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Renaissance Hotel • Long Beach, California USA
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SESSION XV—METHODS & MICROBIOLOGY

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METHODS & MICROBIOLOGY

DIFFERENT MOLECULAR APPROACHES FOR IDENTIFICATION OF ANAEROBES IN ENDODONTIC INFECTIONS

Gomes, B.P.F.A.;*[1] Vianna, M.E.;[1,2] Jacinto, R.C.;[1] Berber, V.B.; Shah, H.N.;[3] Conrads, G.[2]
[1]Endodontic Area, Department of Restorative Dentistry, Piracicaba Dental School, State University of Campinas, Piracicaba, SP Brazil
[2]Division of Oral Microbiology and Immunology, Department of Operative and Preventive Dentistry & Periodontology, and Department of Medical Microbiology, RWTH University Hospital Aachen, Germany
[3]NCTC – Molecular Identification Service Unit, Health Protection Agency, Centre for Infections, London, UK

Microorganisms isolated from anaerobic infections have been traditionally identified by culture. Recently, molecular methods have become available, which have helped clinical management and improved our understanding of primary endodontic infections. The use of several techniques—such as conventional PCR, Real-time PCR, microarrays, clonal analysis, and 16S ribosomal RNA (rRNA) gene sequencing—have emerged as valuable tools for bacterial identification.

Purpose: Our aim is to present a range of molecular approaches that have been used to increase the detection of anaerobes in primary endodontic infection; to discuss the importance of the transport medium and also of the period and conditions of storage in the molecular analysis.

Material and methods: Microbial samples were collected from 20 root canals with necrotic pulp tissues and periapical lesions. Microorganisms were identified by culture and also by a combination of conventional PCR, microarrays, clonal analysis and 16S ribosomal RNA (rRNA) gene sequencing.

Results: The employment of molecular approaches such as 16S rRNA and microarrays to identify microbial species in endodontic samples that had previously been cultured increased the detection of anaerobes from 43.3% (culture = biochemical tests + gene sequencing) to 67.8% (culture + microarrays), and from 74.07% (culture) to 81.16% (culture + PCR + clonal analysis). Conventional PCR and clonal analysis allowed the identification of species that could not be isolated such as *T. forsythia, T. denticola, F. alocis, S. satelles, D. pneumosintes* and *O. profusa*.

Conclusion: Our findings, using a combination of anaerobic culture and molecular identification approaches, confirmed the polymicrobial nature of primary endodontic infections with a predominance of anaerobic bacteria, and showed that molecular techniques provide significant additional information particularly regarding the anaerobic component of the microbial community. Supported by FAPESP (05/53729-1, 06/60500-3, 07/58518-4), CNPq (305437/2006-2, 470820/2006-3) and CAPES (PRODOC).

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METHODS & MICROBIOLOGY

MACRO AND MICRO ARRAY ANALYSIS OF SUBGINGIVAL AND VAGINAL INFECTION IN PREGNANCY

Tanner, A.C.R.*
Harvard University and The Forsyth Institute, Boston, MA USA

Aim: The primary aim was to examine a link between periodontal micro-organisms in subgingival vaginal and cervical samples and low birth weight (LBW) and preterm (PLBW) in infants using DNA probes. Secondary aims evaluated associations between bacteria with gynecological infections linked with prematurity, also compared data from macro and micro array DNA probe formats.

Methods: Periodontal measurements and subgingival, vaginal, and cervical samples were obtained at 15-23 weeks of pregnancy. Samples were assayed using whole genomic probes in a macro array. Vaginal samples were assayed by PCR to *Porphyromonas gingivalis* (*Pg*), and to 350 16S rDNA probes to subgingival species in a micro array (HOMMIM).

Results: The population sampled comprised 231 pregnant women that were predominantly Hispanic (82%), with mean age 26 (18–43) years. Maternal infections were 38% periodontitis: 17% β *Streptococcus* (βstrep) infection, 17% urinary tract infection (UTI), and 11% chorioamnionitis. Infants were 9% LBW, 7% PLBW. No significant associations between periodontitis or subgingival species and prematurity or other infections were observed. By macro array, species in subgingival samples associated with gingivitis included *Eubacterium nodatum, Campylobacter rectus, P. gingivalis, Tannerella forsythia*, and *Treponema denticola*. Subgingival samples harbored at most low levels of cervical/vaginal species.

Cervical and vaginal microbiotas were similar to each other. High levels of *Lactobacillus* and/or *Gardnerella* species, but low levels of subgingival species were detected. *F. nucleatum* subsp. *nucleatum* from vaginal samples was associated with LBW or PLBW. *Pg* by PCR was detected in vaginal and cervical samples, but detection was not associated with LBW/PLBW. Urinary tract infection and chorioamnionitis, but not β strep infections, were associated with LBW. Additional species detected in vaginal or cervical samples were associated with chorioamnionitis, β strep infection, and urinary tract infections. For selected vaginal samples, microarray data using 16S rDNA probes for subgingival species was consistent with macroarray analysis, but the microarray had a higher threshold of species detection.

