Anaerobe • 2008

The 9th Biennial Congress of the Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

SESSION I—INTESTINAL FLORA & INTRA-ABDOMINAL INFECTIONS

SESSION I—INTESTINAL FLORA & INTRA-ABDOMINAL INFECTIONS

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTESTINAL FLORA & INTRA-ABDOMINAL INFECTIONS

IMPORTANCE OF GLYCAN SYNTHESIS AND REGULATION TO COLONIZATION OF THE MAMMALIAN INTESTINE BY *BACTEROIDES FRAGILIS*

Chatzidaki-Livanis, M.*
Channing Laboratory, Harvard Medical School, Boston, MA USA

Bacteroides species are the most abundant Gram negative bacteria of the mammalian intestinal ecosystem. These endogenous organisms synthesize an extensive number of surface polysaccharides, many of which phase vary by DNA inversions of the promoters of their biosynthetic loci. We have found that in addition to invertible promoters, there are other levels of regulation of polysaccharide expression. Each of the eight polysaccharide biosynthesis loci begin with a pair of genes that we have designated *upxY* and *upxZ* (x = a, b, c, d, e, f, g, or h depending on the locus) which have regulatory functions. Based on the presence of *upxY* genes in each of the polysaccharide biosynthesis loci, and the presence of a NusG-like domain, we predicted that these products would function in transcriptional antitermination of their respective loci. Internal non-polar deletions of three *upxY* genes resulted in loss of expression of the expected polysaccharide and, in each case, synthesis was restored when the respective *upxY* was added *in trans*. Synthesis of heterologous polysaccharides was not affected by deletion of the *upxY* gene of another locus, rather each *upxY* gene was only necessary for the synthesis of its own polysaccharide. UpxZs have no homologs in the databases outside of the intestinal *Bacteroidales* species. In order to investigate the role of *upxZ* products, we made internal deletions in three of these genes. Unlike the *upxY* products, UpxZs are not essential for expression of their respective polysaccharide. Rather, overexpression of UpxZs in wild type repressed expression of heterologous polysaccharides. These data reveal a completely novel function for the *upxZ* products and demonstrate that promoter inversion is only one of the factors that regulate polysaccharide expression. Therefore *B. fragilis* has evolved a complex and interconnected regulatory system that ensures that there will always be only one polysaccharide locus transcribed at a time. To understand the biological significance of this elaborate capsular polysaccharide regulation we created two *B. fragilis* mutants; one that is able to synthesize a single phase variable polysaccharide and another that is stably acapsular. We showed that the acapsular mutant was rapidly out-competed; however, synthesis of a single polysaccharide was sufficient for the organism to competitively colonize the gnotobiotic mouse intestine. These data suggest that the synthesis of at least one polysaccharide is required for competitive colonization of the gnotobiotic mouse intestine and that multiple phase-variable polysaccharides are likely important for long term maintenance in the normally complex and competitive human intestinal ecosystem.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTESTINAL FLORA & INTRA-ABDOMINAL INFECTIONS

THE *BACTEROIDES FRAGILIS* OXIDATIVE STRESS RESPONSE DURING INTRA-ABDOMINAL INFECTIONS

Smith, C.J.*
Department of Microbiology & Immunology, Brody School of Medicine, East Carolina University, Greenville, NC USA

Intra-abdominal abscesses are formed in response to contamination of the peritoneal cavity with intestinal bacteria following perforation of the bowel. These are generally polymicrobic infections that induce a multifactorial host response designed to wall off and contain the invading microbes. *Bacteroides fragilis* is the predominant anaerobe associated with intra-abdominal infections and the critical role of its capsular polysaccharide for induction of abscesses has been established. Resistance to oxidative stress and aerotolerance also are expected to be important factors during the initiation and persistence of these infections. This is because relative to the colon, the peritoneal cavity is an oxygenated environment and the recruitment of PMNs to the site of infection will result in exposure to reactive oxygen species. In vitro transcription and protein profiling have established that *B. fragilis* mounts an oxidative stress response that enhances survival during stress. Analysis of the expression patterns suggests that there is a rapid, acute response designed to minimize the immediate effects of oxygen radicals, and studies show that this is necessary for both short-term and long-term resistance to oxidative stress. The transcriptional regulator, OxyR, mediates the response through the induction of several detoxification/protection enzymes which remove reactive oxygen species. This acute response is crucial for in vivo cell survival and abscess formation as indicated by studies with OxyR mutants in the mouse intra-abdominal infection model. On those occasions where the OxyR regulon does not control the stress an expanded metabolic response is induced which encompasses the genes for a wide range of enzymes that can supply reducing power for detoxification and expand energy generating capabilities. Fundamental to the metabolic response is the repression of genes related to translation and biosynthesis which correlate with an immediate reduction in growth rate and entry into a stationary phase-like state. The importance of the metabolic response to long term cell survival and abscess formation is presently not known. Aerotolerance and resistance to oxidative stress are physiological adaptations of *B. fragilis* to its niche which promote its survival in extra-intestinal sites and now can be listed with the capsular polysaccharide as virulence factors important for the development of opportunistic infections.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTESTINAL FLORA & INTRA-ABDOMINAL INFECTIONS

MICROBIOLOGIC ELEMENTS OF THE IDSA/SIS GUIDELINES ON MANAGEMENT INTRA-ABDOMINAL INFECTIONS

Solomkin, J.S.*
University of Cincinnati College of Medicine, Cincinnati OH USA

Evidence-based guidelines for the management of patients with intra-abdominal infections were prepared by an Expert Panel of the Infectious Diseases Society of America and the Surgical Infections Society. This presentation will focus on those elements dealing with microbiologic specimen processing.

Routine cultures from low-risk patients are regarded as optional, based on local practices and concern (C-III). Such cultures may facilitate recognition of local changes in resistance, and thereby optimal selection of empiric antimicrobial agents.

For higher risk patients, cultures should be routinely obtained (C-III), particularly in patients with prior antibiotic exposure, who are more likely to harbor resistant pathogens.

The incidence of organisms in the community with resistance to commonly prescribed agents is rising. If resistance for a given antibiotic is greater than 10-20% for a common intra-abdominal pathogen in the community, use of that agent should be avoided. Because of widespread resistance of *E. coli* to ampicillin/sulbactam, that antibiotic is no longer recommended for routine empiric therapy of complicated intra-abdominal infections.

