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Age-related Acquisition of Oral Yeast Species and Stability of Colonization in Young Children. J. HANNULA[1], M. SAARELA[1], H. JOUSIMIES-SOMER[2], A. TAKALA[2], R. SYRJÄNEN[2], S. ASIKAINEN[1] ([1]Univ. of Helsinki, Helsinki, Finland, [2]National Public Health Institute, Helsinki, Finland).

The aim of the prospective 22-month follow-up study was to determine the occurrence and stability of colonization of oral yeast species and strains in children from the age of 2 months. At baseline salivary samples were cultured from mothers to study the role of the mother as the source of yeasts for the child. 40 healthy children participated in the study at the ages 2, 6, 12, 18 and 24 months. A total of 111 yeast isolates, 26 isolates from mothers and 85 from children, 1-17 isolates per subject (mean 3.0, SD 3.8), were mainly collected from SDA plates, subcultured on CHROMagar Candida Medium plates and AP-PCR genotyped using primer 5'-AGTCAGCCAC-3'. Yeasts were recovered at least once from 17/40 (43%) children during the follow-up. 11/40 (28%) of the children were yeast-positive at multiple samplings. Recovery of yeasts was 18-20% at ages 2, 12 and 18 months and 13% at ages 6 and 24 months. *Candida parapsilosis* was isolated from 18/33 (55%) and *Candida albicans* from 12/33 (36%) of yeast-positive samples from children during the follow-up. *C. albicans* predominated at ages 2 (57%) and 6 (60%) months and *C. parapsilosis* at ages 12 to 24 months (range 63-88%). The same yeast species was rarely detected in the follow-up samples. 20/40 (50%) of the mothers harbored yeasts. *C. albicans* was recovered from 19/20 (95%) of the yeast-positive mothers whereas *C. parapsilosis* from none. 7/20 (35%) of the yeast-positive mothers had a yeast-positive child. In 5/7 (71%) of these mother-child pairs both harbored the same yeast species (*C. albicans*) and in 3/5 (60%) of the pairs the AP-PCR-profiles of the *C. albicans* isolates were identical. In children a significant relationship (χ^2-test, $p<0.05$) was found between recovery of yeasts and dummy sucking and in mothers between recovery of yeasts and use of antibiotics. In conclusion, yeasts seemed to be transient colonizers in the developing oral floras of the children. *C. parapsilosis* and *C. albicans* were the most frequently isolated oral yeast species from the children. In *C. albicans* -positive mother-child pairs the mother was the likely source of oral transmission. Further studies are warranted to investigate the source of *C. parapsilosis*.

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Salivary flow rate, pH and lysozyme levels in HIV-infected individuals in Hong Kong. C.S.TSANG and L.P. SAMARANAYAKE* (Faculty of Dentistry, The University of Hong Kong, Hong Kong, China)

As the quality and the quantity of saliva is critical in maintaining oral health, the salivary flow rate, pH and lysozyme levels in a cohort of 32 HIV-infected individuals in Hong Kong was compared with an equal number of HIV-free, healthy individuals. Particular attention was paid to the salivary lysozyme -a potent antimicrobial agent, and its relationship to the salivary flow rate, pH, CD4+ count, oral yeast and coliform carriage, and the presence of oral lesions in the HIV-infected cohort. Whole, mixed saliva samples were collected by expectoration into a chilled beaker, in a standard manner, over a 3 min period and expressed as mL/min. The pH of saliva was determined immediately using a pH electrode, and the salivary lysozyme level determined using Quantiplate Lysozyme Test (Kallestad Labs. Inc, Chaska, USA). Oral yeast and coliform carriage was assessed using the oral rinse method, and culturing the rinse sample in Sabourauds and MacConkey agar, respectively (Samaranayake *et al. J Oral Pathol* 1986; 15:251-4). Both the flow rate and the pH of whole mixed saliva of HIV-infected individuals were significantly lower when compared with the controls ($p < 0.05$). The salivary lysozyme level of the HIV-infected cohort was 24% higher compared with the controls ($p = 0.0003$). However, no significant relationship existed between the salivary lysozyme level and either the CD4+ count, presence of oral lesions, or oral yeast and coliform carriage. Further controlled studies with a larger cohort is required to confirm the current findings.