Conclusions: The DNA probe assay was valuable to screen for multiple species in over 200 subjects. The macro array confirmed the patterns of species detection using the micro array. Vaginal or cervical infection may be more important rather than subgingival infection in prematurity.
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METHODS & MICROBIOLOGY

NEW METHODS FOR SELECTIVE ISOLATION OF BACTERIAL DNA FROM HUMAN CLINICAL SPECIMENS

Horz, H.P.;[1] Scheer, S;[1] Vianna, M.E.;[1,2] Conrads, G*[1]
[1]Division of Oral Microbiology and Immunology, Department of Operative and Preventive Dentistry & Periodontology, and Department of Medical Microbiology, RWTH University Hospital Aachen, Germany
[2]Endodontic Area, Department of Restorative Dentistry, Piracicaba Dental School, State University of Campinas, Piracicaba, SP Brazil

Separation of bacterial DNA from human DNA in clinical samples may have an important impact on downstream applications, involving microbial diagnostic systems.

Purpose: We evaluated two commercially available reagents (MolYsis®, Molzym GmbH & Co. KG, Bremen, Germany) and Pureprove®, SIRS-Lab GmbH, Jena, Germany) for their potential to isolate and purify bacterial DNA from human DNA. We chose oral samples, which usually contain very high amounts of both human and bacterial cells. Three different DNA preparations each were made from eight caries- and eight periodontal specimens using the two reagents above and a conventional DNA extraction strategy as reference. Based on target-specific RT-PCR assays, we compared the reduction of human DNA versus loss of bacterial DNA. Human DNA was monitored by targeting the β-2-microglobulin gene, while bacteria were monitored by targeting 16S rDNA (total bacteria and *Porphyromonas gingivalis*) or the glycosyltransferase gene (*Streptococcus mutans*).

Results: We found that in most cases at least 90% of human DNA could successfully be removed, with complete removal in eight of 16 cases using MolYsis, and two (of 16) cases using Pureprove. Conversely, detection of bacterial DNA was possible in all cases, with a recovery rate generally ranging from 35% to 50%.

Conclusion: Both strategies have the potential to reduce background interference from the host DNA, which may be of remarkable value for nucleic-acid based microbial diagnostic systems in both aerobic and (mixed-) anaerobic infections.

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THE INTERACTION OF *BACTEROIDES FRAGILIS* AND THE COMPONENTS OF THE HUMAN FIBRINOLYTIC SYSTEM

Ferreira, E.O.;*[1] Carvalho, J.B.;[1] Peixoto, R.J.M.;[1] Dias, B.S.;[1] Lobo, L.A.;[2] Zingalli, R.B.;[3] Smith, J.;[2] Rocha, E.R.;[2] Domingues, R.M.C.P.[1]

[1]Instituto Prof. Paulo de Góes, UFRJ, RJ Brasil

[2]Department of Microbiology and Immunology, East Carolina State University, Greenville, NC USA

[3]Departamento de Bioquímica Médica, UFRJ, RJ Brasil

Bacteroides fragilis is a Gram-negative strictly anaerobic bacterium that represents about 1% of the total *Bacteroides* found in the human large intestinal tract. Although, it is a minority component of the intestinal microbiota, *B. fragilis* is one of the most frequently isolated bacteria from clinical infection, especially from patients suffering of intraabdominal infections and bacteremia. Previously, our group has described the capability of two *B. fragilis* strains, MC2 and 1081, to adhere strongly to Laminin-1 (LMN-1). It was also showed that bacterial molecules involved in such recognition would be possibly presented in the outer membrane proteins OMP extracts. After passing the OMPs of the MC2 strain through an affinity LMN-1 column, the most purified fraction was sent to a Proteomics Core Facility and showed to have 98% of similarity to a putative plasminogen (Plg) binding protein precursor described in *B. fragilis*, Bfp60. Plg is a glycoprotein that is converted to the active serine protease plasmin in the fibrinolytic system. Indiscriminate activation of plasmin can contribute to significant tissue damage, and for that reason, the recruitment and activation of Plg must be well controlled. The bacterial receptors which recognize Plg, bind it and enhance its activation by tissue type Plg activator on the bacterial surface, turning a non-proteolytic bacterium into a proteolytic causing degradation of other substrates, such as the ECM components. The focus of this work was to isolate, clone, express, and characterize this adhesin presented in the OMP extract of a *B. fragilis* strain, by performing adhesion and inhibition assays. The capability of the MC2 and the purified protein convert Plg into plasmin was also tested using an indirect assay. For the following study, a PCR was performed using primers designed with enzyme restriction sites to amplify the gene in the MC2 strain. The gene fragments obtained were cloned in frame with the His-tag sequence presented in the expression vector pET26b+ to produce a recombinant fusion protein, Adhesin-His-tag (Ads-6xHis). Our results showed that the MC2 strain could adhere to LMN-1 and Plg, and this adhesion could be inhibited by these proteins. The Ads-6xHis could also block the adhesion to each protein significantly. Concerning the indirect assay, either the strain or the purified protein could convert Plg into plasmin as strong as the positive control used in the assay, the streptokinase. In conclusion, we present *in vitro* evidence for a pathogenic function of plasminogen receptor in promoting adherence to LMN and also the formation of plasmin in *B. fragilis*.