Interpretation of culture results should be based on pathogenic potential of identified organisms in this setting and quantization. The major pathogens in intra-abdominal infections are based on older antibiotic trials and animal models of intra-abdominal sepsis involving colonic flora showing coliforms (Enterobacteriaceae, especially *E. coli)* and anaerobes (especially *B. fraglis*) are the major pathogens. Pathogens are nearly always present in concentrations of 105/gm of tissue or 105 /ml exudate. This would correspond to moderate or heavy growth on the primary isolation plates. There should be attention to the predominant pathogen since many of these cultures yield mixed flora where it is difficult to distinguish pathogens from commensals. In mixed cultures, microbiologists should be directed to perform susceptibilities for Pseudomonas, Proteus, Acinetobacter, *Staphylococcus aureus*, and predominant (up to two) Enterobacteriaceae, as these species are more likely to yield the resistant strain.

Most intra-abdominal infections involve anaerobic bacteria, but laboratories show great variation in reliably performing in vitro sensitivity tests. Sentinel studies of *B. fragilis*, the major pathogen, show uniform susceptibility to metronidazole, carbapenems and "*B*-lactam/" *B*-lactamase inhibitors. Anaerobic cultures, in most cases of intra-abdominal sepsis, are unnecessary (providing empiric therapy assumes their presence) and therefore are not recommended.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTESTINAL FLORA & INTRA-ABDOMINAL INFECTIONS

MODULATION OF THE GUT MICROBIOTA OF PRETERM INFANTS

Campeotto, F.;[1,3] Suau, A.;[2] Magne, F.;[2] Waligora-Dupriet, A.J.;[3] Ferraris, L.;[3] Kapel, N.;[4] Dupont, C.;[1] Butel, M.J.*[3]

[1]Neonatologie and Gastroenterologie Pédiatrique, Hôpital Saint Vincent-de Paul, Paris, France
[2]Laboratoire de Biologie, Conservatoire des Arts et Métiers, Paris, France
[3]EA 4065, Microbiologie, Université Paris Descartes, Paris, France
[4]Coprologie Fonctionnelle, Groupe Hospitalier Pitié Salpêtrière, Paris, France

The neonatal gut is sterile at birth, but bacterial colonization begins early on the first day of life. By 7-10 days of age, most healthy term infants are colonized with a heterogeneous microbiota including enterobacteria, enterococci, lactobacilli, and bifidobacteria. The intestinal bacterial establishment of preterm neonates differ from that of term infants. Due to a delayed enteral feeding, a relatively sterile environment, and frequently broad spectrum antibiotic courses, the natural colonization process tends to be both delayed and impaired. This abnormal pattern of colonization is thought to render these infants at increased risk of gastrointestinal diseases, including neonatal necrotizing enterocolitis (NEC). Attempts to modify this ecosystem using prebiotic (non-digestible oligosaccharides that beneficially affect the host by selectively stimulating the growth and/or the activity of a limited number of bacteria) or probiotic (live microorganisms that ingested in sufficient quantities exert a positive influence on the host) could be an interesting way of prevention. Recent studies with a prophylactic administration of probiotics containing bifidobacteria reported a reduction in the incidence of NEC. However, there was no indication on the mechanism involved.

Patients and methods. A prospective randomized double blind bicentric study enrolled 58 preterm infants born at a gestational age from 30 to 35 weeks. They were fed either a standard preterm formula or the same formula added with heat-killed *Bifidobacterium breve C50* and *Streptococcus thermophilus*, ie 33 vs 25 infants, of whom respectively 7 and 6 were also fed their mother's milk from day 5 to day 14 according to cases. Faecal samples were collected weekly during the hospital stay, which lasted from 4 to 34 days. Feces were analysed for flora (culture and culture-independent methods) and TNFα, calprotectin, and sIgA content (ELISA).

Results. Neonatal characteristics and anthropometric data during the follow-up were similar between groups. The gut microbiota was poor (both methods). Infants were rapidly colonized by staphylococci whereas enterococci and enterobacteria increased all along the follow-up to reach 50% of the infants at 4 weeks of age. Colonisation by *Bacteroides* and bifidobacteria was noticeably delayed with 9 and 16 of colonized infants, respectively. Clostridial colonization occurred at a high level, increasing from 1/3 to almost all infants at 1 month. No difference was observed between the 2 groups of feeding. Faecal TNFα was undetectable except in 11 infants, with no difference between the 2 groups. As previously described, the calprotectin level was high ranging from 16 to 1240μg/g, with no difference whatever the type of feeding. High levels of sIgA were observed in infants supplemented with mother's milk, significantly more ($p<0.001$) with the formula added with ferments.

Conclusion. Presence of heat-killed ferments and their metabolites in the formula did not affect the gut microbiota balance or the inflammatory markers but seemed to enhance mucosal immunity.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTESTINAL FLORA & INTRA-ABDOMINAL INFECTIONS

INDIGENOUS HUMAN MICROFLORA AND ANAEROBE AGENTS: INTERACTION AND ETIOLOGICAL ROLE IN NOSOCOMIAL DISEASES OF HOSPITALIZED PATIENTS

Petrov, V.O.;[1] Szabo, J.;[2] Kulja, A.;[2] Koval, H.;[1] Kalapos, I.;[2] Boyko, N.V.*[1]
[1]Department of Microbiology, Immunology, Virology and Etiology of Infectious Diseases, Medical Faculty, Uzhhorod National University, Uzhhorod, Ukraine
[2]University of Debrecen, Debrecen, Hungary

In order to improve the strategy for selection of effective means and techniques for prevention and therapy of hospital diseases, the bacteriological assessment of surgical and gastrointestinal units from Ukrainian and Hungarian clinics were performed. All isolates obtained from examined materials were identified by using of API test systems (bioMérieux, France) and automatic system VITEK. Genetic generosity of dominantly persistent species of microorganisms and correspondingly their etiological role in registered nosocomial infections has been confirmed by PCR (with specific primers to vancomycin resistance genes: vanA, vanB, vanC, vanD, vanE and ESBL genes: SHV, TEM, CTX) for Gram-negative bacteria and by PFGE for Gram-positive strains.

