by decreased yields of H_2 and butyrate, products of NADH oxidation. A major mechanism of oxygen metabolism by this spirochaete is NADH oxidase (EC 1.6.99.3), an enzyme that directly couples the reduction of O_2 with the oxidation of NADH. Seven strains of *S. hyodysenteriae* were examined and found to express high levels of oxidase activity (0·8–2·1 μmol NADH oxidised/min/mg cell protein). NADH oxidase purified from strain B204 is a monomeric, FAD-linked enzyme with a molecular mass of 47 kDa. The enzyme yields water from the reduction of oxygen. Every strain (18/18) of *S. hyodysenteriae* that we have examined by Southern blot analysis using an oligodeoxynucleotide probe, 5'-ATGAAAGT(TA)AT(TA)GT(TA)AT(TA)GG-3, contains the NADH oxidase gene (*nox*). The probe is based on the first seven amino acids of purified enzyme and hybridises specifically with the *nox* gene. *S. hyodysenteriae* cells also have protective mechanisms against toxic products of oxygen metabolism. These include superoxide dismutase, NADH proxidase, and catalase activities. The catalase activity is induced by adding H_2O_2 (200 μM, final concentration) to cultures of the spirochaete. *S. hyodysenteriae* cells possess various enzymes for contending with or taking advantage of oxygen and dealing with oxidative stress in their environment. Future research will characterise more fully these activities, study their regulation, and investigate their role in the physiology and ecology of this pathogen.

POLYSACCHARIDE CHARGE MEDIATES ABSCESS FORMATION IN A RAT MODEL OF INTRAABDOMINAL SEPSIS

A. O. Tzianabos, A. B. Onderdonk, R. L. Cisneros, D. L. Kasper. Harvard Medical School, Boston, Massachusetts, USA

The capsular polysaccharide complex (CPC) from *Bacteroides fragilis* promotes the formation of abscesses in an animal model of intraabdominal sepsis. The CPC comprises two distinct high molecular weight polysaccharides, termed polysaccharides A and B, which each possess positive charges (free amino groups) and negative charges (carboxyl or phosphate groups) as part of their respective repeating unit structures. We investigated whether this unusual biological property associated with the CPC is related to the distinct dual charge motif exhibited by these component saccharides. Polysaccharides A and B were each tested in the animal model and found to induce abscesses at very low concentrations. The abscess dose 50 (AD_{50}) of polysaccharides A and B were 0·67 μg and 25 μg, respectively, while the AD_{50} of the CPC was 22 μg. Neutralisation via chemical modification of the positive or negative charges on either polysaccharide A or B abrogated abscess induction in these animals. Polysaccharides derived from other bacteria that possess the dual charge motif exhibited by the *B. fragilis* polysaccharides also induced abscesses in this animal model while polysaccharides that lack charges or have one negative charge per repeating unit failed to do so. Further, a non-abscess-inducing negatively charged bacterial polysaccharide (the Vi antigen derived from *Salmonella typhi*) was converted to an abscess-inducing polysaccharide following addition of a positive charge to this repeating unit structure. This homopolymer, a repeating unit of galactaminuronic acid, was de-N-acetylated to form a structure consisting of free amino groups (positive charge) and carboxyl groups (negative charge). The unmodified polysaccharide did not have abscess-inducing activity while the modified

Vi antigen had an AD_{50} of 16 μg. These results indicate that polysaccharide charge modulates abscess induction in the peritoneal cavity of rats and provides a structural rationale for the unusual biological properties associated with the *B. fragilis* CPC.

IMMUNOASSAYS FOR THE ENTEROTOXIN OF *BACTEROIDES FRAGILIS*

R. L. Van Tassell, D. M. Lyerly, T. D. Wilkins. Department of Anaerobic Microbiology, VPI & SU, Blacksburg, Virginia, USA

Many strains of *B. fragilis* produce an enterotoxin that has been associated with diarrhoea in animals and humans. The enterotoxin, a small acidic protein (20 000 Mr), is cytotoxic on HT-29 cells and induces a fluid response in lamb ileal loops. A major drawback to studying this toxin has been the lack of neutralising antibodies and toxin-specific immunoassays. Consequently, we produced rabbit and goat antisera with high titres against the enterotoxin of *B. fragilis* VPI strain 13784 and developed specific indirect and direct toxin ELISAs which gave good dose responses with purified enterotoxin in the range of 1–1000 ng/ml. Using the toxin ELISAs, we screened: (i) dilutions of 10 normal human faecal specimens, (ii) culture filtrates of 76 strains of *B. fragilis*, and (iii) cultures of over 200 strains of other common intestinal anaerobes, including the seven most common species in the *B. fragilis* group. All faecal specimens were ELISA negative. All 14 known enterotoxigenic strains and four of 62 unknown strains of *B. fragilis* were ELISA positive and produced neutralisable cytotoxic activity, indicating that the enterotoxin is unique in *B. fragilis*. As a follow-up to early observations that rabbits had preimmune anti-enterotoxin serum titres, we screened neutral sera from a variety of other animals and humans using an antibody ELISA in which purified toxin was used as the capture phase. Neutral sera from mice, goats, horse, chickens and humans were negative, however, neutral sera from cows, sheep and rabbits had pre-existing antienterotoxin titres one-tenth that of the vaccinated goats. We are currently optimising the ELISAs for detecting the toxin in stools and other clinical samples. In addition, we are also screening sera from other animals and more humans to determine the extent of this pre-existing antibody phenomenon.

THE 'BASIC SYMBIOTIC UNIT': A CONCEPT OF COMMUNITY ORGANISATION IN ENTERIC MICROBIAL ECOSYSTEMS

L. C. Hoskins. VA Medical Center and Case Western Reserve University, Cleveland, Ohio, USA

The 'Basic Symbiotic Unit' (BSU) concept attempts to meet the need of a conceptual model of enteric microbial ecology. It assumes that competition for nutrients is maximum in climax enteric communities and that the observed population stability results from mutualistic associations among populations. A BSU is defined as the smallest number of strains capable of surviving together under existing conditions where none could grow alone, and it can support other strains. To test the concept we studied in batch and continuous cultures a BSU comprising a faecal *Escherichia coli* and a mucin oligosaccharide-degrading (MOD) anaerobe (*Ruminococcus torques* IX-70) in a medium consisting of faecal ions and purified commercial hog gastric mucin (HGM). The *E. coli* strain utilised acetate, growing little but scavenging O_2 from the medium, permitting growth of the MOD strain, whose constitutive glycosidases degraded HGM oligosaccharides to monosaccharides which both strains utilised. Growth, measured by culture optical density (OD), was rate limited by HGM concentration. Two other MOD strains (*Bifidobacterium* spp.) could replace *R. torques* IX-70 in the mutualistic association with *E. coli*. A *Bacteroides vulgatus* strain inoculated into the mixed culture enhanced growth OD and appeared to contribute to survival of the *R. torques* strain. Addition of a protease-producing *Enterococcus faecalis* strain enhanced mucin protein degradation from $\leqslant 20$ per cent to >60 per cent associated with a three-fold increase in culture OD. The 'MOD strain BSU' is a promising model for studying ecological interactions among enteric bacteria.

CORRELATION BETWEEN THE DIVERSITIES OF THE INTESTINAL COLIFORMS AND THEIR RATE OF TRANSLOCATION IN A RAT MODEL OF HAEMORRHAGIC SHOCK

M. Katouli, T. Bark†, O. Ljungquist†, T. Svenberg†, R. Möllby*. *Department of Bacteriology, Karolinska Institute and †Department of Surgery, Karolinska Hospital, Stockholm, Sweden*

Coliform bacteria are frequently reported to translocate after haemorrhagic stress. We used an automated biochemical fingerprinting method (the PhP system) to compare coliforms found in mesenteric lymph nodes (MLNs) with those found in the caecum of traumatised rats. We also evaluated the correlation between the diversities of the intestinal coliforms and their rate of translocation. Nine rats were subjected to severe haemorrhage (H group) whereas another nine rats were only sham operated (S group) without bleeding. Six not-instrumented rats (N) served as a control group. Forty coliforms from the caecum and 16 from MLNs (where possible) were tested with the PhP system. The phenotypic diversity of the coliform bacteria in each caecal sample was measured as Simpson's diversity index (Di), and the similarities between the bacteria in different samples were measured as population coefficients (Sp). Three rats in group S and seven in group H showed translocation. Identical biochemical phenotypes (BPTs) were found in a caecum and MLNs of each rat. The translocating strains in all rats, when compared pairwise, belonged to only two distinct BPTs (*translocating BPTs*). Rats showing no translocation either did not carry the *translocating BPTs*, or if they did carry them had high coliform diversities in their caecum. Coliform populations with high SP-values yielded similar pattern of translocation, indicating that they shared bacteria with similar BPTs. These results suggest that the translocation of coliforms in a rat model of haemorrhagic stress may be influenced by high diversities of bacteria in the caecum and that the presence of certain BPTs in bacteria in the caecum might be necessary for translocation.