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DETAILED PHENOTYPIC ANALYSIS OF IMPORTANT ANAEROBES

Lei, X.-H.; Bochner, B.R.*
Biolog, Inc., Hayward, CA, USA

The purpose of this project was to extend the application of Phenotype MicroArray (PM) technology to anaerobes by developing a universal protocol for testing anaerobes of importance in human health. PM technology allows a biologist to test nearly 2,000 phenotypes of diverse bacterial species and gain a comprehensive overview of pathway functions. Different species can be compared, as well as different strains, or isogenic strains with gene knockout mutations. The phenotypic assays are designed from a physiological perspective to survey the function of diverse pathways including both metabolic and regulatory pathways. Included in the phenotypes are basic cellular nutritional pathways for C, N, P, and S metabolism (800 tests), pH growth range and regulation of pH control (100 tests), sensitivity to various salts and ions (100 tests), and sensitivity to 240 chemical agents that disrupt various biological pathways (1,000 tests). The information about the cellular physiology and the presence and activities of many pathways provides insight into the environmental niche in which each species competes most successfully and can be integrated with genome sequence information and annotation. Initially, we have focused on 3 important species that can colonize the human intestine: *Clostridium difficile*, a spore forming pathogen; *Bacteroides fragilis*, a predominant commensal that is also a common pathogen; and *Lactobacillus acidophilus*, a probiotic added to foods. These species were selected because of their diverse physiology and oxygen tolerance, as well as their importance in human health. All handling and setup of PM panels was performed in an anaerobic chamber. The bacteria were cultured overnight on Biolog Universal Anaerobe Agar, cell suspensions were prepared in anaerobic inoculating fluid, and then the PM panels were inoculated with 100 µl per well. Each panel was then sealed inside of a transparent gas-proof plastic bag, removed from anaerobic incubation, and loaded into the OmniLog. This instrument reads the phenotypic reactions in up to 5,000 wells every 15 minutes, providing quantitative kinetic assay results for each well. The data from the OmniLog is downloaded at the end of each run and can be compared to runs with other bacteria. We will compare and contrast phenotypes of these 3 bacteria that we have determined using PM analysis.

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EVALUATION OF CRAYFISH CHARCOAL MEDIUM AND THREE TRANSPORT MEDIA FOR PRESERVATION OF ANAEROBIC BACTERIA

Egwari, L.O.;* Akinyoyenu, Y.A.; Nwokoye, N.N.
Department of Botany and Microbiology, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria

Crayfish charcoal medium (formulated) and three other media were assessed as storage media for four genera of anaerobic bacteria (*Porphyromonas, Prevotella, Peptostreptococcus* and *Clostridium*). The anaerobes were not maintained at 6± 2° C for upward of 7 days in all four media. *Porphyromonas gingivalis* survived the first week of storage both at -20° C and room temperature (30 ± 2° C) only in cooked meat medium. Thioglycollate broth maintained *C. perfringens* and *Peptostreptococcus* spp at -20° C for 2 and 4 weeks respectively. *Prevotella bivia, C. perfringens*, and *Peptostreptococcus* spp were maintained at -20° C in Amies, crayfish charcoal medium and cooked meat medium for 3-6 weeks. The degree of survival of the anaerobes at -20° C in the transport media at three weeks of storage showed no significant difference between Amies and cooked meat medium ($p > 0.01$) with a significant difference in preservation between Amies and crayfish charcoal medium ($p < 0.001$). These media can be used for short term storage of anaerobes at room temperature or at -20° C where long term preservation facilities are not available.

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IDENTIFICATION OF *DIALISTER* AND *LEPTOTRICHIA* SPECIES BY PCR-RFLP

Hall, V.*
Anaerobe Reference Laboratory, Cardiff, UK

Members of the genera *Dialister* and *Leptotrichia* isolated from clinical sources are among the diverse range of anaerobic Gram-negative rods referred to the Anaerobe Reference Laboratory for confirmation of identity. In both genera, several novel species have been described in recent years and identification methods must be adapted to encompass taxonomic changes. PCR-RFLP using endonucleases *Hpa*II and *Taq*I to cleave the 16S rDNA gene has proved to be highly discriminatory and practical for identification of *Bacteroides* spp. and some other anaerobic Gram-negative rods.

PCR-RFLP (Stubbs *et al*, J Clin Microbiol 38:3209-13) was applied to isolates presumptively identified as *Dialister* sp. (n=54) or *Leptotrichia* sp. (n=28). GelComparII software was used to compare resulting banding patterns with those of other anaerobic Gram-negative rods in a library constructed in-house. Identities of representative isolates were confirmed by partial (~450bp) 16S rDNA sequence analysis.

