1209 A MADS-Box Binding Site may be the Universal Regulator of Phase-Specific Genes in *Candida albicans*.

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Candida Albicans is an organism that can exist in the oral cavity as either a commensal or a pathogen. With the exponential increase in the number of immunocompromised individuals in the last 10 years, *C. albicans* has become a serious health threat. One of the proposed virulence factors of *C. albicans* which helps it to evade the host immune system is phenotypic switching. Using the white-opaque transition, a major experimental system for studying switching, we found that the promoter of the opaque phase-specific gene *OP4* that controls opaque phase expression was a MADS-box protein consensus binding site which was most closely related to the Mcm1 binding site from *Saccharomyces cerevisiae*. Because Mcm1 is a universal regulatory protein in *S. cerevisiae*, we proposed that it may act as a universal regulator of phase-specific genes in *C. albicans*. We, therefore, used the MADS-box consensus sequence as a probe to identify other phase-specific genes containing that sequence in their promoter. A double stranded oligonucleotide consisting of the 14 nucleotide MADS-box binding site and the 16 preceding and 8 following nucleotides was concatamerized, labeled and used to screen a genomic DNA library from *C. albicans*. Of the 100 initial clones selected as positives, 24 were chosen for Southern analysis, and 14 of them hybridized with the *OP4* open reading frame. From the ten remaining clones we have shown by northern analysis that we have one opaque-specific and one white-enhanced clone. We are currently screening the rest of the clones for more phase-specific genes. The two phase-specific sequences we currently have are being isolated from lambda phage and sequenced. Our results represent the first indication that a number of phase-specific genes are regulated by the same phase-specific trans-acting factors through the MADS-box sequence, and demonstrate the efficacy of this approach for identifying phase- specific genes. This work was supported by NIDR T35 DE07159 and NIH grants DE10758, AI39735

1210 Genetic variation within clinical isolates of *Candida dubliniensis*

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The incidence of oral candidosis continues to increase, largely as a result of the ongoing AIDS epidemic. Although *Candida albicans* remains the most common cause of candidosis, the incidence of infections caused by other species, including the recently discovered species *Candida dubliniensis*, is increasing rapidly. *C. dubliniensis* was first recognised as a separate species in 1995, however, the reasons for its emergence are not clear. In order to investigate the level of genetic variation within clinical isolates and the effect of antifungal agents, such as fluconazole, on this variation we generated DNA fingerprints of multiple single colony isolates from clinical samples and from isolates which were exposed to fluconazole *in vitro*. In four cases of oral candidosis where *C. dubliniensis* was the only species recovered from swabs taken from the dorsum of the tongue, 10-18 colonies were chosen for analysis. Genomic DNA was purified from these isolates. This DNA was digested with the restriction enzyme *EcoR*1 and electrophoresed on agarose gels. The DNA was then transferred to nylon membranes by Southern blotting and subsequently hybridized with the *C. albicans*-specific mid repeat DNA fingerprinting probe, 27A. In addition, genetic variation was examined using pulsed-field gel electrophoresis (PFGE). The effect of isolate exposure to fluconazole on DNA fingerprint patterns was examined by subculturing isolates sequentially on increasing concentrations of drug. Three of the four sets of clinical isolates examined showed significant genetic variation using the 27A DNA fingerprinting probe and by PFGE analysis. In addition, we were also able to induce stable fluconazole resistance (MIC, 16-64 μg ml^{-1}) in what were originally susceptible isolates. These resistant derivatives often showed genetic polymorphisms when compared with the parental isolate. Therefore, clonal populations of *C. dubliniensis* exhibit a high degree of genetic diversity. The ability to generate this diversity may be advantageous in stressful conditions, such as exposure to antifungal drugs, and may at least in part explain the recent emergence of this species as a significant human pathogen. This study was supported by Irish Health Research Board grant No. 41/96