SAFETY, IMMUNOGENICITY AND EFFICACY IN HUMAN VOLUNTEERS OF BIODEGRADABLE, BIOCOMPATIBLE MICROSPHERES CONTAINING COLONISATION FACTOR ANTIGEN/II (CFA/II) AS AN ENTERAL VACCINE AGAINST ENTEROTOXIGENIC *E. COLI* (ETEC)

E. Boedeker, R. Reid*, H. Bhagat†, P. Dalal†, C. Tacket‡, J. Nataro‡, G. Losonsky‡, R. Edelman‡, M. Levine‡*. *Walter Reed Army Institute of Research, Washington, DC, †School of Pharmacy and ‡Center for Vaccine Development, University of Maryland, Baltimore, Maryland, USA*

ETEC are a major cause of travellers' diarrhoea and of infantile diarrhoea in developing countries, however there are no vaccines against these diseases. Protection against ETEC should be provided by mucosal antibody to ETEC virulence factors, particularly fimbrial and fibrillar CFAs, but delivery of these protein antigens through the gastrointestinal tract to inductive sites of the organised gut-associated lymphoid tissues may require specialised delivery systems. To test the safety, immunogenicity and protective efficacy of biodegradable, biocompatible microspheres containing CFA, we incorporated CFA/II (1 per cent core loading) into polylactide-coglycolide microspheres of 5–10 μm diameter. Ten healthy volunteers were given four doses of 1 mg CFA/II (90 per cent CS3, 10 per cent CS1) by intestinal tube on days 0, 7, 14 and 28. Immune responses were measured by determining anti-CFA/II in intestinal fluid and sera and by enumerating anti/CFA/II circulating antibody secreting cells (ASC). Adverse reactions were monitored using a diary of symptoms. Ten vaccinees and 10 control subjects were challenged with 10^9 c.f.u. of ETEC strain E23247-7A (CFA/II (CS1, CS3)+ LT+ST+) and observed for development of diarrhoea. The vaccine was well tolerated. Five of 10 volunteers developed IgA anti-CFA/II ASC. Of these, all had anti-CS3 and three had anti-CS1 ASCs. Five of 10 also showed four-fold or greater rises in intestinal fluid anti-CFA/II IgA. Ten of 10 controls, but only seven of 10 vaccinees developed diarrhoeal illness (30 per cent efficacy). Two of the protected volunters had the highest ASC and sIgA responses. These results indicate the safety and mucosal immunogenicity, in humans, of CFA/II antigens delivered in biodegradable polylactide-coglycolide microspheres, and suggest protective efficacy in those with highest immune responses.

DECREASED EPITHELIAL CELL ADHERENCE OF *ENTAMOEBA HISTOLYTICA* CULTIVATED WITH MUCIN-DEGRADING BACTERIA

E. Variyam, P. Shah. VA Medical Center, and Case Western Reserve University School of Medicine, Cleveland, Ohio, USA

Adherence is a key initial step in *E. histolytica* (EH)-induced epithelial cell injury. *In vitro*, glycosidases produced by mucin-degrading normal enteric bacteria, along with pancreatic proteases, decrease Chinese hamster ovary (CHO) epithelial cell

adherence of EH grown in axenic media. To further investigate this microbial interaction as a potential host defense against invasive amoebiasis, we determined the adherence of EH cultivated along with mucin-degrading bacteria (MDB). EH strain HM1-IMSS was adapted to grow in modified egg slant medium with antibiotic-free and serum-free overlay of gastric mucin, *Escherichia coli* (for oxygen scavenging), and two MDB strains—*Bifidobacterium infantis* and *Ruminococcus torques*—and with or without pancreatic proteases (trypsin and alpha chymotrypsin). CHO cell adherence (i.e. per cent of EH with ≥ 3 adherent CHO cells) of EH trophozoites from these xenic cultures was compared to those of EH grown in axenic TYI-S-33 medium. Adherence (%, mean $\pm$ SEM) of axenic EH was $72{\cdot}6 \pm 0{\cdot}9$. Adherence of EH grown in xenic cultures was $56{\cdot}5 \pm 2{\cdot}2$ ($P < 0{\cdot}05$). Adherence of EH grown in xenic cultures containing pancreatic proteases adherence was $42{\cdot}1 \pm 3{\cdot}5$ ($P < 0{\cdot}01$). EH could be maintained in xenic cultures through three times weekly transfers and in one 4 wk continuous culture. These observations further support a potential protective role for MDB in decreasing EH virulence and the feasibility for *in vivo* studies of EH-MDB interaction.

EFFECTS OF GLUCONIC ACID ON THE HUMAN FAECAL MICROBIOTA

T. Asano, K. Yuasa*, K. Kunugita*, T. Terachi*, T. Mitsuoka†. *Chemicals Research Laboratories, Fujisawa Pharmaceutical Co. Ltd, Tsukuba and †Department of Food Hygiene, Nippon Veterinary and Animal Science University, Musashino, Japan*

Bifidobacteria are the main component of the human intestinal microflora, and may contribute health and nutritional benefits to its host. In Japan various oligosaccharides are used in food products as bifidobacteria growth-promoting factors. Most of these bifidogenic factors belong to sweeteners, and there is no report on acids which promote the growth of bifidobacteria. From among acids we directed our attention to gluconic acids, which include glucono-δ-lactone and calcium gluconate, and investigated the effect on the growth of bifidobacteria. We performed three experiments: measurement of utilisation by intestinal bacteria *in vitro*; measurement of absorption from a ligated small intestinal loop in rat *in situ*, and faecal flora analysis during ingestion of glucono-δ-lactone powder (9 g daily for 2 wk) in human volunteers. In the *in vitro* experiment, gluconate was utilised selectively by the *B. adolescentis* group in the genus *Bifidobacterium*, some of *Lactobacillus* species and Enterobacteriaceae, but was not utilised by most other bacteria including Bacteroidaceae and *Clostridium perfringens*. In the absorption test, only 20 per cent of injected gluconate was absorbed from the ligated loop under the condition that 100 per cent glucose was absorbed. These results suggest that most of ingested gluconate is not absorbed from the small intestine and reaches the large intestine where it is utilised by *B. adolescentis*. In the volunteer test, the number of faecal bifidobacteria increased significantly ($P < 0{\cdot}01$) during ingestion of glucono-δ-lactone, while those of Bacteroidaceae, which are the most predominant bacteria, showed a tendency to decrease slightly. Moreover the number of *C. perfringens*, which produces undesirable substances including toxins and volatile amines, decreased considerably. The number of Enterobacteriaceae, which utilised gluconate *in vitro*, did not increase in human volunteers. The relative percentage of bifidobacteria to total bacterial levels increased from 18 per cent to 46 per cent by ingestion of glucono-δ-lactone. The results show clearly that gluconates promote the growth of *B. adolescentis* which is one of the most abundant bifidobacterial species in the human adults. Gluconates in food products, therefore, may prove very useful for our health, not only for the purpose of food additive.