Isolates of *Dialister pneumosintes* (n=35), *D. micraerophilus* (16), *D. invisus* (2) and *D. propionifaciens* (1) each yielded distinct banding patterns in PCR-RFLP. *D. pneumosintes* isolates were from blood (22), pleural fluid (4) abscess of the head or neck (7) and unspecified abscesses (2). *D. micraerophilus* isolates were from blood (4), breast abscess (6), perianal, pilonidal, pelvic, brain abscess (1 of each) and unspecified wounds (2).

Isolates of *Leptotrichia trevisanii* (n=18), *L. buccalis* (2), *L. goodfellowii* (2), *L. wadei* (1) and unnamed *Leptotrichia* species (5) each yielded distinct PCR-RFLP banding patterns. The majority of these isolates were from blood cultures.

The PCR-RFLP method developed for identification of *Bacteroides* spp. is also practical for the identification of clinical isolates of *Dialister* and *Leptotrichia* spp. Though numbers of isolates are small, these data suggest differing sites of colonization and/or infection for *D. pneumosintes* and *D. micraerophilus*. The recently described *L. trevisannii* was isolated more frequently than the Type species *L. buccalis*.

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RECLASSIFICATION OF *CLOSTRIDIUM COCCOIDES, RUMINOCOCCUS HANSENII, RUMINOCOCCUS HYDROGENOTROPHICUS, RUMINOCOCCUS LUTI, RUMINOCOCCUS PRODUCTUS* AND *RUMINOCOCCUS SCHINKII* AND DESCRIPTION OF *BLAUTIA WEXLERAE SP. NOV.*, ISOLATED FROM HUMAN FECES

Liu, C.;*[1] Lawson, P.A.;[2] Song, Y.;[1] Finegold, S.M.[1]
[1]Research Service, VA Medical Center West Los Angeles, CA USA
[2]Department of Botany & Microbiology, University of Oklahoma, Norman, OK USA

Background: Phenotypic and phylogenetic studies were performed on 15 isolates of an unidentified Gram-positive, anaerobic, non-sporulating coccobacillus-shaped bacterium isolated from human feces. The organisms were catalase-negative, indole-negative, and produced acetate and succinate as end-products of metabolism. Comparative 16S rRNA gene sequencing demonstrated that the 15 isolates were highly related to each other and form a hitherto unknown sub-line within the clostridial rRNA cluster XIVa of organisms. The organism formed a robust phylogenetic group with a number of organisms that included *Clostridium coccoides, Ruminococcus luti, Ruminococcus obeum*, and a number of other misclassified ruminococci.

Methods: In the present study, 15 isolates isolated from human fecal specimens were characterized, using phenotypic and molecular taxonomic methods.

Results: The isolates were strictly anaerobic, gram-positive, non-sporing, 1.0-1.5 x 1-3 μm coccobacillary shaped. All of the isolates were negative for lecithinase, lipase, catalase, and indole, but positive for urease. They were capable of hydrolysing esculin, but not gelatin. The utilization of amygdalin, cellobiose, fructose, lactose, maltose, mannitol, melezitose, melibiose, raffinose, rhamnose, salicin, sorbitol, sucrose, and trehalose was variable. Using the API ZYM and rapid ID 32A systems, all isolates of the same group produced a similar profile. Positive reactions were obtained for α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase and α-fucosidase by both systems. With the rapid ID 32A system, α-arabinosidase was positive, and acid phosphatase was detected by the API ZYM system. Urease results were variable tested by the rapid ID 32A system and esterase (C4), esterase lipase (C8), naphthol-AS-Bl-phosphohydrolase were variable tested by the API ZYM system. All the other tests were negative. The isolates were sensitive to vancomycin (5 μg) and kanamycin (1000 μg), but resistant to colistin (10 μg) identification disks.

Conclusion: On the basis of these studies, we propose a novel genus, *Blautia* gen. nov., is proposed and that, *Clostridium coccoides, Ruminococcus hansenii, Ruminococcus hydrogenotrophicus, Ruminococcus luti, Ruminococcus productus*, and *Ruminococcus schinkii* be transferred to this genus as *Blautia coccoides* comb. nov., *Blautia hansenii* comb. nov., *Blautia hydrogenotrophicus* comb. nov., *Blautia luti* comb. nov., *Blautia productus* comb. nov. and *Blautia schinkii* comb. nov. The hitherto unknown coccus-shaped bacterium WAL 14507^T (ATCC BAA-1564^T and DSM 19850) is proposed as the type strain of a new species *Bautia wexlera* sp.nov.

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DIFFERENTIATION OF THE MEMBERS OF *STREPTOCOCCUS ANGINOSUS* GROUP

Molitoris, D.;*[1] Wooton, J.;[1] Rowlinson, M-C.;[1] Summanen, P.H.;[1] Finegold, S.M.[2,3,4]
[1]Research, and [2]Infectious Diseases Section, VAMCWLA, Los Angeles, CA USA
[3]Dept of Medicine and [4]Dept of Microbiology, Immunology and Molecular Genetics, UCLA School of Medicine, Los Angeles, CA USA

The genus *Streptococcus* comprises gram-positive cocci that are facultative anaerobes, many species of which are common residents of the skin and mouth. They are frequently isolated from a myriad of wound infections, mostly in mixed bacterial infections. In a study of more than 400 wound infections, we found that species from the *Streptococcus anginosus* group (*S. anginosus, S. intermedius* and *S. constellatus*) were the most predominant of this genus; they were isolated from 59 specimens.