For gastroenterology unit, *Staphylococcus hominis, S. aureus, Enterobacter cloaceae* and *Klebsiella pneumoniae* were defined as agents of nosocomial diseases, whereas *Proteus morganii, Acinetobacter spp., S. aureus, Pseudomonas aeruginosa, K. pneumoniae* and *E. cloaceae* – for surgical unit. Anaerobes were presented by strains of *Bacteroides spp., Fusibacterium spp., Peptostreptococcus anaerobius* and *Peptococcus niger*. *Clostridium difficile* was not found. Indigenous gut microflora of patients was limited by species from *Enterococcus* and *Escherichia genera*, no lactobacilli or bifidobacteria were detected. The strong correlation between observed disorders in indigenous microflora' homeostasis and frequency of nosocomial infections was noted. The level of serum Igs of patients with symptoms of nosocomial infections significantly differ from normal indices.

Selected strains of normal gut microflora were separately tested in vivo on its efficacy against agents of nosocomial diseases by using of appropriate mice models. *Lactobacillus salivarius, Schaedler Escherichia coli, Morganella morganii* and *Bacillus subtilis* demonstrated both synergic stimulatory effect on host immune system functions and high inhibitory properties against *K. pneumoniae, E. cloaceae,* and *S. aureus* but not to *P. morganii* and *P. aeruginosa*.

The bacterial cocktail including *Bacteroides distasonis* and other commensals of intestinal mucosal surfaces was established. Bacterial association orally administered to mice prior to their following challenge with pathogens (LD_{100}) protects the infectious diseases developing in most cases up to 70% of infected animals. Currently, we demonstrated that expression of Reg III b and g genes in colon together with arising of number of IFN-g producing NK cells in IE space of SCID mice was significantly increased under influence of certain commensal bacteria.

The experimental data obtained are background for the new strategy developing for patients' protection from nosocomial infections in clinical units.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTESTINAL FLORA & INTRA-ABDOMINAL INFECTIONS

INHIBITORY EFFECT OF *GARCINIA KOLA* ON *CLOSTRIDIUM DIFFICILE* TOXIN INDUCED MOTILITY OF RAT COLON

Egwuatu, T.O.;*[1] Ogunsola, F.T.;[1] Anigbogu, C.N.;[2] Banjo, A.A.F.[3]
[1]Department of Medical Microbiology and Parasitology
[2]Department of Physiology
[3]Department of Morbid Anatomy College of Medicine, University of Lagos, Lagos, Nigeria

Garcinia kola is a plant widely used among local people in Nigeria as an appetite stimulant and as therapy for common cold, abdominal colic, bronchitis, dysentery, and diarrhea. We investigated the effect of methanol and aqueous extracts of *Garcinia kola* on gastrointestinal smooth muscle contractility of rats challenged with *Clostridium difficile*. Twenty five Sprague-Dawley rats weighing 70-100g were used for this study. These animals were divided into a control and experimental groups 'A-E' of five rats each. Two experimental groups, 'D and E' were pretreated with 0.2ml of 200mg/ml of the methanol and aqueous extracts respectively. Studies were carried out on 1.25cm sections of rat colon suspended in Tyrode's solution in 50ml organ baths. The preparations were bubbled with oxygen and maintained at a temperature of 37°C. Isotonic recordings of contraction were carried out using a force displacement transducer. The effects of the various *Garcinia kola* extract on contractility of the rat colon were compared to atropine. The methanol extract reduced the contractility of the rat colon to the same degree as atropine ($p<0.05$), and this was still significant when the effect of the methanol was corrected for ($p<0.02$). The aqueous extract also inhibited the gastrointestinal motility but was much less effective, and the difference between the effect of the methanol and aqueous extracts was significant ($p<0.001$). Both methanol and aqueous extracts of *Garcinia kola* have atropine-like antimotility effect on the rat colon and may therefore explain in part its antidiarrhoeal effect. More studies are therefore proposed to determine the anti inflammatory effect of *Garcinia kola* on *Clostridium difficile* associated colitis.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTESTINAL FLORA & INTRA-ABDOMINAL INFECTIONS

EXAMINATION OF THE CONTRIBUTIONS OF DPS TO THE OXIDATIVE STRESS RESPONSE AND PATHOGENESIS OF THE OPPORTUNISTIC PATHOGEN, *BACTEROIDES FRAGILIS*

Gee, J.M.;* Rocha, E.R.; Smith, C.J.
Department of Microbiology and Immunology, East Carolina University, Greenville, NC USA

The obligate anaerobe, *Bacteroides fragilis*, is a highly aerotolerant intestinal tract organism and is isolated from nearly 60% of all anaerobic infections. Aerotolerance may contribute to the virulence of this organism by allowing it to survive the relatively high oxygen concentrations found outside its normal anaerobic niche, thereby conferring a selective advantage in establishing infections in extra intestinal sites. In fact, *B. fragilis* has evolved a complex oxidative stress response that induces the expression of greater than 28 proteins in response to oxygen or hydrogen peroxide exposure. Many of these genes are regulated by the LysR-type redox responsive transcriptional regulator OxyR. However, a *B. fragilis oxyR* mutant strain maintains a relatively high level of aerotolerance, suggesting additional oxidative stress resistance pathways are present. One highly oxygen induced protein is Dps, a DNA binding protein, that displays both OxyR-dependent and independent regulation in response to oxidative stress. In many aerobic bacteria, Dps protects DNA from oxidative damage and, in some cases, provides a ferritin-like function by storing excess iron. In this study, a *B. fragilis dps* mutant was examined to determine its affect on aerotolerance and the ability to form abscesses in a mouse model of infection. The *dps* mutant was attenuated in vivo, and in-vitro analysis suggested that this protein plays several roles in contributing to the overall fitness of *B. fragilis* in response to exposure to different kinds of environmental stress. The *dps* mutant was sensitive to exposure to several oxidative stress-generating compounds, acidic pH, and DNA damaging agents. In addition, Dps may contribute to iron homeostasis, as the mutant strain was more sensitive to the antibiotic streptonigrin and the iron chelator dipyridyl and was unable to persist as well as the parent strain under conditions of iron starvation. Transcriptional analysis of *dps* indicated that the gene is induced in response to oxygen exposure and iron starvation, suggesting that this protein has an important bifunctional role, not only in aertolerance, but also in iron homeostasis. In addition, we have identified a putative ecf-type sigma factor which appears to have some role in the OxyR-independent regulation of *dps* expression. Current studies are underway to further characterize the phenotypic contributions of this important gene product.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTESTINAL FLORA & INTRA-ABDOMINAL INFECTIONS