SOLUBLE FIBRE PREVENTS TOXIN PRODUCTION AND INTESTINAL TISSUE DAMAGE CAUSED BY *CLOSTRIDIUM DIFFICILE*

T. May, R. I. Mackie*, K. A. Garleb†. *Department of Animal Sciences, University of Illinois at Urbana-Champaign, Illinois and †Ross Laboratories, Columbus, Ohio, USA*

Clostridium difficile and its toxins are causative agents of pseudomembranous colitis in humans. Intestinal colonisation by healthy gut microbiota normally suppresses growth of toxigenic *C. difficile*. Two studies were conducted with the purpose of examining the relationship between substrate fermentability and short chain fatty acid (SCFA) production on the growth of normal intestinal microbiota, and numbers of and toxin production by *C. difficile*. In the first study, faecal inoculum was derived from pigs which allowed precise control of diet composition and intake. Results showed that fermentable fibre sources sustained the *in vitro* growth of indigenous organisms yielding SCFA

which effectively controlled growth of and toxin production by *C. difficile*. In the second study, an antibiotic-compromised murine model was developed using BALB/c mice. Mice were fed fermentable fibre sources, dosed with cefoxitin and then challenged with *C. difficile* VPI 10463. Mice fed the fermentable fibre sources had the highest faecal SCFA concentrations compared with control mice fed no fibre. Histopathological examination of the gastrointestinal tracts showed that the worst lesions occurred in the caecum of mice consuming the control diet; fermentable fibre inclusion in the diet reduced the severity of these lesions. Although the mechanisms are not clear, the growth promotion of the normal gastrointestinal microbiota by these fibre sources results in reduced growth and toxin production by *C. difficile* and hence reduced damage to intestinal epithelial tissue. Further studies aimed at elucidation of the ecological and immunological mechanisms which serve to control *C. difficile* colonisation are underway.

PLASMID TRANSFER IN THE GASTROINTESTINAL TRACT OF GERM-FREE ANIMALS BETWEEN LACTOCOCCI

P. Saadbye, J. Schlundt, B. Lohman. Institute of Toxicology, National Food Agency, Søborg, Denmark

The use of genetically engineered lactococci in dairy products will soon be a reality. Little is known about the fate of non-indigenous bacteria in the gut and almost nothing is known about their potential for plasmid transfer. We have tested the potential for gene transfer in conventional rats and in germ-free rats with almost identical strains of lactococci. The spatial distribution in the gut of donors, recipients and transconjugants were also examined. In conventional rats the donor and recipient were quickly eliminated and transconjugants were not detected. In germ-free rats, we examined two different experimental designs. Group 1 was dosed with the recipient strain MG1614 and 29 d later the donor NCDO712(pAMβ1) was administered and observed for 28 d. In group 2, the donor MG1614(pAMβ1) and the recipient MG1363-1 were dosed simultaneously and observed for 12 d. At the end of the experiment the animals of both groups were sacrificed and samples of lumenal contents were taken from duodenum, jejunum, caecum and colon. In group 1, the recipient colonised at 10^9 c.f.u./g faeces and remained at this level throughout the experiment. After repeated dosing, the donor was eliminated after 10 d. Transconjugants were detected 24 h after dosing and reached a level of 10^4 c.f.u./g which remained constant throughout the experiment. In group 2, both the donor and recipient established at 10^8 c.f.u./g and the transconjugants quickly established at 10^3–10^4 c.f.u./g. The concentration of transconjugants in both groups reached approx. 10^4 c.f.u./g in jejunum and remained at this level throughout the large intestine. In contrast, the concentration of recipients (and donors in group 2) increased from 10^4 c.f.u./g in the small intestine to 10^9 in the large intestine. The transconjugants seem to have a competitive advantage in the jejunum but not in the large intestine. This shows that faecal samples may not represent the situation in the gastrointestinal tract.

Poster Presentations

BACTERIAL VAGINOSIS DURING PREGNANCY

R. Mandar, H. Saag†, P. Peil†, M. Mikelsaar*. *Institute of Microbiology, University of Tartu and †Tartu Women's Hospital, Tartu, Estonia*

Rather few comparative data are available concerning the quantitative composition of vaginal microbiota and bacterial vaginosis (BV) confirmed by slides during the course of pregnancy. The aim of our study was to find the correlation between the occurrence of BV during pregnancy with the incidence and amount of lactobacilli. Forty-two pregnant women were repeatedly examined during a prospective study at the Tartu Women's Hospital. Each woman was investigated four to seven times, altogether 229 investigations were performed. Quantitative seedings of the vaginal swabs were made aerobically and anaerobically onto different media: the prereduced blood-thioglycollate-agar-medium, and the blood-agar, MRS-4-agar, Endo and Sabouraud media. In the case of each woman we calculated the total counts of microorganisms per swab and the relative amount (percentage) of lactobacilli in the vaginal microbial community. Gram-stained smears of the initial material were read for BV, using the scoring system (0 ... 10) of Nugent *et al.* (1991). We found bacterial vaginosis in 31 per cent of 229 investigations. At least one episode of BV during pregnancy was present in 20 women out of 42. In 14 of them BV was revealed in most of their samples while seven of them had BV in

all the samples. Nearly half of all the investigations showed intermediate and only 19 per cent normal vaginal microbiota. Lactobacilli were found in 39 of 42 women and in 139 of 229 samples. Their median relative amount (percentage) in the microbiota did not differ in cases of normal and intermediate vaginal microbiota, but it was 0 in all scores of BV. The incidence and the relative amount of lactobacilli in vaginal microbiota increased during pregnancy ($r = 0{\cdot}90$) while that of BV decreased ($r = -0{\cdot}62$). Thus, a close relationship was found between the incidence of BV and occurrence of lactobacilli during pregnancy. The frequent persistence of BV during pregnancy should make clinicians aware of the possible danger for adverse pregnancy outcome.

QUANTITATIVE EFFECT OF VARIOUS NON-MEDICATED DOUCHE PREPARATIONS ON VAGINAL MICROBIOTA

M. L. Delaney, A. B. Onderdonk. Brigham & Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA

Previous studies in our laboratory have examined the effect of douching with a solution containing povidone-iodine on the quantitative and qualitative make-up of the vaginal microbiota. Analysis of these data revealed a transient, but significant reduction in total levels following use of the medicated douche and little change in the qualitative make-up. The present study examined the effect of repeated use of non-medicated douche solutions on the total bacterial counts of the vaginal microbiota. The result of washing the surface of the vaginal vault with a solution of physiological saline was determined first. The repeated use of the various douche preparations was then evaluated to determine whether additional alterations of the vaginal microbiota occurred. Duplicate vaginal swab samples were obtained at predetermined intervals from 35 healthy volunteers for three sampling cycles before and after the use of douche products for various periods of time. Samples were analysed for both total facultative and obligately anaerobic bacterial populations. Results indicate that the use of non-medicated douche preparations caused a modest transient reduction of total bacteria, with most of the change attributable to the effect of washing the surface of the vaginal vault.

MICROBIAL FLORA OF THE VAGINA

A. Mehta, J. Talwalkar, C. V. Shetty, N. D. Motashaw. Departments of Microbiology and Gynaecology, Seth G.S. Medical College, K.E.M. Hospital and P.D. Hinduja National Hospital, Bombay, India

The vaginal flora from 66 women of child-bearing age (Group 1) and 20 postmenopausal asymptomatic women (Group 2) were studied. Vaginal secretions were obtained before pelvic examination on two sterile swabs from the posterior fornix of the vagina after insertion of Cusco's (bivalve) speculum without the application of any kind of lubricant or antiseptic. The secretions were cultured for aerobes and anaerobes by standard techniques. Twenty-seven of the Group 1 women were in secretory phase and 39 were in the proliferative phase. A total of 169 anaerobic and 76 aerobic isolates were recovered from the Group 1 women, whereas in Group 2 women, the number of aerobic and anaerobic isolates were 41 and 25 respectively. Lactobacilli were the most predominant bacteria in the microflora of the human vaginal tract, isolated in 61·53 per cent and 29·63 per cent of women, in the first and second half of the menstrual cycle respectively. *Bacteroides fragilis*, *B. melaninogenicus*, peptostreptococci, staphylococci, etc. were also present in the normal vagina. The aerobes observed in this study were *Staphylococcus epidermidis*, diphtheroids, alpha and beta haemolytic streptococci. Besides these non-haemolytic streptococci, *E. coli*, *Klebsiella* spp., *Proteus* spp. etc. were also found. In the first half of the menstrual cycle the maximum number of anaerobes were isolated but in the second half of the cycle and in the postmenopausal subjects the rate of isolation of anaerobes was low. While the aerobes were isolated in somewhat similar numbers in both halves of the menstrual cycle, they were found to be predominant in postmenopausal women.

