2657 Immunofluorescent Localization of Rab3B, 3D, Lingual Lipase and Amylase in Rat Exocrine Glands.

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Small GTP-binding proteins (SGPs) have been implicated as important factors in the regulation of secretion. To investigate the role of SGPs in exocrine secretion, we compared three exocrine glands, von Ebner's (VE), parotid (par) and pancreas (pan), for the presence of two SGPs, rab3B and rab3D, with lingual lipase (LL) and amylase (Amy) as controls. GTP-overlay experiments [method of Ambudkar, et al. *Biochim Biophys Acta* (1990) 1055, 259-264] revealed at least two SGPs in von Ebner's glands. Fasted rats were injected ip with isoproterenol (Iso) (30 mg/kg, β-adrenergic) or pilocarpine (Pilo) (30 mg/kg, cholinergic) 1 hour prior to dissection of the glands. Tissues were fixed in glutaraldehyde-picric acid, embedded in LR White and 1μ sections were treated with rabbit polyclonal antibodies to rab3B, rab3D (gift of R. Raffaniello), LL, or Amy. FITC labled mouse-anti-rabbit followed by rabbit-anti-mouse secondary antibodies were used for detection. Anti-rab3B labeled secretory granules (sg) in pancreatic islets, but not in exocrine (ex) pan, VE or par. Anti-rab3D labeled sg in VE, par and ex. pan. As expected, anti-Amy labeled sg in all three glands, but anti-LL labeled sg only in VE glands. There was no cross-reaction of LL and pancreatic lipase. Compared with controls, areas occupied by sg decreased in the order Iso > Pilo in the par, and Pilo > Iso in VE and pan. Luminal membranes were not labeled. These results indicate that rab3D may have a function in the regulation of exocrine secretion. Supported by the Department of Veterans Affairs.

2658 Cariogenicity of *Streptococcus mutans* Isolated from Infective Endocarditis Patients.

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Streptococcus mutans is occasionally isolated from the blood of patients with bacteremia and infective endocarditis, and the possibility that it could be a pathogen for these diseases has been discussed. Three strains (TW871, TW964, and TW1378) were isolated from patient blood and determined as *S. mutans* from their biochemical and genetic properties. The serotype of TW871 could not be classified and, thus, was determined as being untypable. On the other hand, TW964 and TW1378 were determined as serotypes *f* and *e*, respectively, by immunodiffusion method. The purpose of the present study was to examine the cariogenic properties of these three *S. mutans* strains. Their activities in producing acid from glucose, synthesizing insoluble glucan from sucrose, and adhering to a glass surface in sucrose medium were similar to those of *S. mutans* MT8148R (serotype c). However, the ability of the serologically untypable strain, TW871, to adsorb saliva-coated hydroxyapatite was significantly lower than that of the other strains. Accordingly, the caries inducing activity of these strains was examined in SPF SD rats fed the caries-inducing diet 2000. The caries score of the untypable strain (TW871) was significantly lower than that of MT8148R, and the bacterial recovery of TW871 from the mandible was also significantly lower than MT8148R. These results suggest that the cariogenicity of untypable strain of *S. mutans*, isolated from the blood of patient with infective endocarditis, may become lower with changes in cell surface proteins and polysaccharide antigens under different conditions from the oral cavity.

2659 *Candida albicans* biotypes in persistent root canal infections in a Finnish population.

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Thirty-seven *C. albicans* strains isolated from persistent root canal infections in Finland were biotyped. The biotyping method was based on the presence of five different enzymes, assimilation of eleven different carbohydrates and resistance to boric acid (Williamson *et al.* 1987). The presence of enzymes and assimilation of carbohydrates were determined using commercially available API ZYM and API 20 C test kits. The resistance of the isolates to boric acid was tested by their ability to grow on yeast-nitrogen-agar with incorporated boric acid (1.8 mg ml^{-1}). Combining of the tests resulted in a total of 14 different biotypes. The majority of the isolates, 26 strains, were classifiable into three major biotypes: 16 isolates (43.2%) belonged to biotype A1R, six (16.2%) to A1S and four (10.8%) to B1S. The remaining 11 biotypes represented only a single isolate each. The dominance of the biotype A1R was comparable to previous reports of strains isolated in Europe. Circumstances in necrotic root canal did not seem to favor biotypes different from strains isolated from other oral or non-oral sources. Genotyping of the strains is under process.