A SURVEY ON METABOLISM OF SUDAN AZO DYES BY PREDOMINANT INTESTINAL MICROORGANISMS

Chen, H.;* Xu, H.; Heinze, T.M.; Paine, D.D.; Cerniglia, C.E.
National Center for Toxicological Research, Jefferson, AR USA

There is evidence that Sudan azo dyes (1-amino-2-naphthol-based azo dyes) have genotoxic effects and that ingestion of food products contaminated with Sudan I, II, III, IV, and Para Red could lead to exposure in the human gastrointestinal tract. The ingested Sudan azo dyes are mainly metabolized by intestinal microflora to colorless aromatic amines by NAD(P)H-dependent azoreductases. Our previous results demonstrated Sudan azo dyes can be biodegraded by human intestinal microflora, and the metabolites were recognized and confirmed by using HPLC and LC/ESI-MS/MS. In this study, we examined 30 predominant species of human intestinal bacteria, which include 7 *Bacteroides spp*., 5 *Bifidobacterium spp*., 7 *Clostridium spp*., 3 *Eubacterium spp*., 2 *Enterococcus spp*., 2 *Fusobacterium spp*., 2 *Ruminococcus spp*., *Peptostreptococcus magnus*, and *Escherichia coli*, to evaluate their capacity to degrade Sudan azo dyes. Among these tested bacterial strains, 20, 5, 23, 21 and 22 species were able to metabolize Sudan I, II, III, IV, and Para Red, respectively. *Bifidobacterium infantis, Clostridium indolis, Clostridium clostridioforme*, and *Ruminococcus obeum* were able to metabolize all five tested Sudan azo dyes. However, *E. coli* was the only strain which is not able to degrade any tested Sudan azo dyes to any significant extent. Metabolites of the degradation of the tested Sudan azo dyes by *B. infantis* and *Enterococcus faecalis* were isolated, identified, and quantified through HPLC and LC/ESI-MS/MS analyses and compared with authentic standards. These results indicate that the degradation of the tested Sudan azo dyes depends upon the characteristics of each individual species regardless of its genera or Gram staining. It also suggests that most of the intestinal bacteria are able to metabolize the tested Sudan azo dyes except Sudan II.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTESTINAL FLORA & INTRA-ABDOMINAL INFECTIONS

EXPRESSION OF *BACTEROIDES FRAGILIS* HEMOLYSINS IN VIVO AND ROLE OF HlyBA IN AN EXPERIMENTAL INTRA-ABDOMINAL INFECTION MODEL

Lobo, L.A.;*[1] Alligood, R.B.;[1] Jenkins, A.L.;[2] Rocha, E.R.[1]
[1]Department of Microbiology and Immunology
[2]Department of Comparative Medicine
East Carolina University Brody School of Medicine, Greenville, NC USA

Bacteroides fragilis is the most frequent opportunistic pathogen isolated from anaerobic infections. The success of *B. fragilis* pathogenicity is still not completely understood, but virulence factors such as capsular polysaccharides, adhesin and neuraminidase play an important role. Another potential virulence factor that has received little attention is the ability of *B. fragilis* to produce hemolysins. Thus to initiate studies to elucidate the role of hemolysin production in *B. fragilis* infections, we have assessed the expression and regulation of *hlyA* through *hlyG* and *hlyIII* using an intra-abdominal implantation of the perforated table tennis "ping-pong" ball to establish an in vivo tissue cage rat model of infection. Bacteria were injected into the tissue cage ball at approximately $1x10^8$ cfu/ml. Aspirates from the tissue cage were taken at 1, 4, and 8 days post-infection for cfu determination and total bacterial RNA extractions. Samples were immediately mixed with bacterial RNA protect reagent and after differential cell lysis to remove host cells, total bacterial RNA was extracted. Real-time RT-PCR was used to quantify the expression of *hlyA*, *B, C, D, E, F, G,* and *III* mRNAs normalized to the levels of s34 mRNA. The *hlyA* mRNA was induced approximately 6-fold after 4 days post-infection compared to the mRNA levels in the inoculum culture prior to infection. The *hlyB* mRNA increased approximately 3-fold after 1 day, 6-fold after 4 days and 12-fold after 8 days post-infection. Expression of *hlyC* mRNA increased 6-fold after 1 day, 45-fold after 4 days and 16-fold after 8 days post infection respectively. Levels of mRNA for *hlyD* and *hlyE* were not altered after 1 day post-infection but were induced approximately 40-fold after 4-days post-infection. These finding suggest that *B. fragilis* hemolysins are induced and differentially regulated in vivo. To determine whether hemolysins play a role in the establishment of *B. fragilis* infection, the dual component *hlyBA* mutant strain was used in an experimental infection and the cfu was compared to the parent strain. Both parent and mutant strains reached levels of approximately $3\text{-}8x10^9$ cfu/ml after 1 day post-infection. However, the *hlyBA* mutant strain lost 2 logs in viable cell counts, compared to the parent strain after 8 days post–infection. Taken together, these findings show for the first time that the HlyBA play a role in survival of *B. fragilis* in an intra-abdominal infection model.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTESTINAL FLORA & INTRA-ABDOMINAL INFECTIONS

COMPARATIVE STUDY OF SPECIES AND STRAIN DISTRIBUTION OF BIFIDOBACTERIA IN THE FECES OF BREAST-FED INFANTS AND THEIR MOTHERS

Kulagina, E.V.;* Shkoporov, A.N.; Donskikh, E.E.; Khokhlova, E.V.; Kafarskaia, L.I.; Efimov, B.A.
Department of Microbiology and Virology, Russian State Medical University, Moscow, Russian Federation

Aim of the research: To study and to compare qualitative and quantitative composition of cultivable numerically predominant *Bifidobacterium* genus bacteria in the feces of mothers and their breast-fed infants, to reveal possible identical strains of bifidobacteria in the mother-infant pairs.