QUALITATIVE AND QUANTITATIVE ASSESSMENT OF VAGINAL MICROBIOTA FOLLOWING USE OF COTTON TAMPONS FOR 2 OR 12 H

M. L. Delaney, A. M. DuBois. Brigham & Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA

Previous studies have examined the quantitative and qualitative effects of vaginal tampons composed of various fibres on the vaginal microbiota during menstruation. The present study was designed to

determine the effect of length of tampon use on vaginal microbiota. Total bacterial and individual species counts were obtained following the use of all cotton tampons for 2 or 12 h. Tampon and vaginal swab samples were obtained from 12 subjects for three menstrual cycles. Samples were analysed for total facultative and anaerobic bacterial levels and the predominant species were identified. Comparison of the 2 h and 12 h total anaerobe levels for concomitant swab and tampon samples showed no significant difference, however at 12 h total aerobes were significantly higher when compared to both the concomitant swab samples and the 2 h tampon samples. Qualitative analysis demonstrated significantly higher levels for coagulase-negative *Staphylococcus* spp., *Corynebacterium* spp., and aerobic *Streptococcus* spp. in the 12 h tampon samples when compared to the 2 h samples. Vaginal swab samples yielded similar counts at both time points, suggesting that no biologically significant change in the vaginal microbiota had occurred.

USE OF LACTOBACILLI TO PREVENT ADHESION OF PATHOGENS TO MEDICAL DEVICES: *IN VITRO* DATA

G. Reid, A. W. Bruce, C. Tieszer. Department of Microbiology and Immunology, University of Western Ontario, London; and Division of Urology, University of Toronto, Canada

Pathogenic microorganisms utilise various biomaterial substrata to adhere, form biofilms and gain access to tissue sites of the urogenital tract, where they cause infection. This is responsible for 100 000 catheter-related deaths in the USA per year, and a bladder infection rate of 40 per cent in people using incontinence pads. Hydrophobic and hydrophilic lactobacilli, indigenous to the healthy female urogenital tract, have been found *in vitro* to bind to polymers, catheters and diaper material, and exclude adhesion of over 80 per cent of *Escherichia coli*, *Staphylococcus epidermidis* and *Enterococcus faecalis*. In addition, the lactobacilli displaced a net 82 per cent of pathogens from the catheter surface. Furthermore, the mere presence of lactobacilli in the suspending fluid reduced the uropathogenic adhesion by up to 86 per cent. The adhesiveness and ability of selected strains of lactobacilli to interfere with pathogenic adhesion to biomaterials and tissue cells suggests a potential biotherapeutic usage. Further *in vivo* experiments will determine if, rather than eradicating the microbiota with broad-spectrum antibiotics, it could be possible to supplement or stimulate the lactobacilli for protective purposes.

USE OF PCR FOR DIRECT DETECTION OF TOXIGENIC STRAINS OF *C. DIFFICILE* IN HUMAN FAECES

G. Trani, A. Arzese, C. Pipan, G. A. Botta. Institute of Microbiology, Udine Medical School and University Hospital, Udine, Italy

C. difficile is the major causative agent of pseudomembranous colitis in humans. The organism produces two toxins (toxin A and toxin B), thought to play a major role in the diarrhoea and colitis. The aetiology can be confirmed by the detection of the toxins and/or by the isolation of the organism from the stool specimen in patients who develop diarrhoea during or after treatment with antimicrobial agents. However these procedures are time-consuming and it takes up to 5 d to isolate and identify *C. difficile* and to differentiate between toxigenic and non-toxigenic strains. Here we report on the use of PCR to amplify segments of non-repeating sequence of the *C. difficile* toxin A gene (proposed by Kato) using as starting material heated faecal samples. Two sets of primers specific for the toxin A gene and derived from non-repeating sequences were used to avoid lack of discrimination with *C. sordelli*. This method was applied in a surveillance study of chronic care facilities with high circulation of *C. difficile*. The method was proven to be simple and rapid. Toxigenic *C. difficile* strains can be identified in faeces in 5 h. A good agreement of PCR with clinical state was evident and in a few asymptomatic patients PCR was positive: these probably represent *true* healthy carriers. In conclusion direct detection of the gene by PCR helps overcome difficulties, greatly shortening the time required to send out a clinically relevant report, although one should bear in mind that in specific situations the *tox*A gene, albeit present, is not expressed.

ACKNOWLEDGEMENTS

Experiments reported here were made possible by Grant CNR.92.00027.PF41. and MURST 40 per cent. Thanks to Tiziana Galai for skilful technical assistance and to the student Lorena Riul for participation in the experiments.

CHEMOTAXIS BY *CLOSTRIDIUM DIFFICILE*

S. P. Borriello, M. P. R. G. Bhatt. Department of Microbiology, Queens Medical Centre, Nottingham, UK

For some enteric pathogens chemotaxis has been shown to be important in pathogenesis (e.g. *Vibrio cholerae* and *Campylobacter jejuni*). This is a little studied aspect of colonisation/virulence in pathogens and had not been examined in *Clostridium difficile*. In the first instance putative chemoattractants (taxins) were used, i.e. the known nutrients proteose peptone, hyaluronic acid and *N*-acetylglucoseamine, which were positive in that order of taxinicity. In experiments with 50 per cent colonic mucus as taxin it was demonstrated that *C. difficile* was attracted to rabbit, hamster, mouse, adult and neonatal human mucus. There was little difference in chemotactic activity between strains that adhered well and those that adhered poorly *in vivo*. This indicates that differences in the ability to adhere are reflective of differences in adhesion and not due to differing abilities to move to mucus for adhesion to occur.

INVOLVEMENT OF CAPSAICIN-SENSITIVE SUBSTANCE P CONTAINING SENSORY NEURONES IN THE INTESTINAL EFFECTS OF *CLOSTRIDIUM DIFFICILE* TOXIN A, BUT NOT CHOLERA TOXIN

C. Pothoulakis, I. Castagliuolo, A. Jaffer, C. P. Kelly, J. T. LaMont, S. Leeman. Sections of Gastroenterology and Pharmacology, Boston University School of Medicine, Boston, Massachusetts, USA

C. difficile toxin A (TxA) causes acute inflammation and fluid secretion when injected into ileal loops, while cholera toxin (CT) stimulates secretion, without causing inflammation. Previous studies indicated that primary sensory neurones containing the peptide substance P (SP) are involved in intestinal inflammation. In this study, we determined whether pretreatment with capsaicin, a primary sensory neurone desensitizer, or with an SP receptor antagonist, would alter the intestinal effects of TxA and CT. Adult rats were pretreated with either capsaicin (35 mg/kg/d × 3 d, s.c. with a 2 wk rest before subsequent experimentation) or with the SP receptor antagonist CP 96 345 (2·5 mg/kg, i.p.), or the same dose of its inactive enantiomer CP 96 344, IP, 10 min before administration of the toxins. Rat ileal loops were then injected with TxA (5 μg) or CT (25 μg) of buffer and 4 h later enterotoxicity was assessed by fluid secretion, blood-to-lumen excretion of mannitol and histological grading of enteritis. Pretreatment with capsaicin completely inhibited TxA-mediated secretion (P0·01) and reduced mannitol permeability by 88 per cent, P0·01) and neutrophil infiltration (by 91 per cent, P0·05). Pretreatment with CP 96 345, but not with CP 96 344 significantly reduced TxA-induced fluid secretion (by 89 per cent, P0·01), mannitol permeability (by 93 per cent, P0·01) and neutrophil infiltration (by 86 per cent, P0·01). In contrast, capsaicin and the SP antagonist had no effect on intestinal secretion caused by CT. These results indicate that sensory afferent neurones containing SP participate in the secretory response to TxA, but not to that of CT.

IN VITRO STUDIES ON THE MECHANISM OF ACTION OF THE CYTOTOXIC NECROTISING FACTOR 1 FROM PATHOGENIC *ESCHERICHIA COLI*

G. Donelli, C. Fiorentini*, L. Falzano*, P. Boquet†. *Department od Ultrastructures, Istituto Superiore di Sanità, Rome, Italy; †Unité des Toxines Microbiennes, Institut Pasteur, Paris, France*

A number of pathogenic *E. coli* strains produce the cytotoxic necrotising factor 1 (CNF1), a protein toxin which causes necrosis in rabbit skin and multinucleation in cultured cells. CNF1 was first revealed in clinical isolates from children with diarrhoea and, subsequently, from patients with extraintestinal infections. Cultured epithelial cells exposed to the toxin displayed time- and dose-dependent effects, mainly consisting of the enlargement and flattening of the cell body accompanied by an increased amount of stress fibres and intense membrane ruffling. Ultrastructural observations showed a remarkable heterogeneity in nuclear morphology, whereas other cytosolic organelles appeared to be unaffected. Often, it was possible to observe the presence of multipolar mitosis. Very recently, we observed that the profound reorganisation of the actin cytoskeleton into prominent stress fibres and membrane ruffles is accompanied by a potent phagocytic-like activity. CNF1-treated HEp-2 cells acquired progressively the ability to ingest latex beads as well as non-invasive bacteria such as *Listeria innocua* and the CNF1-producing *E. coli* strain BM2-1. Our results suggest the possibility that, *in vivo*, pathogenic CNF1-producing *E. coli*

strains may invade epithelial cells by this induced pathogocytic-like mechanism.