Identification of the *S. anginosus* group to the species level by conventional methods is a challenge, and they are often reported out in the clinical microbiology laboratory as "viridans streptococci." We analyzed isolates from wound infections using 16S rRNA sequence analysis but found that this method was also unable to accurately speciate within this closely-related phylogenetic group. Similarly, the three species could not be separated with the API 20 Strep identification kit. The *tuf* gene has been used for differentiation within this group with some success, and we found that it was more discriminatory than the 16S rRNA gene. Nine strains whose identities within the *S. anginosus* group were not distinguishable using the 16S gene resulted in two to three percentage point difference in sequence similarity between species when tested using the tuf gene.

We analyzed which *S. anginosus* group species were more commonly present in the wound infections and determined whether this prevalence was related to specimen type.

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METHODS & MICROBIOLOGY

USE OF MATRIX-ASSISTED LASER DESORPTION IONIZATION- TIME OF FLIGHT MASS SPECTROMETRY FOR SPECIES IDENTIFICATION OF *BACTEROIDES* SPECIES

Nagy, E.;*[1] Kostrzewa, M.;[2] Maier, T.;[2] Urbán, E.;[1] Terhes, G.[1]
[1]Institute of Clinical Microbiology University of Szeged, Hungary
[2]Bruker Daltonik GmbH, Leipzig, Germany

Bacteroides fragilis and related species are important commensalists in the lower intestinal tract of mammals and are also important opportunistic anaerobic pathogens causing sever infections including intra-abdominal, pelvic, lung and brain abscesses, peritonitis, and sepsis. Correct identification is necessary as resistance to different anti-anaerobic drugs may differ according to the species. Several taxonomic changes have occurred in this group of anaerobic bacteria during the past years, and new species were found and accepted to belong the genus. The phenotypic identification of members of genus *Bacteroides* similarly to other anaerobic bacteria is difficult not only because many anaerobes are rather inactive in biochemical tests, but also because the slow growth of bacteria is influencing their identification, especially by commercially available identification kits.

Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry has been shown to be a useful and simple method for rapid identification microorganisms associated with infectious diseases and also to discriminate among different subtypes of pathogens. In this study, our aim was to set up a database for the most frequently isolated anaerobic bacterial species belonging to the genus *Bacteroides*. 222 clinical isolates identified in routine laboratories as different species of the genus *Bacteroides*, collected from different European countries (Sweden, France, Croatia, Belgium, Germany and Hungary), were identified by MALDI-TOF MS and the dedicated BioTyper software using ATCC reference strains as references. The phenotypic identification was carried out by classical biochemical tests and by rapid ID 32 A (ATB) and API20 ANA (BioMerieux). *B. fragilis* could be clearly identified with both methods whereas subtype differentiation were detected by mass spectrometry. The discriminatory power and identification accuracy of MALDI-TOF was superior to biochemical tests in the case of *B. theaiotaomicron, B. ovatus* and *B. uniformis*. Newly accepted species as *B. salyersae,* and *B. nordii* could be identified by the MALDI-TOF.

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CHARACTERIZATION OF A FIBRONECTIN-BINDING MOLECULE IN *BACTEROIDES FRAGILIS*

Pauer, H.;[1] Ferreira, E.O.;*[1] Portela, M.B.;[1] Guimarães, P.R.;[2] Zingalli, R.;[2] Soares, R.M.A.;[1] Domingues, R.M.C.P.[1]
[1]Instituto de Microbiologia Professor Paulo de Góes, UFRJ, Rio de Janeiro, RJ Brasil
[2]Instituto de Bioquímica, UFRJ, Rio de Janeiro, RJ Brasil

Adherence of pathogenic microorganisms to host cells and molecules is considered to be the first step in establishing infection in the host, and it is an important virulence factor. The bacterial adherence involves surface components, called adhesins, that recognize and bind to cell surface molecules and host extracellular matrix (ECM). *Bacteroides fragilis* is the Gram-negative strictly anaerobic bacterium most frequently isolated from infectious processes, especially from patients suffering from intraabdominal infections and bacteremia. These bacteria have a lot of adhesins able to bind to ECM components. In this study, strains isolated from bacteremia were cultivated in a synthetic media which variations in the cysteine concentrations determine alterations in the oxidation-reduction potential (Eh). All the assayed strains were capable of adhering to fibronectin when cultivated in both conditions (oxidized and reduced). The 1405 strain was the one that showed the largest distinction in the adherence capacity to fibronectin when cultivated in the oxidizing condition, comparing to the reducing condition. Considering this results, additional tests were made with this strain in order to characterize the possible structure involved in this mechanism. Chemical treatments suggested the involvement of a proteic molecule in the interactions between *B. fragilis* and fibronectin. Analyses of the outer membrane proteins (OMPs) expressions through SDS-PAGE demonstrated differences among extracts obtained from cultures in this two conditions; it was detected bands with stronger expressions in the oxidizing than in reducing conditions. The Dot blotting analyses showed a better recognition of the fibronectin with OMPs, when they were obtained from cultures with higher Eh. Western blotting assays revealed two band of approximately 102 and 30 kDa. The band with ca. 102 kDa was sequenced and defined as a putative TonB-dependent OMP. Our results suggest that the expression of *B. fragilis* surface components able to recognize fibronectin can be associated with Eh variations.