1211 Molecular types of oral *Candida albicans* isolates and relatedness to phenotypic features

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Oral candidosis a common oral manifestation of compromised patients and *Candida albicans*, is the most frequently isolated species in this condition. Molecular typing and phenotypic characterisation of *C. albicans* is thus vital for better understanding, diagnosis and the management of oral candidosis. Hence genotypic and phenotypic features of 10 oral isolates of *C. albicans* were characterized to ascertain the relationship between these two distinct parameters. The isolates were molecular typed by Pulse Field Gel Electrophoresis (PFGE) using a hexagonal electrode. The number of chromosomes varied from 6 to 7 and their sizes ranged from 1000Kbp to 3200Kbp, giving 4 different karyotypes. *EcoRI* Restriction Fragment Length Polymorphisms (RFLP), yielded 7 different genotypes of *C. albicans* while DNA fingerprinting by Randomly Amplified Polymorphic DNA (RAPD) with primers OBU1 (5'CACATGCTT3'), OBU2 (5'CACATGCCT3'), OBU3 (5'CGCATGCTT3') and RSD6 (5'GCGATCCCCA3') resulted in 4, 3, 6 and 4 different genotypes, respectively. These characteristics were compared with different phenotypic features such as adherence to buccal epithelial cells, cell surface hydrophobicity and the rate of germ tube formation. A relatively close relationship was observed between the latter phenotypic characteristics and the genotypic features of the studied isolates, an observation which has hithertofore been sparsely described in oral isolates of *Candida albicans*. Supported by the CRCG, University of Hong Kong, Hong Kong

1212 Proteinase Activity and Switching of *Candida* Varies in HIV+ Patients

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Candida albicans is one of the most common causes of opportunistic infections among immunocompromised patients. Many virulence factors have been attributed to *Candida* pathogenicity. Among the most important are proteinase activity and phenotypic switching. It was the purpose of this study to evaluate differences in proteinase activity and phenotypic switching among yeast strains isolated from HIV+ individuals collected longitudinally before the first episode of thrush, during an episode of oral thrush and after antifungal treatment. Controls consisted of healthy uninfected individuals matched for age and sex. Aspartyl proteinase activity was evaluated using 2 different methods. 1) BSA plates inoculated with 1x10^{8}cfu of yeast were incubated at 37°C for 96 hours, stained with 0.6% naphtol blue, destained and examined under backlighting for clear proteolytic zones around the colonies. The clear zone was measured from the edge of the colony to the outside border of the clear area using a Boley gauge. Measurements were expressed in mm. 2) An FITC-casein assay was used to evaluate proteolytic activity of supernatants from cultures grown in proteinase inducing medium. Phenotypic switching was assessed by spreading 100μl of cells (app. 100 cells) onto 10 modified Lee's agar plates, incubating for 7 days at 25°C and scoring for colony phenotype. Statistical analysis was done using a one-way ANOVA and Tukey post-hoc tests. The results showed: a) proteinase production was significantly increased ($p<0.05$) in the isolates obtained after antifungal therapy when compared to those obtained either before or during an episode of thrush, b) high-frequency phenotypic switching was greatly increased in the HIV+ group when compared to control subjects ($p<0.05$) and c) variability was found in proteinase activity of different switched phenotypes. These results suggest that there may be a preferential selection of strains with a higher overall level of secreted proteinase activity as the disease progresses and that switching plays an important role in pathogenesis and must be taken into consideration during evaluation for treatment. This research project was supported by NIH grant DE00364

1213 Oral Yeast and Coliforms Carriage of Tibetans in Lhasa.

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We investigated the oral colonization of yeast and coliform bacteria in a cohort of Tibetans living in Lhasa City, Tibet Autonomous Region, China. Random samples of children (n=50) and adults (n=40) were selected from a pool of 340, 11-13 years old (from 2 primary schools) or 160, 25-44 years old (from 4 governmental or local agencies), respectively. These subjects were recruits of a concurrent epidemiological survey. Mean age for children/adults were 11.6±0.9 year/36.5±6.9 years and 42%/65% were female. All subjects lived in Tibet since birth. Oral rinse samples were collected as described previously (Samaranayake et al. J Oral Pathol Med, 1986, 15, 386) spiral plated and cultured using conventional techniques. Oral yeast and coliform bacteria isolation rates for children/adult groups were 16%/49% or 78%/33% respectively. Mean yeast and coliforms colony forming unit (cfu) per mL oral rinse from children/adult groups was found to be 1.2±5.7 cfu/5.0±10.9 cfu or 13.9±20.4 cfu/13.0±70.8 cfu per mL respectively. There was no statistical significant difference between the count (cfu) of yeast or coliforms per mL oral rinse isolated from female or male in both children and adult groups. This preliminary study showed that native Tibet inhabitants especially children appeared to have high oral carriage rate of coliform bacteria. We postulate that this might be due to their unique dietary habits and/or lower standards of general hygiene.