Materials and methods: The research was conducted in the group of 13 mother-infant pairs. Mean age of the infants was 5.5 month, and of the mothers – 26.2 years. *Bifidobacterium* strains were isolated from the feces by plating serially diluted homogenates onto selective media. Species identification of pure cultures was accomplished by PCR with *Bifidobacterium* species-specific primers, and by partial 16S rDNA sequencing. Comparison of strains was conducted by means of REP-PCR (repetitive element PCR).

Results: It was found, that the most frequent dominant *Bifidobacterium* species isolated from the mothers were *B. longum* and *B. adolescentis* (occurrence rates 92.3% and 76.9% respectively). *B. bifidum, B.catenulatum*, and *B.breve* were detected less frequently (46.2%, 30.8%, and 7.7% respectively). The average number of different *Bifidobacterium* species per sample was 2.7±1.1. The infants were colonized mostly by *B. bifidum* (61.5%) and *B. longum* (53.8%), but *B. longum bv. infantis* (30.7%), *B. breve, B. adolescentis, B. dentium* (15.4% for each species), and *B. angulatum* (7.7%) were also detected. The average number of different *Bifidobacterium* species per sample was 1.9±1.0. In 9 of 13 cases both mother and her infant were colonized by strains (totally 34), which belonged to the same species (*B. longum, B. bifidum, B. adolescentis* or *B. breve*). But only in 5 (38.5%) cases these pairs of strains from the mothers and their infants were found to be identical by their REP-PCR signatures. These strains belonged to *B. longum* in one case, *B. bifidum* in three cases, and *B. adolescentis* in one more case.

Conclusion: Our results provide initial experimental support for the previous hypothesis of early colonization of infants with maternal *Bifidobacterium* strains.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTESTINAL FLORA & INTRA-ABDOMINAL INFECTIONS

INCIDENCE OF ENTEROTOXIGENIC AND NON-TOXIGENIC *BACTEROIDES FRAGILIS* PATTERNS IN STRAINS ISOLATED FROM BRAZIL AND A HOSPITAL IN FRANCE

Miranda, K.R.;* Dias, M.F.; Guimarães, P.L.S.; Boente, R.F.; Pauer, H.; Balassiano, I.T.; Antunes, E.N.F.; Falcão, L.S.; Ferreira, E.O.; Ferreira, L.Q.; Paula, G.R.; Santos-Filho, J.; Domingues, R.M.C.P.
Instituto de Microbiologia Professor Paulo de Góes, UFRJ, Rio de Janeiro, RJ Brasil

Enterotoxigenic *Bacteroides fragilis* (ETBF) strains, harboring a pathogenicity island (BfPAI), has been associated to diarrhoeal illness in humans and animals. Non-toxigenic *Bacteroides fragilis* strains (NTBF) are subdivided in at least two major patterns: pattern II, which lacks both BfPAI and a 12-kb fragment flanking this region, and pattern III, which contains this flanking region, but lacks the island. Recently, was determined that the flanking regions of the BfPAI in ETBF 86-5443-2-2 and a related genetic element in NTBF III NCTC 9343 are putative conjugative transposons (CTns), designated CTn86 and CTn9343, respectively. CTns are the major contributors to the spread of resistance genes among *Bacteroides* species and the finding of the *bft* gene in a CTn suggests that this toxin can be transmitted from ETBF to NTBF strains by horizontal gene transfer events. In this study, we investigated the distribution of these patterns among *B. fragilis* strains isolated from 1980 to 2007 in order to verify eventual changes in enterovirulence over the years. We analyzed a total of 114 *B. fragilis* strains, 96 isolated in Brazil and 18 isolated in a hospital in France. Although any ETBF strains were detected among the strains from Brazil, 11.11% (2/18) of France strains harbored the *bft* gene (ETBF - pattern I) and 38.88% (7/18) were classified as NTBF pattern III. In Brazil, 50% (48/96) belonged to the pattern III. This study has shown that in Brazil no changes in ETBF strains patterns have occurred. Otherwise, we also demonstrate that ETBF strains are still circulating in France at rates previously documented. Based on the high percentage of NTBF III and assuming the possible horizontal transfer, further investigations are required to monitorate possible changes in the enterovirulence of this microorganism.
Financial support: MCT/CNPq, MCT/PRONEX/FAPERJ and Faperj

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTESTINAL FLORA & INTRA-ABDOMINAL INFECTIONS

THIOREDOXINS IN REDOX MAINTENANCE AND SURVIVAL DURING OXIDATIVE STRESS OF *BACTEROIDES FRAGILIS*

Reott, M.A.;* Rocha, E.R.; Parker, A.C.; Smith, C.J.
Department of Microbiology and Immunology, East Carolina University, Brody School of Medicine, Greenville, NC USA

Bacteroides fragilis is a gram-negative, obligate anaerobe native to the colon. Under normal circumstances, this symbiotic organism is beneficial, breaking down complex polysaccharides, providing otherwise unavailable nutrients to the host. However, following injury to the colon, *B. fragilis* can migrate to the intraperitoneal space, where it frequently is involved in intra-abdominal infections. The ability to colonize this space suggests the presence of a highly effective oxidative stress response (OSR) that allows *B. fragilis* to survive and persist in initially aerobic regions outside of the anaerobic gut environment. The components of *B. fragilis* OSR involve a wide array of genes associated with detoxification, protection, and metabolism that may contribute to the ability to survive the stress and persist in opportunistic infections. One such set of OSR components which could play a crucial role in survival is the thioredoxin family of proteins. Since their initial discovery, thioredoxins have been shown to be important in diverse cellular processes; including roles as a reductant for a wide array of enzymes, as a redox mediator in the cytoplasm, as a protein regulator, and in defense thioredoxin genes; *trxA, trxC, trxD, trxE, trxF, trxG*, and the thioredoxin reductase gene, *trxB*. Mutational analysis was used to establish a correlation between specific *trx* genes and protection against oxidative stress. Thus, for example, *trxD* was shown to be specifically and significantly upregulated during treatment with the thiol-specific oxidant, diamide, suggesting a role in the OSR during exposure. In the context of a functional OxyR system including catalase and AhpC/F, the thioredoxins did not provide additional protection from peroxide stress, implying that these proteins may function as part of a separate arm of the OSR. Additionally, both *trxD*, and *trxE*, were shown to rescue a *trx*-depleted *E. coli* strain, suggesting their importance in acting as electron donors for ribonucleotide reductase in aerobic conditions. We also have evidence suggesting *trxA* is the only *trx* required for growth of *B. fragilis*, and the only one expressed constitutively at high levels, regardless of the oxidative stress. These and further studies will contribute to our understanding of how this anaerobic bacterium is extraordinarily capable of tolerating oxidative stress, and the roles thioredoxins play in bacterial processes.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTESTINAL FLORA & INTRA-ABDOMINAL INFECTIONS