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METHODS & MICROBIOLOGY

THE ROLE OF PUTATIVE *BACTEROIDES FRAGILIS* 638R RecQ HOMOLOGUES IN DNA REPAIR AND GENOME STABILITY

Paul, L.V.;* Abratt, V.R.
Molecular and Cell Biology, University of Cape Town, Cape Town, South Africa

The RecQ protein is an ATP-dependent DNA helicase belonging to the Superfamily 2 of helicases. Eukaryote organisms code for multiple RecQ homologues, whereas prokaryotes generally code for a single RecQ protein. *B. fragilis* 638R is, however, of interest because it encodes three putative RecQ helicases, BF638R3282, BF638R3781 and BF638R3932. The various functions of these proteins in *B. fragilis* 638R were explored in this study. RecQ helicases are highly conserved from prokaryotes to eukaryotes. Bio-informatic analysis of the putative *B. fragilis* RecQ proteins identified the presence of the 3 conserved domains of this group, namely the helicase, the recQCt, and the HRDC domains in BF638R3282 and BF638R3932, but showed that BF638R3781 lacked an HRDC domain. Mutations in RecQ proteins reportedly cause increased sensitivity to DNA damage and increased genome instability through an increase in the rate of secondary recombination events and mutations, as well as an increase in illegitimate recombination between non-homologous sequences. In this study, insertional inactivation of the individual *B. fragilis* 638R *recQ* genes was done, and shown to cause increased sensitivity of *B. fragilis* 638R to metronidazole-induced DNA strand break damage, as well as to mitomycin C-induced DNA adducts. The levels of sensitivity varied between the respective mutants. This indicates that the 3 RecQ proteins are involved in repairing different types of DNA damage, and that they may contribute differentially to the levels of strand break repair. The presence of secondary recombination events in the RecQ mutants was also demonstrated, indicating increased genome instability or mutational potential in the absence of certain RecQ proteins. In addition, cellular elongation was observed in two of the mutants (BF638R3781 and BF638R3932), indicating impaired cellular division and/or DNA replication in recQ mutants. The results of this study indicate that the roles of *B. fragilis* RecQ helicases are similar to those described in other bacteria and eukaryotic organisms.

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METHODS & MICROBIOLOGY

THE ROLE OF BACTERIAL MATRIX IN NITRO-CELLULOSE TRANSFORMATION

Petrova, O.E.;* Tarasova, N.B.
Kazan Institute of Biochemistry and Biophysics, Russian Academy of Science, Kazan, Russia

Bacterial adsorption on solid matter is the important property for microbe survival in the environment. It alleviates the concentration of microorganisms and their communication and allows the formation of such complex structures as biofilm or matrix. Matrix formation by sulfate reducing bacteria *Desulfovibrio desulfuricans* 1388 and its influence on nitro-cellulose (NC) transformation was Nitrocellulose is insoluble synthetic polymer with porous surface. Bacterial growth in the presence of NC was accompanied by formation of the slimy clot consisted of polymer particles, bacterial cells and their extracellular metabolism products. About 2% of bacterial cells were yet connected with the clot after twice washing. It was established that round the clot all hydrogen sulfide produced by bacteria was collected and the concentration of nitrate-ions was 3 times higher than in culture medium. It was supposed that *D. desulfuricans* 1388 formed the structure that was modeled on bacterial matrix. Its formation was connected with termination of active bacterial growth and with the advent of stationary phase. Bacterial cells were adsorbed on porous NC surface and could penetrate between polymer particles. The extracellular polymeric compounds synthesized by bacteria surrounded NC deposit. The following NC transformation by *Desulfovibrio desulfuricans* 1388 proceeded in the limited volume of clot, in direct contact of bacteria with NC. The efficiency of NC transformation in matrix increased essentially as compared with NC transformation in active growth phase. The cell growth under stirring conditions that prevented matrix formation caused only 25% reduction in cell yield but 10-fold decrease in NC transformation.

It is obvious that matrix formation is beneficial for microbial trans-formation of insoluble compounds.