1214 Acquisition of *L. acidophilus* and *L. casei* in infants

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Lactobacilli are members of the indigenous oral microbiota associated with occlusal, root and recurrent caries, and are secondary invaders in smooth surface lesions. Although this large genus consists of about 60 species, few studies have been done on the natural history of the acquisition of lactobacilli at species level. Therefore, this longitudinal study aimed to delineate the natural history of the acquisition of *L. acidophilus* and *L. casei* in infants. Fifty-two infants (26 female, 26 male) and their mothers were followed from delivery to 24-month at 3-month intervals. Salivary (411 from babies, 437 from mothers) and plaque (46 from babies) samples were collected and cultivated on Rogosa SL agar. *L. acidophilus* and *L. casei* were quantitated and the tooth emergence status was recorded. During the 24-month period, we found 65% of infants had no detectable level of *L. acidophilus*, 23% of them have *L. acidophilus* detected only at one visit and 8% of them only at early visits (before 6-month). Fifty-six percent of the infants had no detectable level of *L. casei* and 27% were detected only once. The status of mothers' *L. acidophilus* and *L. casei* and gender of the infants had no effects on the acquisition of *L. acidophilus* or *L. casei* (gender RR=1.4, 95%CI=0.7-3.1, RR=1.6, 95%CI=0.8-2.9, respectively). Tooth emergence for children older than 6-month did not correlate with the acquisition of *L. acidophilus* or *L. casei* (RR=0.9, 95%CI=0.1-9.2, RR=1.6, 95%CI=0.4-6.8 respectively). The results of this study suggest that *L. acidophilus* and *L. casei* may be only transients in the oral cavity in the early childhood prior to the initiation of caries lesions. This study was supported by the NIDR, Grant DE 11147

1215 The Effect of Stannous Fluoride on Intracellular Polysaccharide Production in *L. salivarius*

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The production of intracellular polysaccharide (IPS) from bacteria in dental plaque has been suggested to have a significant cariogenic effect. The effect of stannous fluoride on IPS production in *Lactobacillus salivarius* was investigated by the method of Dipersio et al. (1974) and confirmed by electron microscopy. *L. salivarius* was cultured in MRS growth medium with 2% glucose. *L. acidophilus* was used as a negative control for the purpose of comparison. Five separate trials were conducted with 0, 25, and 50 PPM of stannous fluoride. At stannous fluoride concentrations of 500 PPM the growth rates were reduced by 65% and 99% for *L. salivarius* and *L. acidophilus*, respectively. The results further demonstrated that, during the early stationary phase, a 50-PPM stannous fluoride solution reduced IPS production by an average of 47.5%. Linear regression analysis indicated that the decrease in IPS production by SnF_2 was statistically significant ($P=0.014$). The regression analysis did not rule out the possibility of a slight degree of curvature in the dose- response relationship. Electron microscopy cytochemistry showed a reduction in intracellular glycogen-like electron dense granules at 50 PPM of SnF_2. **It is concluded that 50 PPM significantly reduces IPS production in *L. Salivarius*.**

1216 Phospholipid analogue profiles of some IE and non-IE *Lactobacillus* isolates

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Previously, we have used Fast Atom Bombardment Mass Spectrometry (FAB MS) to examine distribution of analogues of phospholipids (PL) in representative strains of the genus *Lactobacillus*[1]. Although *Lactobacillus* is frequently regarded as a non-pathogenic genus, it has been associated with Infective Endocarditis (IE)[2] and of course dental caries. DNA methods have not been able to differentiate between IE and non-IE strains. The aim of this study was to see whether PL analogue profiling could differentiate between IE and non-IE strains. Of *L. rhamnosus*, we examined 10 IE and 4 non-IE strains, of *L. paracasei* ssp *paracasei*, 6 IE and 3 non-IE strains. Lipids were extracted with chloroform-methanol and examined by FAB MS in negative-ion mode, using published methods[1]. The major PL analogues were with m/z 719, 745, 747, 761 and 773, tentatively identified as PG(32:1), PG(34:2), PG(34:1), PG(35:1) and PG(36:2). The following less intense peaks were seen, with putative ID: m/z 717, PG(32:2), 731, PG(33:2); 733, PG(33:1), 771, PG(36:3), 775, PG(36:1), 787, PG(37:2), 801, PG(38:2). Objective comparison of spectra was made using SPSS/PC+ to calculate Pearson coefficients of Linear correlation. It was possible to distinguish between the species in the majority of cases but not between IE and non-IE strains. We conclude that phospholipid analogue distributions are not predictors of IE status but that different species of *Lactobacillus* may have profiles that are the same qualitatively but differ quantitatively.

[1] Drucker *et al* (1995) Phospholipids of *Lactobacillus* spp. J Bacteriol 177 6304-6308
[2] Harty *et al* (1994). Pathogenic potential of lactobacilli. Int J.Fd Microbiol 24 179-189