POLARIZED HEMOLYTIC ACTIVITY AND ALTERED PROTEIN PROFILE WITH DIFFERENTIAL IMMNO- REACTIVITY TO SERUM ANTIBODIES FROM CROHN'S DISEASE ARE INDUCED IN CO-CULTURES OF *BACTEROIDES FRAGILIS* AND A COLITIS-ASSOCIATED *BACTEROIDES VULGATUS* STRAIN

Rocha, E.R.;*[1] Smith, C.J.;[1] Sartor, R.B.[2]
[1]Department of Microbiology and Immunology, East Carolina University Brody School of Medicine, Greenville, NC USA
[2]Department of Medicine, CGIBD, University of North Carolina at Chapel Hill, NC USA

Bacteroides spp. are commensal colonic resident bacteria. However, several lines of evidence support the role of *B. vulgatus* and *B. fragilis* in inflammatory bowel diseases (IBD), which comprise both ulcerative colitis (UC) and Crohn's disease (CD). Very little is known about the pathophysiology of *Bacteroides spp*. in the disruption of the epithelial barrier which can contribute to inflammation of the intestinal tract. In this study, we show that a *B. vulgatus* strain associated with experimental colitis, but not *B. vulgatus* isolated from normal flora, was found to induce a polarized hemolytic activity in strains of *B. fragilis* cultured upon blood agar plates. Other *Bacteroides spp.* failed to demonstrate a similar synergism suggesting that this interaction is strain specific. Analysis of hemolysin (*hly*) expression was carried out by Real-Time RT-PCR using total RNA isolated from both proximal and distal growth of *B. fragilis* relative to *B. vulgatus* cross-growth. It revealed that *hlyA* mRNA was up-regulated approximately 8-fold in proximal cells of *B. fragilis* compared to the *hlyA* mRNA levels in the distal cells. In addition, comparison of whole cell protein extracts and cell-free culture supernatants obtained from *B. fragilis* and *B. vulgatus* grown either in single or mixed cultures revealed a significant change in total protein profile as determined by SDS-PAGE. Western blot analysis of crude extracts from mid-log phase *B. vulgatus* with serum from a CD patient, but not with normal human serum, revealed the presence of a protein with an estimated MW of 55 kDa. This immunoreactive protein was not expressed in *B. vulgatus* grown into stationary phase. Taken together, these findings suggest that there are specific cell interactions among *Bacteroides spp.* such as induction of Hly protein production. Moreover, these interactions lead to cellular responses that affect the expression of growth phase dependent bacterial antigens which are differentially recognized by normal human serum versus CD serum. Further studies may help us to understand the role bacterial cell communication plays in the pathophysiology of *Bacteroides spp*. in the intestinal tract.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTESTINAL FLORA & INTRA-ABDOMINAL INFECTIONS

CHARACTERIZATION OF STRICTLY ANAEROBIC STRAINS OF *ESCHERICHIA COLI* RECOVERED FROM INTRA-ABDOMINAL INFECTIONS: ABSENCE OF *hemB* GENE

Tyrrell, K.L.;* Warren, Y.A.; Citron, D.M.; Goldstein, E.J.C.
R. M. Alden Research Lab, Culver City, CA USA

Three strains of presumptive *Fusobacterium* species isolated from abdominal and pelvic specimens were identified by phenotypic methods including special potency disks (5μg vancomycin, resistant; 1 mg kanamycin, and 10 μg colistin, sensitive), indole positive, and catalase negative. Two were identified as *F. mortiferum* and one as *Fusobacterium sp*. using pre-reduced peptone-yeast sugars. However, although the sugar patterns were in agreement with *F. mortiferum*, these strains were nitrate positive and shared unusual antimicrobial susceptibility profiles for fusobacteria, such as metronidazole resistance, therefore they were further characterized.

Methods: These strains were reidentified using16S rDNA sequencing and found to match *Escherichia coli,* with a 99.9% confidence level. We then re-examined phenotypic characteristics of these three strains, as well as the aerobic counterpart strains of *E. coli* isolated from the same specimens, using tests such as API 20E and RapID ANA II. We also examined molecular characteristics of these aerobic and anaerobic strains, focusing on the genes involved in catalase regulation including *hemB, hemD, katG, oxyR, oxyS, sodA, sodB, soxR,* and *soxS*. The anaerobic strains were compared with their facultative counterparts by amplifying specific lengths of these genes with gene specific primers. The presence or absence of the target sequences was determined by gel electrophoresis.

Results: The API 20E identified the anaerobic, catalase-negative strains with high confidence as *E. coli;* however, the profiles did not match any of the profiles obtained for the aerobic strains. The RapID ANA II was not able to generate identification on any of anaerobic strains. Of the catalase regulation genes that were examined, only one gene had an anomaly. The 249bp target segment of *hemB* that was amplified was absent in all three anaerobic strains. Amplified in its place was a molecule roughly twice the size of the *hemB* target sequence, ~500bp, suggesting a mutation in this gene that is likely responsible for the loss of catalase activity.