THE ROLE OF THE INTESTINAL MUCOSA IN MICROBIAL VIRULENCE

A. Erdener, L. L. Go, P. J. Healey, C. T. Albanese, S. C. Watkins, R. L. Simmons, M. I. Rowe. Departments of Surgery, Children's Hospital of Pittsburgh and University of Pittsburgh, Pittsburgh, Pennsylvania, USA

There are differences in the ability of enteric microbes to translocate across the gastrointestinal tract. It is unknown, however, whether these differences are due to microbial species-specific effects on the intestinal mucosa. We studied the relationship of the intestinal mucosa to microbial virulence by exposing mucosa mounted in an Ussing chamber to different microbes and examining mucosal histology and transmucosal microbial passage. Rat terminal ileum stripped of serosa was mounted in an Ussing chamber. Following exposure of the mucosal surface to *Escherichia coli* (two strains), *Listeria monocytogenes* (two strains), *Vibrio cholerae*, *Enterococcus* spp., *Candida albicans* or coagulase-negative staphylococci, the transmucosal passage of these microbes was monitored by serial cultures of the submucosal perfusate. The membranes were then fixed and examined with light microscopy by a blinded investigator.

Organism	Transmucosal microbial passage	Mucosal histology
E. coli C-25	28/78 (35%)	Normal
E. coli K-1	11/20 (55%)	Normal
Listeria (virulent)	8/17* (47%)	Damage
Listeria (non-virulent)	1/13 (7%)	Normal
Vibrio cholerae	4/11 (36%)	Damage
Enterococcus sp.	4/12 (33%)	Normal
Candida	2/10 (20%)	Damage
Coagulant-negative staphylococci	2/12 (16%)	Damage

*$P < 0.05$ compared to virulent *Listera*.

With the exception of the virulent strain of *Listeria*, which showed a significantly greater ability to pass through the mucosa compared to the avirulent strain, the other microbes studied did not show significant differences in the incidence of transmucosal passage. In addition, there appeared to be no correlation between the incidence of passage and the degree of mucosal injury. We conclude that, although microbes vary in their ability to translocate across the intestine, the difference in translocation rates are not due solely to a direct effect on the intestinal mucosa.

INSTABILITY OF THE POUCH FLORA: CAUSE OF POUCHITIS?

J. G. H. Ruseler-van Embden, M. P. Hazenberg, L. M. C. van Lieshout, W. R. Schouten. Department of Immunology, Erasmus University, Rotterdam, The Netherlands

For patients requiring proctocolectomy the continent ileostomy and the ileoanal anastomosis have evolved into attractive alternatives for the permanent Brooke ileostomy. However, some patients will develop a clinical syndrome known as pouchitis. To gain more understanding in mechanisms that might control the generation of pouch-ileitis we compared the following parameters in the output from patients with a good-functioning pouch and patients with pouchitis: the composition of the pouch microbiota, the activity of bacterial enzymes with the potency to degrade intestinal mucus, pH and proteolytic activity. The microorganisms of patients with pouchitis had increased numbers of aerobes, a decreased ratio of anaerobes to aerobes, less bifidobacteria and anaerobic lactobacilli, more *Clostridium perfringens* and several species that were never found in control patients (e.g. fungi). Furthermore, the pH was significantly higher in patients with pouchitis (median value 6·5) than in control patients (5·4). If the pH is a controlling mechanism in the ileal pouch, it might be of influence in the degradation of intestinal mucus glycoproteins. Therefore the effect of pH on glycosidases and proteases present in pouch output and on the breakdown of hog gastric mucine by pouch microorganisms, was tested. Some glycosidases were inhibited, others were stimulated by a low pH (5·2), however in each sample the proteolytic activity was inhibited by 75 per cent at pH 5·2 as compared with pH 6·8 and 7·6. Mucin degradation by the pouch microbiota was a very active process at pH 7·2: within 2–4 h of incubation more than half of the mucin was degraded. At pH 5·2 it took twice as long. We concluded that pouchitis may be the result of instability of the microorganisms in the pouch,

by which homeostasis disappears (dysbiosis) and the protection of the pouch epithelium by the mucus layer becomes affected by increased activity of bacterial and host-derived enzymes.

POSITION OF *ESCHERICHIA COLI* CELLS IN THE GASTROINTESTINAL TRACT OF RATS DETERMINED BY USE OF rRNA PROBES

E. M. Nielsen. Institute of Toxicology, National Food Agency, Søborg, Denmark

The mechanisms of colonisation and specific adhesion in the gastrointestinal tract of most well-known pathogenic microorganisms have been studied fairly extensively. However at present, the corresponding knowledge for most non-pathogenic bacteria colonising the intestinal tract is very limited. We have studied the physical position and metabolic activity of an *E. coli* strain, BJ20, during the colonisation process in the gastrointestinal tract of gnotobiotic and streptomycin treated rats. The rats were dosed p.o. with 10^5 c.f.u. of the *E. coli* strain, and animals were killed from 4 h up to 15 d after dosing. Tissue from jejunum, ileum, caecum and colon was quick-frozen and 8 μm thick sections were made. The sections were mucin-stained and hybridised with a fluorophore-labelled probe specific for *E. coli* rRNA. Following hybridisation, single cells of *E. coli* were identified using epifluorescence microscopy, and the physical location was assessed in relation to the epithelium, the mucus layer and the intestinal content. Dense areas of cells were seen in the mucus layer lining the epithelial cells but apparently not in direct contact with the epithelium or in the mucus in the intestinal crypts. The picture was almost the same 4 h after dosing compared to the animals killed 1–15 d after dosing. Since the cellular content of ribosomes is correlated to metabolic activity, the relative growth rate of single cells is correlated to the fluorescence intensity. The preliminary results show a generally high intensity in all locations of the gastrointestinal tract. At killing, the concentration of *E. coli* in the lumenal contents of the same parts of the gastrointestinal tract were determined by plating. The concentration of *E. coli* BJ20 indicated some multiplication after 4 h, and after 24 h the populations in the different parts of the gastrointestinal tract had reached their maximal sizes, and these were kept constant throughout the experiment. This study suggests that the use of rRNA probes on frozen sections of intestinal tissue can bring new information about subpopulations, location, and metabolic activity of microorganisms in the gastrointestinal tract.

PREMENSTRUAL SYNDROME (PMS): EFFECT OF PROBIOTIC SUPPLEMENT AND *S*-ADENOSYL-L-METHIONINE (SAMe) ON INTESTINAL MICROORGANISMS

M. E. Bertazzoni, A. Benini†, L. Vicentini*, P. Grussu†, R. Cerutti†. *Institute of Pharmacology, and †Department of Obstetrics and Gynaecology, University of Verona, Padova, Italy*

PMS is a complex pathology with psychosomatic, gastrointestinal and hormonal features (altered metabolism of sexual hormones). Intestinal microflora of women with PMS showed microbial fluctuations with increase in different enterobacteria. We can hypothesise that the microbial flora may be an additional factor in determining PMS, with other phenomena such as the modifications of sex-steroid hormones pattern. In order to correct gastrointestinal symptoms (constipation, diarrhoea, abdominal pain and microflora alterations), we administered to 16 young women (range 28–35 yr) with severe PMS a supplement of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* ($4 \times 10^8 + 4 \times 10^8$ bacteria/g) in capsule form three a day for 2 mth and SAMe, 800 mg twice a day. SAMe is a methyl donor which participates in hepatic biochemical reactions and may favour the metabolism of sex-steroid hormones during the enterohepatic cycle. Eight healthy young women were included as controls. Faecal samples were collected on the 11th and 23rd d of their cycle before the starting therapy and on the 23rd d during therapy. Microflora composition was performed by standard methods. Women with PMS showed low bacterial levels in comparison to the healthy controls. *E. cloacae*, *Hafnia* spp., and *Citrobacter freundii* were isolated in the majority of PMS women before treatment, but decreased or disappeared after 2 mth of therapy. Aerobic lactobacilli increased 1 log. The mean levels of clostridia and bacteroides were not significantly altered. Different clostridia species (*C. fallax*, *C. bifermentans*, *C. histolyticum*) appeared during treatment. Anaerobic gram-positive non-spore-forming rods increased in species number; *E. limosum*, and *Propionibacterium acnes* appeared as new species. Moreover we noted a relief of some symptoms of PMS in studied women, i.e. minor abdominal distress and pain, no constipation and better psychological behaviour.