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METHODS & MICROBIOLOGY

NUCLEOTIDE SEQUENCE AND CHARACTERISTICS OF THE CODED ORFS OF A FREQUENT, 5.6 KB PLASMID OF *BACTEROIDES* SPP

Sóki, J.;*[1] Wareham, D.W.;[2] Rátkai, C.;[1] Nagy, E.[1]
[1]Institute of Clinical Microbiology, University of Szeged, Szeged, Hungary
[2]Centre for Infectious Disease, Institute of Cell and Molecular Science, Queen Mary's School of Medicine and Dentistry, London, UK

Objective: Our aim was to determine the nucleotide sequence and infer the possible function of pBFP35, a member of Class III *Bacteroides* plasmids frequently found in clinical isolates in Europe, and previously regarded to be cryptic.

Methods: pBFP35 was cloned in the pUC19 vector, and the resulting construct mutagenized using a TGS Kit (Finnzymes) and sequenced with M13 and TGS sequencing primers and primers designed from the partial sequences. Bioinformatic analysis was done to find ORFs whose probable functions were identified using BLAST similarity searches and pattern recognition.

Results: pBFP35 is 5594 basepairs long, and its G+C% is 39.61. It contains 7 ORFs longer than 100 codons, and 7 ORFs longer than 50 codons of which only 1 may be functional. The putatively assigned functions and names of the 7 large ORFs are as follows: an NBU-type mobilization protein (416 aa, *mobA*$_{P35}$), a rolling-cycle-type replication protein (349 aa, *repA*$_{P35}$), a putative lipoprotein (256 aa, *orf1*), a putative integral membrane protein (192 aa, *orf2*), a protein homologous to a *B. thetaiotaomicron* genomic ORF with unknown function (132 aa), a *mazF* homologue, potentially participating in plasmid stability (106 aa, *mazF*$_{P35}$) and an orphan ORF in the coding region of *mobA*$_{P35}$ (104 aa). Out of the small ORFs only one had a homologue, an 82 aa long ORF (*mazE*$_{P35}$) to *mazE* proteins. Additionally nucleotide sequence motifs participating in replication and mobilization initiation were found. In Orf1 a sequence repeat and an ADP-ribosylation motif were revealed. The functional analysis of the maintanance (MazEF) and the toxin activities (Orf1, Orf2) are being carried out in our laboratories.

Conclusion: The Class III *Bacteroides* plasmids encode proteins other than the regular maintenance (replication, mobilization, stability) functions so are not really cryptic. Additionally, the amino acid sequences implicate a role in interaction with the host organism.

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METHODS & MICROBIOLOGY

PORPHYROMONAS BENNONIS SP. NOV., ISOLATED FROM HUMAN CLINICAL SPECIMENS

Summanen, P.H.;*[1] Liu, C.;[1] Molitoris, D.;[1] Rowlinson, M-C.;[1] Ulger-Toprak, N.;[1,2] Finegold, S.M.[3,4,5]
[1]Research, and [3]Infectious Diseases Section, VAMCWLA, Los Angeles, CA USA
[2]Marmara University, Dept of Microbiology, Istanbul, Turkey
[4]Dept of Medicine and [5]Dept of Microbiology, Immunology and Molecular Genetics, UCLA School of Medicine, Los Angeles, CA USA

During our investigation of the bacteriology of human wound infections and abscesses, a novel anaerobic, nonsporeforming, gram-negative bacillus distantly related to *Porphyromonas somerae* (89-90% sequence similarity) was isolated. Comparative 16S rRNA gene sequencing demonstrated that the novel strains were genealogically homogeneous, clustering within the *Porphyromonas* genus.

Strains. Fourteen strains isolated at the Wadsworth Medical Center were included. The specimen sources included skin and soft tissue abscesses above the diaphragm (2) and on the lower back (1), buttock/groin/perirectal area abscesses (7), axillary abscess (1), and breast abscess (3). *P. somerae* ATCC BAA-1230 was included for comparison. Testing was done from 24 to 72 hour pure cultures on Brucella or CDC blood agar.

Methods. Strains were characterized by 16S rRNA sequencing and conventional biochemical methods.

Results. The novel species was the most frequently isolated anaerobic gram-negative rod from a study of skin and soft tissue infections: 5% (19/394) of specimens yielded this strain, predominantly from patients with perirectal/buttock/groin area abscess (63%). The most common predisposing condition was diabetes mellitus. At 2 days, the colonies were small and slightly β-hemolytic, and were grey to cream. After 10 days incubation the colonies were weakly pigmented (beige to tan), and showed no red fluorescence under UV light. The strains were resistant to colistin and kanamycin, and 9 of the 14 strains were sensitive to the vancomycin special potency disks. All isolates were indole-, lipase-, and nitrate-negative and sensitive to bile. Catalase was variable. All strains hydrolyzed gelatin. All were asaccharolytic and produced acetic and succinic, and occasionally trace amounts of iso-valeric and lactic acids as metabolic end-products. The strains were negative for α-fucosidase, trypsin, and esculin, but positive for
α-glucosidase, N-acetyl-β-glucosaminidase, and ONPG.