Conclusion: Strictly anaerobic strains of catalase-negative *E. coli* can be mis-identified as the more saccharolytic members of the *Fusobacterium mortiferum-varium* group.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTESTINAL FLORA & INTRA-ABDOMINAL INFECTIONS

IMMUNOSTIMULANT CpG MOTIFS IN BIFIDOBACTERIAL DNA: DISTRIBUTION AMONG STRAINS ISOLATED FROM INFANTS

Ménard, O.; Butel, M.J.; Waligora-Dupriet, A.J.*
EA 4065, Microbiologie, Université Paris Descartes, Paris, France

Bifidobacterium is a dominant genus of infants' fecal flora and can be used as probiotics. This genus has shown preventing beneficial effects in various pathologies including allergic diseases, but their role in immunity has so far been little known. Numerous studies have shown the crucial role of the initial intestinal colonization in the development of the intestinal immune system and bifidobacteria could play a major role in this process. For a better understanding of the effect on the immune system of *Bifidobacterium*, we aimed at determining the presence of CpG motifs in *Bifidobacterium* DNA, their impact on immunity, and their conservation among *Bifidobacterium* species. We studied 27 strains of *Bifidobacterium: B. longum* NCC2705, whose genome had been entirely sequenced, and 26 strains isolated from infant's fecal flora and belonging to 6 species. Working *in silico* on *B. longum* NCC2705 genome, we identified 5 DNA fragments with a GC% between 56 and 68 and including 6 to 16 CpG motifs, i.e., 5'-pur-pur-CG-pyr-pyr-3' or 5'-pur-TCG-pyr-pyr-3' motifs. These DNA sequences were amplified and their immuno-stimulant properties were studied on mice splenocytes through cytokines production. The presence or absence of these sequences in the genome of the other strains was checked by PCR. The distribution of these sequences among other species, among strains isolated in breast-fed vs. formula fed infants, and among strains isolated from allergic vs. healthy infants is currently analysed. The number of sequences was significantly higher among *B. longum* strains than *B. breve* strains ($p=0.024$). Moreover, among *B. longum* strains, strains isolated from the fecal flora of allergic infants have a lower number of sequences in their genome than strains isolated from healthy infants ($p=0.027$). These results allow a better understanding in the capacity of *Bifidobacterium* to stimulate immunity, a capacity which was shown to be strain-dependant. Moreover, detection of CpG motifs could be criteria for selection of probiotic strains.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTESTINAL FLORA & INTRA-ABDOMINAL INFECTIONS

REIDENTIFICATION OF INTRA-ABDOMINAL ISOLATES OF "*BACTEROIDES PUTREDINIS*-LIKE" ORGANISMS AS OTHER *ALISTIPES* SPECIES: PIGMENT PRODUCTION IS NOT A PRACTICAL TEST

Warren, Y.A.;* Citron, D.M.; Goldstein E.J.C.
R.M. Alden Research Lab, Culver City, CA USA

Background: *Alistipes* is a newly described genus of strictly anaerobic gram-negative rods that are weakly saccharolytic, bile-resistant, indole-positive, and with the exception of *A. putredinis*, produce brown pigment in four days and appear black in 10 days under UV light on laked rabbit blood agar. Currently, the genus includes four species: *A. finegoldii, A. putredinis, A. shahii*, and *A. onderdonkii*. Using standard phenotypic tests including resistance to kanamycin, colistin, and vancomycin special-potency discs, weak carbohydrate fermentation, positive test for indole, growth on BBE, and preformed enzyme kits, we had identified eight organisms in our culture collection as *B. putredinis* and six others that were phenotypically similar but were identified as *Bacteroides* sp. None of the strains produced pigment on routine media, even after prolonged incubation. We further characterized them using 16S rRNA gene sequencing, which placed them in *Alistipes,* but species other than *A. putredinis*.

Methods: Since pigment was not demonstrated for any of these isolates on routine agar media, we sought other media that might show pigment production. Using a standard streaking procedure, each isolate was inoculated on to different anaerobic plates, including Brucella sheep blood agar (BBA), laked BBA, laked rabbit BBA, BBA with phenyl-ethyl alcohol, CDC BA, laked BBA with KV, freshly made Columbia agar with laked blood, and Brucella agar with laked rabbit blood—all supplemented with vitamin K1 and hemin. The plates were incubated at 37ºC in the anaerobic chamber for seven days and observed daily for pigment. On the seventh day, they were removed from the chamber to be checked for a black appearance under UV light.

Results: All strains produced recognizable light brown pigment and appeared black under UV light, but only after seven days of incubation and only on freshly made Brucella agar with laked rabbit blood.

Conclusion: Since pigment production cannot be demonstrated on readily available media, such as LKV or Brucella blood agar, and requires prolonged incubation on freshly prepared plates with laked rabbit blood, pigment-production is not a useful characteristic for initial recognition and identification for *Alistipes* spp. None of the strains in our collection were *A. putredinis*.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTESTINAL FLORA & INTRA-ABDOMINAL INFECTIONS

INTERFERENCE OF THE TRANSCRIPTIONAL REGULATOR OxyR AND CATALASE IN *BACTEROIDES FRAGILIS* SURVIVAL WITHIN PERITONEAL MACROPHAGES

Ferreira, L.Q.;*[1] Dias, M.F.;[1] Carvalho, J.B.;[1] Peixoto, R.J.M.;[1] Vommaro, R.C.;[2] Santos-Filho, J.;[1] Américo, A.A.;[1] Fracallanzza, S.E.L.;[1] Rocha, E.R.;[3] Domingues, R.M.C.P.[1]

[1]Instituto de Microbiologia Professor Paulo de Góes, UFRJ, Rio de Janeiro, RJ Brasil

[2]Instituto de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro, RJ Brasil

[3]Department of Microbiology and Immunology, East Carolina University Brody School of Medicine, Greenville, NC USA

Bacteroides fragilis is an obligate anaerobic Gram negative bacterium which is able to survive in the presence of O_2 for approximately 48h without significant loss of viability. To survive an oxidative stress, *B. fragilis* must defend itself against the damage caused by reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide, and hydroxyl radicals. ROS activates a complex mechanism of transcription regulation and the synthesis of 28 proteins, among them catalase (*kat*B), superoxide dismutase (*sod*), alkyl hydroperoxide reductase (*ahp*CF), and a DNA-binding protein (*dps*). These genes are coordinately regulated at the transcriptional level by OxyR. In the presence of H_2O_2, the OxyR activation occurs by conformational changes that alter the interactions with DNA, and therefore with RNA pol, leading to transcriptional activation of antioxidant genes. Thus, the aim of this study was to evaluate if OxyR and catalase affects, somehow, *B. fragilis* interaction with peritoneal macrophages. To analyze the behavior of *B. fragilis* mutant strains IB298 (Δ*oxy*R), IB260 (Δ*kat*B) and the parent strain 638R, they were all grown under aerobic atmosphere by shaking the cultures. Aliquots were taken at different time points for cell viable counts. Parental and *kat*B mutant strains did not show a decrease in cell viability after been exposed to O_2 for 24h. In contrast, the *oxy*R mutant lost 50% in cell viable counts. To evaluate the survival of these strains within peritoneal macrophages, a bacteria:macrophages interaction assay was performed. The strain IB298 showed a greater susceptibility to macrophages killing (34.6%). In contrast, the strain IB260 and 638R seemed to be less sensitive (19.7% and 9.3%, respectively). Taken together, these data reinforced that OxyR and catalase play a role in *B. fragilis* survival in environments with high levels of reactive oxygen species. Recently, it was revealed that under *in vivo* conditions OxyR regulon can interfere with abscesses induction capacity of this species. Peritoneal macrophages are important cells in the first line of host defense and these cells had also been implicated with the abscess formation. Nevertheless, the oxidative burst is considered critical for the bacterial action of phagocytes. So, the interference of OxyR with *B. fragilis* virulence may be also related with the survival within these cells.