EFFICACY OF PASSIVE TRANSFER OF PGG-GLUCAN-INDUCED PROTECTION AGAINST MORTALITY ASSOCIATED WITH EXPERIMENTAL INTRAABDOMINAL SEPSIS

R. L. Cisneros, A. G. Carayannopoulos, A. B. Onderdonk. Brigham & Women's Hospital and Harvard Medical School, Boston, USA

Previous studies in our laboratory have established the efficacy of poly-*B*-(1-6)-glucotriosyl-*B*-(1-3)-glucopyranose, PGG-glucan, a biological response modifier, during experimental peritonitis in a rat model for intraabdominal sepsis. These studies documented that the i.m. injection of PGG-glucan 24 h and 4 h prior to challenge resulted in a significant reduction in mortality. Quantitative peripheral blood and peritoneal fluid cultures indicated that there were significantly lower numbers of bacteria in the PGG-glucan-treated animals. In addition we have shown that PGG-glucan treatment results in an increase in the absolute granulocyte and monocyte numbers in peripheral blood. To furthr explore the mechanism(s) by which this agent elicits protection, studies were performed to examine whether protection could be transferred from animals treated with PGG-glucan to naive recipients via spleen cells or spleen cell lysates. Passive transfer experiments indicate that the responsible factor(s) is transferable via whole spleen• cells, spleen cell lysates, and peripheral WBCs from treated animals. Furthermore, the transferable factor(s) is resistant to pronase and trypsin digestion, is not heat labile at 56 or 80°C, and is not removed by NH_4SO_4 precipitation. Further analysis is planned for this potential adjunctive for treatment of serious polymicrobic infections.

BIOCHEMICAL FINGERPRINTING AS A TOOL TO STUDY THE DIVERSITY, STABILITY, AND METABOLIC CAPACITY OF INTESTINAL BACTERIA

I. Kühn, M. Katouli, R. Möllby. Department of Bacteriology, Karolinska Institute, Stockholm, Sweden

Studies of the composition and activity of intestinal bacteria are often hampered by the lack of simple methods. We have developed a system based on biochemical fingerprinting of single isolates and of whole communities. The test is performed in prefabricated microplates with 12 or 48 different dehydrated reagents. All test results are read by an optical microplate reader and transferred to a computer which performs all necessary calculations. Using this assay we are able to study some important parameters of intestinal microbial communities.

A. The **phenotypic diversity**, described as Simpson's diversity index (D_i), is measured by assaying at least 24 isolates with a set of 12 highly discriminating reagents.
B. The **stability**, described by the aid of the Population Similarity Coefficient (S_p), is measured by assaying at least 24 isolates from each sample with a set of 12 highly discriminating reagents.
C. The **metabolic capacity** (MC) is measured as the total activity of the whole bacterial population on a set of 48 different reagents.

We have used this assay, e.g., to study the intestinal coliforms in six piglets 0–3 mth of age, and two sows, with sampling every week, and we found that the communities varied greatly between sampling occasions, and that the D_i decreased during an outbreak of diarrhoea in the herd. We suggest that this method is a simple and useful aid in the study of intestinal bacteria in both man and animals.

CRITERIA FOR EVALUATION OF FAECAL MICROBIOTA

M. Mikelsaar, E. Sepp*, R. Mändar*, A. Ormisson†. *Institute of Microbiology and †Children's Hospital of Tartu University, Tartu, Estonia*

Large individual differences both in the qualitative and quantitative composition of intestinal microbiota (IMB) have created the need for new criteria in evaluating its state. The aim of the study was to compare the quantitative composition of faecal MB of preterm and term newborn on different feeding by developed novel criteria and the use of a computer program 'BioQuant'. The faecal MB of 18 preterm neonates (Group I) of birth weight below 2000 g, born in Tartu Maternity Hospital and 25 term neonates (Group II), born in Tampere University Hospital was investigated at the age of 5–7 d. Out of preterm neonates 11 were fed by a specific formula 'pretutteli' (I PT; Valio Ltd, Finland) and seven with other formulas (I OF); out of term babies 10 were breast-fed (II BF) and 15 received additionally *Lactobacillus casei* strain GG

supplement (II BF+GG; Valio Ltd, Finland). In faecal samples 12 groups of aerobic and anaerobic microorganisms were quantified in $\log_{10}$ c.f.u./g. The percentage of subordinate microbes and their sum percentages in the total count (SSM%), calculated by 'BioQuant', enabled us to evaluate the IMB. Several changes of the quantitative composition of faecal MB could be found in different groups of investigated neonates like the significant increase of SSM% in Group I in comparison with Group II, showing the impaired establishment of normal IMB in preterm neonates. In Group I OF the relative share of staphylococci was increased in comparison with Group I PT, suggesting a threat of systemic infections with staphylococci. In Group II BF+GG the relative share of lactobacilli increased in comparison with Group II BF, showing the possibility for establishment of a lactobacillus rich MB by administration of GG-probiotic. We suggest use of the developed matrices in evaluation of the IMB imbalance and its changes under different regimens.

COMPARISON OF MICROBIAL COMPOSITION AND METABOLISM IN HUMAN FAECAL BACTERIA-ASSOCIATED (HFA) MICE INOCULATED WITH HUMAN FAECES

K. Kirayama, K. Itoh, E. Takahashi. Department of Veterinary Public Health, University of Tokyo, Tokyo, Japan

Germ-free mice and rats associated with human faecal bacteria have been used as one method for studying human intestinal microbiota. In this study, the composition of faecal bacteria, production of short chain fatty acids and putrefactive products, and the activities of bacterial biotransformation enzymes in faeces of HFA mice inoculated with faeces from six different humans were studied to compare their variations among HFA mice. The composition of faecal microbiota of HFA mice was similar to that of the original human faeces with respect to the major components except for bifidobacteria. Bacterial enzyme activities in faeces of HFA mice were similar to those in human faeces or between those in human faeces and conventional (CV) mouse faeces. Concentrations and composition of putrefactive products in the faeces of HFA mice were different from those in human faeces and similar to those in CV mouse faeces. The composition of short chain fatty acids in the faeces of HFA mice was similar to that in human faeces when compared with that in CV mouse faeces, although the concentration was lower than that in human faeces. These characteristics in the faeces of each group of HFA mice were maintained in their offspring. There were no significant differences in characteristics in the faeces among the six different groups of HFA mice and individual variations observed in the original human faeces were not distinct among HFA mouse groups. These results indicate that HFA mice provide a stable and valuable tool for studying the human gut ecosystem and metabolism, but they have some limitations as a model.

INFLUENCE OF ROXITHROMYCIN ON THE FAECAL TRYPTIC ACTIVITY

E. Bezirtzoglou, E. Norin†, T. Midvedt†. *Medical School of Ioannina University, Department of Microbiology, Ioannina, Greece; †Department of Medical Microbial Ecology, Karolinska Institute, Stockholm, Sweden*

Germ-free (GF) rats and mice demonstrate several structures and functions reflecting absence of a functional intestinal microflora (Germfree Animal Characteristics, GACs). When a microflora is present, these functions are converted to Microflora Associated Characteristics (MACs). By feeding of antibiotics to conventional animals, some GACs can be generated. In order to evaluate the influence of a new macrolide, roxithromycin (Roussel), on one parameter, the faecal tryptic activity, eight conventional rats of the AGUS strain were given roxithromycin (125 mg/kg) intragastrically for 5 d. Faecal samples were collected every second hour for periods of 18 h prior to, daily during and then on the 4th and 11th d after the drug was given. Thereafter, in order to ensure that the function was re-established, an enema, consisting of 10 per cent dilution of caecum content from conventional rats, was given on day 16, followed by faecal sampling on d 20. A strong effect was observed upon occurrence of faecal tryptic activity after administration of the drug. It is important to mention that the faecal tryptic activity remains at 'high' levels even after 4 wk following administration of the full flora enema. In conclusion, administration of roxithromycin to conventional rats gave rise to significant alterations in the intestinal tryptic activity.