2660 Multiplex PCR Assay without DNA Extraction for *Prevotella intermedia* and *Prevotella nigrescens*.

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Bacteria of the *Prevotella intermedia / nigrescens* group are anaerobic black-pigmented gram-negative rods, whose differentiation is difficult to perform because they exhibit close phenotypical similarity. Several methods have been utilized to distinguish *P. intermedia (P.i.)* from *P. nigrescens (P.n.)* but there still is a need for a simple, rapid and reliable differentiation assay. Our aim was to develop a rapid multiplex PCR assay based on specific primers to discriminate *P.i.* and *P.n.* We studied various regions of 16S rRNA sequences in the GenBank database to find primer pairs which would be able to recognize all known strains of *P.i.* and *P.n.* The selected primers hybridized with their specific target sequences and they were optimized to function in the same reaction. No DNA extraction is required prior to the PCR amplification, as the assay can be made directly from a part of a bacterial colony from the original culture plate. 22 reference strains including 8 strains of other *Prevotella* species were used for specificity testing. Sensitivity of the method was tested by viable counts of the bacteria after one week culture. 100 clinical isolates, which were identified as *P.i./P.n.* in our laboratory by colony morphology and biochemical standard methods, were also tested. Sensitivity of the assay was < 10 cfu/sample. *P.i.* and *P.n.* reference strains gave expected PCR products, where as none of the other species tested gave positive results. Of the 100 tested clinical isolates 47 % were identified as *P.i.* and 53 % as *P.n.* while no isolates remained unidentified. The presented PCR assay is a rapid and realibe method to distinguish *P. intermedia* from *P. nigrescens* directly from a bacterial colony without DNA extraction.

2661 *Prevotella pallens* has a unique phospholipid analogue profile.

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P. pallens is a recently described[1], weakly-pigmenting, *Prevotella* which differs phenotypically and genotypically from related species. The aim of this study was to analyse phospholipid analogue distributions of *P. pallens* in order to see whether they were novel. Twelve strains were analysed following growth on blood-FAA plates in an anaerobe cabinet. Lipids were extracted using chloroform-methanol and purified extracts analysed by Fast atom bombardment-mass spectrometry (FAB MS) in negative-ion mode by our published methods[2]. All experiments were repeated. Results showed that most *P. pallens* strains have major phospholipid analogue peaks with mass-to-charge, m/z, 677, 678, 691, 693 and 694 which may be putatively assigned PG(29:1), first isotope peak of PG(29:1), PG(30:1), PG(30:0) and first isotope peak of PG(30:0). In this context, PG(29:1) is a phosphatidylglycerol molecular species having two fatty acyl substituents with a total of 29 carbons and 1 unsaturation in the alkyl side chain. Related species have major peaks with slightly different m/z values. We conclude that *P. pallens* isolates are chemically different from related species and that their unique phospholipid analogue distributions are of chemotaxonomic value.

[1]Könönen, E. *et al.* (1998). Int. J. Syst. Bact. 48: 47-51.
[2]Drucker, D.B. *et al.* (1995). J. Bact. 177: 6304-6308.

2662 Genotypic Stability of *Streptococcus mutans* in Children.

B.J. ROBINSON*, Y. LI, H.W. WIENER, Z. LU, and P.W. CAUFIELD. (Specialized Caries Research Center, University of Alabama at Birmingham, Birmingham, AL).