Financial Supports: MCT/CNPq, MCT/PRONEX/FAPERJ and FAPERJ

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTESTINAL FLORA & INTRA-ABDOMINAL INFECTIONS

CHARACTERIZATION OF *FUSOBACTERIA NUCLEATUM* ISOLATED FROM HUMAN INTESTINAL BIOPSIES – ARE STRAINS FROM INFLAMMATORY BOWEL DISEASE PATIENTS MORE VIRULENT?

Strauss, J.C.;*[1] Sibley, C.;[2] Kaplan, G.G.;[2] Rioux, K.P.;[2] Panaccione, R.;[2] Beck, P.L.;[2] Lynch, T.;[2] Surette, M.G.;[2] Allen-Vercoe, E.[1]
[1]University of Guelph, Guelph, Ontario, Canada
[2]University of Calgary, Calgary, Alberta, Canada

Inflammatory Bowel Disease (IBD) is an umbrella term used to describe a group of chronic, relapsing/remitting disorders of the gastrointestinal tract. While the precise aetiology of IBD is unknown, dysbiosis, an imbalance between beneficial and harmful bacterial species in the gut, is a leading hypothesis for the role of bacteria in IBD. Thus the search for potentially pathogenic microbial residents of the GI tract is a current research focus.

The Gram-negative human gut resident *Fusobacterium varium* has been previously demonstrated to play a role in the exacerbation of ulcerative colitis, a type of IBD. We were interested to determine whether the related species, *F. nucleatum*, which is a known invasive and pro-inflammatory pathogen in the human mouth and is thought to be an occasional resident of the human gut, might also be associated with IBD pathogenesis.

Using selective media under strict anaerobic conditions, we isolated *F. nucleatum* from human intestinal biopsy samples taken from IBD patients or control healthy patients undergoing colon cancer screening, and characterized them phenotypically, using a variety of biochemical and morphological markers. Invasion of cultured Caco-2 cells by our *F. nucleatum* intestinal isolates was characterized with immunofluorescence microscopy. Additionally, we used a high-throughput screening assay to measure isolate production of autoinducer-2 (AI-2), a molecule which has been proposed to be a universal signal for interspecies communication and has been shown to control gene expression for numerous virulence factors in a number of important enteric pathogens. We found that *F. nucleatum* could be isolated from IBD patients at a significantly greater frequency than from control patients, and that IBD-associated strains were often more invasive than isolates retrieved from healthy persons. AI-2 production was highly variable among the different *F. nucleatum* isolates, however more invasive strains tended to produce greater amounts of this key auto-inducer molecule. This correlation between invasiveness and AI-2 production is intriguing and studies are underway to further elucidate the relationship between AI-2 production and virulence of the *F. nucleatum* isolates. In summary, our data indicate that *F. nucleatum* could potentially play a key role as a pathogenic factor in cases of IBD.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTESTINAL FLORA & INTRA-ABDOMINAL INFECTIONS

BACTEROIDES FRAGILIS AND OTHER *BACTEROIDES* SPECIES DEMONSTRATE MARKED AEROTOLERANCE

Vangelakos, C.G.;* Rhee, K-J.; Wu, S.; Gan, C.; Sears, C.L.
Johns Hopkins University School of Medicine, Baltimore, MD USA

Although *Bacteroides* are considered obligate anaerobes, *B. fragilis* and other *Bacteroides* species are known to be aerotolerant, such that nanomolar levels of dissolved O_2 have been shown to promote *B. fragilis* growth. Similarly, the temporary and nonreplicative characteristics of oxygen survival reported for *B. fragilis* experimentally contrast with the clinical observations that *B. fragilis* are recovered in both aerobic and anaerobic blood culture bottles; recovery in blood cultures signifies *B. fragilis* replication under aerobic conditions, and is consistent with the enhanced morbidity and mortality noted in human disease when *Bacteroides* are recovered in bloodstream infections. Thus, we hypothesized that *B. fragilis* and other *Bacteroides* species possess the capacity to replicate under aerobic conditions. To test this hypothesis, *B. fragilis* (N=4 strains) and other *Bacteroides* (N= 6 strains) were grown under varying O_2 conditions. As expected, when anaerobic BHI broth was inoculated with a single *Bacteroides* colony or subcultured from an anaerobically grown BHI broth culture, all strains showed robust growth (OD > 0.5). However, consistent with our hypothesis, subculture of a BHI broth culture grown anaerobically to a BHI broth culture maintained only in aerobic conditions also yielded robust growth in 8 of 9 trials. Further, inoculation of a single colony from a BHI agar plate grown anaerobically to aerobic BHI broth also yielded growth at 24 hrs, albeit less consistently. The aerotolerance of *B. fragilis* strains was confirmed by serial aerobic stationary subcultures for up to 3 generations. In contrast, no *Bacteroides* strain grew aerobically on agar plates. The identity of aerobically grown *Bacteroides* strains was confirmed by *Bacteroides*-specific PCR. Cell-free culture supernatants of enterotoxigenic *B. fragilis* (ETBF) strains grown under anaerobic or aerobic conditions to similar OD readings and tested for biologic toxin activity on HT29/C1 cells demonstrated that expression of the *B. fragilis* toxin (BFT) was similar whether the ETBF strain was grown anaerobically or aerobically. Our data indicates that *Bacteroides* species and, in particular, *B. fragilis* can replicate aerobically and, in the case of ETBF, without modification of expression of a virulence gene, suggesting that the classification of these bacteria as strict anaerobes may require revision.