ROLE OF ENZYMES FROM HUMAN INTESTINAL BACTERIA IN THE REDUCTION OF AZO DYES AND NITRO-POLYCYCLIC AROMATIC HYDROCARBONS

F. Rafii, C. E. Cerniglia. Division of Microbiology, National Center for Toxicological Research, FDA, Jefferson, Arkansas, USA

Azo dyes and nitrated polycyclic aromatic hydrocarbons (nitro-PAHs), which are abundant in our environment, are reduced to aromatic amines by bacterial and liver enzymes. Bacteria from the human intestinal microbiota capable of reducing azo dyes and nitropolycyclic aromatic hydrocarbons (nitro-PAHs) were identified. They belonged predominantly to the genera *Clostridium* and *Eubacterium*. Azoreductase and nitroreductase both occur as only one isozyme in each bacterium; different forms of the azoreductase and nitroreductase were produced constitutively by several species. An antibody against *C. perfringens* azoreductase had immunological cross-reactivity with those of other azoreductases, suggesting that these enzymes share structural similarities and may be considered as a single related group of enzymes with regard to their function and antigenicity. Immunological, electrophoretic and biochemical assays indicated that one enzyme was probably involved in the reduction of both nitro-PAHs and azo dyes. Azoreductases and nitroreductases from several bacteria had the same electrophoretic mobilities as certain dehydrogenases from these bacteria, indicating that azo dyes and nitro-PAHs are reduced by the same enzyme whose main function may be in cellular electron transfer. Immunoelectron microscopy showed that azoreductase were scattered in the cytoplasm and secreted without prior accumulation.

ENDOGENOUS GENERATION OF N-NITROSO COMPOUNDS BY HUMAN GUT MICROORGANISMS AND THE INFLUENCE OF SOYBEAN OLIGOSACCHARIDES

C. J. Rumney, I. R. Rowland*, Y. Saito†, T. Hirahara†. *BIBRA Toxicology International, Surrey, UK; †Calpis Food Industry Co., Ltd, Shibuya, Japan*

The present work forms part of a programme investigating dietary factors involved in endogenous production of N-nitroso compounds (many of which possess potent carcinogenic and mutagenic activity). We have previously demonstrated in rats that endogenous nitrosation depends on the presence of nitrate and gut bacteria and have confirmed the requirement for nitrate in apparent total N-nitroso compound (ATNC) formation in humans. Purified soybean oligosaccharides (SOR) have been shown to alter the composition of the gut microbiota and tend to increase the proportion of bifidobacteria. SOR were fed at dietary concentrations of 3 per cent and 6 per cent w/w to human-gut microbe-associated (HFA) rats for 4 wk, together with drinking water containing 500 mg nitrate/l and ATNC were analysed in caecal contents. Consumption of diet containing 3 per cent SOR was associated with a significant ($P \leqslant 0{\cdot}01$) decrease in caecal ATNC concentration (0·19 μg [N-NO]/g caecal contents) in comparison with the SOR-free diet (0·68 μg/g), the effect being more pronounced in female than in male rats. A smaller decrease in caecal ATNC concentration was observed in rats fed 6 per cent SOR (0·40 μg/g), indicating the lack of a dose-related increase in the apparent inhibitory effect of SOR. Similarly, caecal pH was lower in both groups of SOR-fed rats than in controls, but the decrease was greater at 3 per cent SOR. In order to investigate further the possible mechanisms involved in the inhibitory effect of SOR, it was necessary to be able to follow nitrosation by gut bacteria *in vitro*. A fresh faecal sample was obtained from a healthy female volunteer who had consumed a diet low in nitrate and ATNC for 5 d. A 20 per cent faecal suspension was prepared in 0·1 M phosphate buffer, pH 7·0, and duplicate, anaerobic incubations in the presence of 0, 10, 50 and 100 mM nitrate were performed *in vitro* at 37°C. The ATNC content of samples taken at 0, 4, 8, 12 and 24 h were determined. In the presence of 10, 50 or 100 mM nitrate, ATNC concentrations increased with time and nitrate concentration, whilst no increase was observed in the absence of nitrate. We are currently investigating the effect of bifidobacteria concentration and pH on *in vitro* nitrosation by human gut microorganisms.

EFFICACY OF *LACTOBACILLUS REUTERI* AS A PROBIOTIC FOR CHICKENS AND TURKEYS

H. J. Dunham, F. W. Edens†, I. A. Casas‡, W. J. Dobrogosz*. *Department of Microbiology and †Department of Poultry Science, North Carolina State University; ‡BioGaia Biologics Inc., Raleigh, North Carolina, USA*

Lactobacillus reuteri colonises the gastrointestinal (GI) tract of healthy animals and humans. Mothers in particular, but other adults as well, are believed to be the primary source for transmission of this and other enteric bacterial species to newborns. In the poultry industry, however, this natural transfer process is interrupted owing to the absence of adult birds during hatching, and *L. reuteri* is found in only approximately 20 per cent of birds hatched and reared under commercial conditions. We have shown that essentially all birds become colonised by probiotic treatments with host-specific strains of *L. reuteri*, delivered by *in ovo* injections, by spraying newly hatched birds, and by addition to their feed. Laboratory and large-scale field tests have shown that significant benefits are derived from these treatments, particularly when the animals are subjected to environmental and/or pathogenic stressors such as mild cold stress and/or challenge with enteropathogens such as *Salmonella* spp. *L. reuteri*-treated flocks consistently exhibit fewer deaths, more rapid body weight growth, and enhanced feed efficiencies. Recent studies have shown that *in ovo* colonisation of the GI tract with *L. reuteri* also dramatically inhibits hatchling mortality caused by horizontal transmission of salmonellae from infected to non-infected birds during the post-hatch period. There are preliminary indications that *L. reuteri* confers these benefits through competitive exclusion mechanisms and by modulating the newborn's immune response. In comparison to controls, treated birds exhibit: (i) longer ileal villi and deeper crypts, a response associated with enhanced T-cell function; (ii) suppressed PHA-induced epidermal DTH reaction; and (iii) increased production of serum anti-salmonella IgM antibodies. Sections of splenic, bursal and GI tissues obtained from control and treated birds have been immunoperoxidase stained using chick monoclonal antibodies to lymphocyte subsets: CD4, CD8, CD3, $\alpha\beta$, $\gamma\delta$, and Bu-1a/Bu-1b. Their relative frequency in these tissues is being determined.

EFFECT OF CULTURE CONDENSATE OF *BIFIDOBACTERIUM LONGUM* ON BACTERIAL TRANSLOCATION

T. Suzuki, K. Itoh†, T. Kaneko*, H. Suzuki*. *Central Research Institute, Meiji Milk Products, and †Department of Vet. Publ. Health, University of Tokyo, Tokyo, Japan*

Bacterial translocation (BTL) is defined as the passage of viable bacteria from the gastrointestinal (GI) tract through the mucosa to the mesenteric lymph nodes (MLN) and other organs. BTL is induced by bacterial overgrowth in the intestine, immunosuppressive conditions and physical disruption of the mucosal barrier in the GI tract. It has been reported that the physical condition of the intestinal epithelia of SPF rats fed a diet including 1 per cent of a culture condensate of *B. longum* (CCB) was improved, and cells of *B. longum* stimulated the non-specific immune system. In this paper, the effects of CCB diet on BTL were studied using *Escherichia coli* C25 monoassociated SPF mice receiving 30 per cent burns and injected with zymosan. Body weight gain was better in the CCB diet group than in the control diet group. In both the CCB and control diet groups, burn stress promoted BTL of *E. coli* C25 from the GI tract to the MLN and peritoneal cavity. In non-burn control mice, however, BTL was inhibited by the CCB diet. Although BTL was not inhibited by the CCB diet in mice receiving burn stress, mortality and the positive rate of BTL in the peritoneal cavity tended to be lower in the CCB diet group than the control diet group. In the zymosan (0·1 mg/g body wt, i.p. injection) induced mouse model, the translocation of *E. coli* C25 from the GI tract to the MLN was inhibited in the CCB diet group. These results indicate that the CCB diet is able to inhibit BTL induced by bacterial overgrowth in the GI tract and stressful conditions.