Discussion. The strains cluster within the genus *Porphyromonas*, but are phenotypically and genetically different from other described *Porphyromonas* species. We propose the name *Porphyromonas bennonis* to encompass this novel species. *P. bennonis* is isolated in mixed culture from various skin and soft tissue infections of non-oral origin, mainly from buttock and groin area abscesses.

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METHODS & MICROBIOLOGY

PEPTONIPHILUS ALLENII SP. NOV., *PEPTONIPHILUS DUERDENII* SP. *NOV. PEPTONIPHILUS KONONENAE* SP. NOV., AND *PEPTONIPHILUS MASSILIENSIS* SP. NOV. ISOLATED FROM CLINICAL SPECIMENS OF HUMAN ORIGIN

Ulger-Toprak, N.;[*1,2] Liu, C.;[1] Molitoris, D.;[1] Rowlinson, M-C.;[1] Summanen, P.H.;[1] Finegold, S.M.[3,4,5]
[1]Research, and [3]Infectious Diseases Section, VAMCWLA, Los Angeles, CA USA
[2]Marmara University, Dept of Microbiology, Istanbul, Turkey
[4]Dept of Medicine and [5]Dept of Microbiology, Immunology and Molecular Genetics, UCLA School of Medicine, Los Angeles, CA USA

Four strains of previously unknown gram-positive, anaerobic, coccus-shaped bacteria from human wound specimens were characterized. Comparative 16S rRNA gene sequencing and biochemical studies demonstrated that these organisms were genotypically and phenotypically distinct and most closely related to *Peptoniphilus* species (88-95% sequence similarity).

Strains. Four novel bacterial strains isolated from human ear-cheek infection (WAL 1768N), vaginal abscess (WAL1998-L), buttock abscesses (WAL2037I) and axillary abscess (WAL1804I) and the phylogenetically most closely related reference strains; *P. asaccharolyticus* (CCUG 9988), *P. lacrimalis* (CCUG 31350), *P. harei* (CCUG 38491), *P. indolicus* (CCUG 17639), *P. ivorii* (CCUG 38492), *P. gorbachii* (CCUG 53341), and *P. olsenii* (CCUG53342) were included. Testing was done from 24 to 72 hour pure cultures on Brucella blood agar.

Methods. Strains were characterized by 16S rRNA sequencing and conventional biochemical methods.

Results. The novel species were isolated together with other anaerobes and/or aerobes, usually in heavy growth (>10^8 cfu/ml). Typical cells of all strains were ≥ 0.7µm in diameter. Colonies at 5 days were 1-2 mm in diameter, grey, circular, and opaque (WAL 1768N, 1998L, 2037I) or 2-3 mm, cream-white, and opaque (WAL 1804I). All were sensitive to kanamycin and vancomycin and resistant to colistin special potency disks. Strain WAL 1804I was resistant to bile and intermediate (8mm) to sodium polyanethol sulfonate (SPS) identification disks, but the other novel strains were sensitive to bile and resistant to SPS. The strains were catalase, urease and nitrate negative; spot indole was variable. All were asaccharolytic and produced major to trace amounts of acetic, butyric, and propionic acids as metabolic end-products. Rapid ID 32A was useful in differentiating the isolates from each other and other *Peptinophilus* spp.

Discussion. Based on both phenotypic and genotypic evidence, we propose four new species of the genus *Peptoniphilus: Peptoniphilus allenii* sp. nov., *Peptoniphilus duerdenii* sp. nov., *Peptoniphilus kononenae* sp. nov., and *Peptoniphilus massiliensis*.

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METHODS & MICROBIOLOGY

COLLAGENASE ACTIVITY IN *BACTEROIDES FRAGILIS* 638R

Galvão, B.P.G.V.;*[1] Abratt, V.R.[1]
Department of Molecular and Cell Biology, University of Cape Town, Cape Town South Africa

Bacteroides fragilis is a normal inhabitant of the human gut, which becomes an opportunistic pathogen if it enters other body locations. Bacterial proteases can act as virulence factors by facilitating tissue invasion and inactivating components of host immune system. In this study, we report on the presence and cellular location of collagenase activity in *B. fragilis*. Proteins showing this activity were identified by means of mass spectroscopy (MALDI). The study also looks at a putative protease gene that was identified by bioinformatic analysis of the *B. fragilis* genome. This gene has high amino acid similarity (67% identity) to the PrtC collagenase of *Porphyromonas gingivalis*. The *B. fragilis prtC* gene was mutated by insertional inactivation and the gene disruption was confirmed by genomic PCR and Southern hybridization. The collagenase activity of the mutant and wild type (WT) was assayed using collagen zymograms as well as the collagen synthetic substrates Azocoll and Pz-peptide. Reverse Transcriptase PCR done on the WT RNA proved that the putative *prtC* gene was part of an operon. One of the genes in the operon, which is downstream of the mutated *prtC* gene, was shown to have similarities to a putative DNA processing Smf-like protein. The possible involvement of this gene and its product in improving cell survival following metronidazole-induced DNA damage may provide an interesting link between *B. fragilis* virulence and drug resistance.

Keywords: *Bacteroides fragilis*, proteases, collagen