Our working hypothesis, based upon a limited set of observations, is that the pioneer mutans streptococci populations colonizing the oral cavities of infants remain stable over time. The objective of this study was to investigate the longitudinal stability of *S. mutans* in young children. Ten African-American children were followed from birth to 3 years old. Saliva, plaque, and swab samples of those children were collected every three months and cultivated on the selective MSB medium for detecting the present of *S. mutans*. Altogether 133 *S. mutans* isolates were obtained from these children over a 6 to 18 month period. Genomic DNA from *S. mutans* isolates was purified using Promega Wizard® purification procedure, then subjected to arbitrarily primed polymerase chain reaction (AP-PCR) genotyping. Variables such as the gender, the number of teeth, the level of *S. mutans*, and eating habits were included in the analyses. We found that among the ten children, the mean age of *S. mutans* acquisition was 20.1 month; one child acquired *S. mutans* at age of 15 mo., five at 18 mo., and four at 24 mo. Genotyping *S. mutans* strains using AP-PCR showed that, on the average, the children acquired between 1 to 3 genotypes of *S. mutans* strains and 92% of them maintained at least one identical strain over a 6 to 18 month period. This pilot study suggested that the stability of *S. mutans* among the children was independent of the gender, the availability of the tooth surfaces, the level of *S. mutans*, and changing eating habits during the study period. Supported by the NIH/NIDCR Grants T35HL07473, T32DE07026, and DE11147.

2663 Characterization and Diversity of *S. sanguis* in Infants.

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The colonization of *S. sanguis* may play an antagonistic role in *S. mutans* infection and the caries process, so accurate characterization of strains is paramount. Here, we ascertain both the phenotype and genotype of *S. sanguis* and other closely related species, and then analyze their distribution in infants. During a 3-year longitudinal study, 35 infants were sampled every 3 months. Based upon distinctive colony morphology on MM10 medium, we obtained 258 isolates of colonies presumed to be *S. sanguis*. From genomic DNA extracts, an AP-PCR fingerprint method for *S. sanguis* was developed. Thirty random primers were screened for their suitability under various conditions. Based on the Beighton's scheme for the identification of *S. sanguis* (1991), we found that among the 258 isolates, 99% of them were *S. sanguis*; 14.1% were biotype I; 57.4% as biotype II; and 28.5% as biotype III. Two isolates (0.8%) were identified as *S. gordonii*. On average, 7.4 isolates were obtained from each individual. 48.5% of the infants harbored one biotype of *S. sanguis*, 40.0% and 11.4% of them harbored 2 and 3 biotypes of *S. sanguis* respectively. AP-PCR fingerprinting using primer OPA02 differentiated the genotypes of *S. sanguis* among the 35 infants. We found that infants harbored 1 to 5 genotypes of *S. sanguis* (mean = 2.4±1.4). In addition, AP-PCR from *S. sanguis* DNA yielded a unique fragment of 1.6 kb compared to other oral streptococci. The results demonstrate that MM10 medium is reliable for isolating *S. sanguis*. AP-PCR was able to further discriminate the number of genotypes per infant compared to biotypes. A PCR fragment unique to *S. sanguis* may provide an alternative method for identifying *S. sanguis*. Supported by NIH/NIDCR grant DE-11147.

2664 Acquisition of *Streptococcus mutans* in Children Delivered by Caesarian Section.

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Previously, we showed that gender and race of the infant were correlated with fidelity of *S. mutans* transmission. The objective of this study was to investigate whether other factors influenced the acquisition of *S. mutans*. Forty-one African-American mother-child pairs were followed from the 3rd trimester of pregnancy until the child was three years old. During this period, information concerning infants was gathered including gender, birth status and birth weight, medical history of infectious diseases, and antibiotic usage. For the mother's group, information on pregnancy, breast-feeding experience, *S. mutans* level, antibiotic usage, and caries status was collected. Oral bacterial samples were collected every 3 months and cultivated on several media, including MSB for isolating *S. mutans*. A total of 1,644 *S. mutans* isolates were obtained and genotyped via AP-PCR. We found that the mean age of acquisition of *S. mutans* was 24.4±8 months. 76% of the children were infected with *S. mutans* by the age of three. Children born by Caesarian section (N = 6) acquired *S. mutans* significantly earlier than vaginally-delivered children (N = 35) (Non-parametric Survival Analyses, $p<0.05$). The study also found that treatment with antibiotics for either mothers ($p< 0.05$) or children ($p < 0.01$) during the first two years significantly delayed the initial acquisition of *S. mutans* by the children. The study suggests that Caesarian section and antibiotic applications can affect *S. mutans* transmission from mother to her child. Also, this study reaffirmed our previous observations that *S. mutans* transmission is acquired through a discrete "window of infectivity." Supported by the NIH Grants T32DE07026 and DE11147.