LACTOBACILLUS RHAMNOSUS AND *L. PARACASEI* AS STARTER CULTURES AND POTENTIAL PATHOGENS

G. Klein, B. Hack†, K. Zimmermann‡, V. Rusch‡, G. Reuter*. *Institute of Meat Hygiene, Free University Berlin, Germany; †Institute of Microbiology and ‡Institute of Microecology, Herborn, Germany*

Lactobacillus rhamnosus and *L. paracasei paracasei* are members of the autochthonous human microbiota. Especially *L. rhamnosus* is often used in milk starter cultures and pharmaceutical preparations. On the other hand these species are considered to be potential pathogens and according to national regulations in the case of *L. rhamnosus* listed as a hazardous organism. Therefore 11 biotechnologically used (including type strains) and 16 clinical isolates (including two *L. paracasei paracasei* and one isolate of human faeces) were screened for biochemical properties, antimicrobial susceptibility and soluble cytoplasmic protein pattern. The aim was to find out

criteria for potential pathogenic lactobacilli of the oral cavity and to evaluate their risk for public health. Twenty-seven biochemical substances and physiological properties were investigated in macrotubes and 22 antimicrobial substances were tested in the agar diffusion test according to NCCLS guidelines and earlier determined breakpoints. Protein patterns were obtained by SDS–polyacrylamide gel electrophoresis and silver staining. Biochemical properties and antimicrobial susceptibility patterns were not able to detect intraspecies differences in general but were sensitive enough for interspecies differentiation. Only patterns of the soluble cytoplasmic proteins allowed in most cases to differentiate clearly between clinical and technologically used strains. Protein pattern preparation could be one suitable method to solve the problem of characterisation of potential pathogenic lactobacilli and to differentiate them from those of biotechnological interest. It is concluded that the normal inhabitants of the gastrointestinal tract and lactobacilli in food products are not to be considered as a hazard in general.

ORAL BACTERIOTHERAPY IN PAEDIATRIC PRACTICE

E. Isolauri, M. Malin, M. Kaila, W. H. Ling, H. Mykkänen, S. Salminen. Departments of Clinical Medicine, University of Tampere, Clinical Nutrition, University of Kuopio; Applied Chemistry and Microbiology, University of Helsinki, Finland

Gut microorganisms are an important constituent in the gastrointestinal defense barrier. The balance of this ecosystem can be indirectly assessed by measuring bacterial enzyme activities. We studied the effect of orally administered *Lactobacillus casei* strain GG (10^{10} c.f.u. twice daily) on bacterial enzyme activities in faeces in patients with acute rotavirus diarrhoea aged 5–28 mth, and in patients with Crohn's disease and their controls aged 2–17 yr. Urease, β-glucuronidase, β-glucosidase and glycocholic acid hydrolase activities in faeces were assessed before and after oral bacteriotherapy. Duration of diarrhoea was recorded. In patients with rotavirus diarrhoea, oral bacteriotherapy significantly shortened the diarrhoeal phase. Dietary supplementation with lactobacilli for 5 d significantly influenced the bacterial enzyme profile. Urease activity during diarrhoea transiently increased in controls not given lactobacilli ($n=21$), but not in those given *Lactobacillus casei* GG ($F=8{\cdot}6$, $P=0{\cdot}01$). No intergroup differences were found in β-glucuronidase, β-glucosidase and glycocholic acid hydrolase levels. In patients with Crohn's disease, oral bacteriotherapy for 10 d showed parallel effects in all bacterial enzymes studied. Oral bacteriotherapy may be a useful tool in re-establishing intestinal integrity in patients with acute and chronic gastrointestinal disease. By reinforcing the mucosal barrier, oral bacteriotherapy may promote host defense.

IMPACT OF A LACTOBACILLUS PROBIOTIC ON FAECAL MICROBIOTA IN CHILDREN WITH SHIGELLOSIS

E. Sepp, E. Tamm†, S. Torm†, I. Lutsar†, M. Mikelsaar*, S. Salminen‡. *Institute of Microbiology and †Children's Hospital of Tartu University, Tartu, Estonia; ‡Foundation for Nutritional Research, Helsinki, Finland*

The impact of *Lactobacillus casei* strain GG (LB GG) administration on clinical course and composition of faecal microbiota (MF) has been investigated in children's shigellosis. Twenty-four consecutive children (2–11 yr, mean 4·5 yr) with acute shigellosis of moderate severity, admitted to the Children's Hospital of Tartu University, were studied. Five children were treated with LB GG powder (LB GG; $\log_{10}$ 10–11 c.f.u./g; Valio Dairies Ltd) alone (Group I); 13 children got trimethoprim-sulphamethoxazole (TMP-SMX; 36 mg/kg for 5 d) and LB GG (Group II): six children were treated with TMP-SMX (Group III). LB GG was resistant *in vitro* to TMP-SMX. In 71 faecal samples from days 1, 5 and 10, 12 groups of aerobic and anaerobic microorganisms were quantified. The sum percentage of subordinate microbes in total counts (SSM%) was calculated. We did not find any differences in the duration of fever, diarrhoea or anorexia between study groups after treatment. At admission the SSM% of all patients was significantly higher (92–95 per cent) as compared to the normal value of SSM% (< 15 per cent) of healthy Estonian infants. Thus, before treatment a severe intestinal microbiota imbalance could be found. After administration of the probiotic the SSM% significantly decreased in Group I and II (33–35 per cent, $P<0{\cdot}05$) and the state of intestinal microbiota improved. Consequently, the symptomatic treatment of shigellosis with live lactobacilli, though not influencing the clinical course of disease, seems to be promising in improving the imbalance of intestinal microbiota.

SEPTICAEMIA, JOINT INFECTION, AND VERTEBRAL OSTEOMYELITIS CAUSED BY *KINGELLA KINGAE*

V. Velculescu, J. Wong. Kaiser Regional Laboratory and California State Department of Health Services, Berkeley, California, USA*

Kingella kingae, a member of the *Neisseriaceae*, is known as a normal commensal of the oral mucous membranes but has been associated with endocarditis, and bone and joint infections. It is universally accepted as being susceptible to penicillin. Four new cases of infection, septic arthritis, vertebral osteomyelitis, and septicaemia caused by *K. kingae* are reported. For isolation, the specimens were cultured on blood, chocolate and EMB agar. Blood samples and vertebral disk aspirate were inoculated into BACTEC blood culture bottles. Three strains were isolated from BACTEC blood bottles by blind subculture or by sampling of bottles with growth index greater than 30 U. One strain grew only after 4 d on chocolate agar media. All four strains were β-haemolytic, gram-negative diplobacilli, non-motile, oxidase-positive, catalase-negative, and produced acid from glucose and maltose. Susceptibility testing was performed for all strains by standard KB disk diffusion, β-lactamase and MIC 'E' test. Out of four isolates only one was a β-lactamase producer. We wish to call attention to the first incidence of β-lactamase producing *K. kingae* and the importance of β-lactamase testing for all *K. kingae* isolates, although the clinical relevance of β-lactamase production is unknown. BACTEC blood culture system utilisation and blind subculture enhances the recovery of *K. kingae* from joint and bone aspirations, and improves bacteriological diagnosis.

A NEW AND RAPID METHOD FOR IDENTIFICATION OF *C. PERFRINGENS* IN CAVE WATERS

E. Bezirtzoglou, A. Panagiou*, J. Savaidis†, D. Theodorou*, O. Tsolas†, G. Antoniadis*. University of Ioannina Medical School, Laboratory of *Microbiology and †Biological Chemistry, Ioannina, Greece*

Little is known about the species composition and variability of natural bacterial communities in caves. In order to evaluate the degree of bacterial pollution, we chose *Clostridium perfringens* as a point source indicator. For this purpose, we utilised a new medium, called lactose-sulphite (LS) broth, proposed for rapid enumeration and identification of *C. perfringens* without the necessity of further confirmatory tests. Membrane filtration equipment was used on site. All samples were alternatively passed through two membrane filters (20–25 μm pore size). One filter was used for retention of the abundant phytoplankton, and the other (porosity 0·45 μm) for *C. perfringens*. The growth medium employed was the LS broth. Membranes were placed into the first tube of 10-fold dilutions from 10^1 to 10^4 and incubated aerobically in a waterbath at 46°C for 24 h. The levels of Ca^{2+} and Mg^{2+} ions were determined in water samples using atomic absorption spectroscopy. The count of *C. perfringens* revealed fluctuations depending on the sampling station. In samples of water where *C. perfringens* was isolated, we found high levels of magnesium. No correlation was obtained with calcium levels. The LS broth not only allows the detection of small numbers of *C. perfringens*, but also permits detection and rapid estimation of its numbers within 24 h. It could be a reliable technique for estimation of *C. perfringens* as an indicator of water pollution.