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The effect of urea containing chewing gum on plaque acidogenicity. C SMITH* and J VERRAN (Dept. Biological Sciences, Manchester Metropolitan University, UK)

The acidogenic ratio (AR) is the ratio of acid producing microorganisms to the total number of microorganisms in plaque and is obtained using bromocresol purple agar. Acid production is indicated by a yellow halo around acidogenic colonies. A high AR has been related to caries experience or susceptibility. A small study was performed to determine the effect of urea containing gum on AR. The ARs of plaque taken from ten subjects were measured over a 10 week period, which included 'control' weeks (1,2,4,5,8,10 - normal dietary regime), raised sucrose intake (six boiled sweets or mints per day: week 3), chewing gum +/- urea (5 panellists per group, gum chewed after meals; weeks 6 and 8). In week 9, a control regime was re-established for the urea gum group and the previously urea free gum group chewed urea gum. Plaque was also tested weekly for acidogenicity by incubation with 0.1M sucrose at 37C for 90 minutes. When the mean ARs of the +/- urea gum groups were compared, the urea gum group has a lower AR at weeks 6 and 7. In week 9, the use of urea gum again produced a lower mean AR. For a panellist with DMFS of 99, plaque acidogenicity was highest in week 3 (sucrose), and was reduced during weeks 6 and 7. For a panellist with DMFS of 2, plaque acidogenicity was always slight. However, in this small study, with little time allowed between treatments for stabilisation of AR, any differences were rarely significant. The AR provides a potential means for indicating plaque acidogenicity. Regular use of a urea containing gum may reduce plaque AR.

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Acid Tolerance of Microflora from Caries and Healthy Tooth Sites. S. EDWARDSSON*, G. SVENSÄTER, M. BORGSTRÖM and I. R. HAMILTON (Oral Microbiology, U. Lund, Malmö, Sweden/Oral Biology, U. Manitoba, Canada).

Knowledge of the acid-tolerant (AT) bacterial community in dental plaque and its role in the development of caries is limited. Our purpose in this study was to isolate and identify AT bacteria from caries and healthy tooth sites using non-selective media supporting growth of bacteria with various degrees of tolerance to acid. Todd Hewitt broth with agar, buffered at pH 7.0, 6.0, 5.5, 5.0 or 4.5, was chosen for culturing plaque samples collected from approximal tooth lesions of caries-positive subjects and from similar sites of caries-free subjects. The proportion of AT bacteria from caries sites was higher than that of healthy sites. Lactobacilli were almost exclusively present on the pH 4.5-agar, while Gram-positive pleomorphic rods and streptococci dominated at pH 5.5 and 5.0 with the latter agar supporting the growth of *S. anginosus, S. constellatus, S. gordonli, S. intermedius, S. mitis, S. mutans S. oralis, S. salivarius, S. sanguis* and *S. sobrinus*. Acid killing curves (pH 7.5 - 2.5 for 3 h) of selected streptococcal isolates from healthy and caries pH 5.5 agar plates indicated that those from caries sites survived to lower pH than corresponding strains from healthy sites. However, once the caries isolates were transferred to pH 7.0, acid tolerance was significantly reduced. The results indicate that caries sites harbour a more acid tolerant microflora than healthy sites with acid tolerance a property of several species. Acid tolerance by the streptococci isolated at low pH is reduced following growth at neutral pH indicating deadaptation. (Supported by Medical Research Councils of Canada (MT-3546) and Sweden (K97-24X-12266-01).

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Growth Phase and the Acid Tolerance Response by *Streptococcus mutans*. I. R. HAMILTON* and G. SVENSÄTER (Oral Biology, U. Manitoba, Winnipeg, Canada and Oral Microbiology, U. Lund, Malmö, Sweden).

When transferred from pH 7.5 to 5.5, log-phase cells of *S. mutans* induce an acid tolerance response (ATR) that enhances survival at a pH killing unadapted pH 7.5 cells. We have now examined strains for the presence of a stationary phase ATR (SP-ATR) typical of enteric bacteria. Four strains of *Streptococcus mutans*, growing in tryptone-yeast extract-glucose (TYEG) broth at pH 7.5 and 5.5, were employed in two types of acid survival experiments: batch growth cultures in which cells were tested for survival at a killing pH (3.5 -3.0 for 3 h) during transition from log to SP, and 'simulated' SP in which survival of pH 7.5-log phase cells was tested following transfer to glucose-free TYE at pH 7.5 and 5.5 for 2 h. During growth, the pH 7.5 (unadapted) cells exhibited no log or SP-ATR, but exhibited enhanced survival in the transition between log and SP only. As expected, pH 5.5 adapted cells exhibited a log-phase ATR, however, the capacity to survive at low pH was lost on entry into stationary phase. The transfer of log-phase cells to TYE-G showed two strains (LT11 and Ingbritt) had no SP-ATR at either pH, strain BM71 exhibited a SP-ATR at pH 5.5, but not 7.5., while strain H7 had a SP-ATR at both pH. Survival with these latter two strains was inhibited by iodoacetate, NaF and the ionophore, nigericin. The results suggest that pH-dependent (BM71/H7) and pH-independent (H7) SP-ATRs are exhibited by some strains, but not others, and SP-ATR appears to be linked to normal pH homeostatic mechanisms sustained by endogenous metabolism. (Supported by MRC Canada (MT-3546) and Sweden (K97-24X-12266-01).

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Turnover of GTFs of *S. mutans* growing at stationary phase. T. OGAWA* (Dept. of Dental Hygiene, Saitama College of Health, Saitama, Japan)

Glucosyltransferases (GTFs), the important cariogenic factors of *Streptococcus mutans* are synthesized extracellularly and cell associated form. They are produced as major extracellular proteins at exponential growth phase. When bacterial growth stops as carbon source limitation, GTFs syntheses stop too. So extracellular GTFs in medium at stationary phase are previous phase synthesized products. In previous reports, GTFs in medium at stationary phase gradually associate to cell surface, but degradations as proteolytic events are not examined. So, GTFs turnover was studied by addition of ^{35}S-labelled GTFs at stationary phase. From autoradiographic profile of SDSPAGE, all extracellular GTFs were disappeared, and about 20% of labelled GTFs were recovered as associated form on cell surface. This phenomenon suggested that GTFs turnover at stationary phase involves some degradation events.

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Nitrate and Nitrite Reduction by Some Strains of Oral Streptococci. J. D. RUDNEY and R. M. DeCAMP* (Department of Oral Science, School of Dentistry, University of Minnesota, Minneapolis, Minnesota 55455, U.S.A.).

Salivary glands take up large amounts of dietary nitrate. Salivary nitrate then is reduced to nitrite by oral bacteria. Nitrite may contribute to the formation of potentially carcinogenic nitrosamines, or to the release of potentially antimicrobial nitric oxide. A number of gram-negative oral anaerobes reduce nitrate. However, streptococci predominate at many oral sites, and their potential for nitrate and nitrite reduction has not been investigated. This study evaluated 10 *Streptococcus sanguis*, 4 *S. gordonii*, 4 *S. parasanguis*, 2 *S. crista*, 16 *S. oralis*, 14 *S. mitis* biovar 1, and 12 *S. mitis* Biovar 2. *Actinobacillus actinomycetemcomitans* ATCC 29522 and 29524 were used as positive controls. Species identifications were based on previous phenotypic and genotypic tests (ribotyping and AP-PCR). Streptococcal stocks were plated on blood and mitis-salivarius agar (MSA). Purity was confirmed by colony-morphology and gram-staining. Colonies from MSA were inoculated in Todd-Hewitt broth containing 200 µM KNO_3, and incubated anaerobically for 18 h at 37° C. Plating and gram-staining then were repeated; broth pH and OD at 650 nm also were recorded. Supernatant nitrite was determined by the Greiss reaction; residual nitrate was found by treating an aliquot with nitrate reductase and co-factors at pH 7.4, followed by stop solution and Greiss reagents. KNO_3 broth without bacteria was serially diluted and treated with nitrate reductase to provide a standard curve. Positive control strain 29524 reduced nitrate to nitrite, while 29522 completely reduced nitrate and nitrite. Four streptococci consistently showed the same pattern as 29522. The remainder displayed variable 0-55% reductions in nitrate, again with no nitrite present. Nitrate reduction was greatest when final broth pH was high ($r = 0.53$, $p < 0.001$); there was no association with final OD. Results suggest that less acidogenic streptococci variably express nitrate and nitrite reductases. The association with final broth pH is consistent with high pH optima for other bacterial nitrate reductases. Further work is needed to determine streptococcal contributions to total oral nitrate and nitrite reduction. Supported by NIH grant 2 R01 DE 07233-11.

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Cell Vitality of *Streptococcus sanguis* in Growth Cultures E.-M. DECKER*and R. WEIGER, (School of Dental Medicine, University of Tübingen, Germany).

The aim of the study was to characterize the growth pattern of *S. sanguis* as early colonizer by metabolic monitoring and to compare these data with the results of four different fluorescent staining procedures. *S. sanguis* (DSM 20068) was cultivated in Schaedler infusion broth for 73 hours. The following parameters were recorded after 2, 4, 6, 8, 10, 12, 25, 49 and 73 hours: optical density at 540 nm (OD), pH, total bacterial counts/mL (BC), colony forming units/mL (Schaedler agar; CFU) and the percentage of vital bacteria (PVB) determined after fluorescent labelling. Three series of tests were carried out. For the measurement of cellular vitality four combinations of vital/dead stainings were used: F1 (fluorescein-diacetate/ ethidiumbromide), F2 (fluorescein-diacetate/ propidium iodide), F3 (Syto 9/ propidium iodide), F4 (tetrafluoro-fluorescein-diacetate/ propidium iodide). During the first 25 hours parameters of growth and vitality (OD, BC, CFU, PVB) increased to the maximum while the pH values dropped simultaneously. In the following up to 49 hours the ability of *S. sanguis* to grow decreased to 0. At that time fluorescent vitality stainings differed considerably: whereas F1 provided insufficient data during the exponential growth stage (PVB_{max}: 48 %), F2 – F4 reflected more equivalent values corresponding to the other growth parameters (PVB_{max}: F2 - 87 %, F3 - 96 %, F4 - 74 %). F3 was the most sensitive, brilliant and established fluorescent label. The assessment of bacterial viability by cultivation alone as the only paramter can lead to false negative results because this method can not detect viable but nongrowing cells. A sensitive fluorescent marking system like F3 can assist in the comprehension of bacterial growth features.

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