1489 Purification and Characterization of *Campylobacter rectus* S layer Proteins. H. NITTA*, J.L. EBERSOLE AND S.C. HOLT (University of Texas Health Science Center at San Antonio, 78284-7894, USA).

C. rectus (Cr) is a putative periodontopathogen which expresses a proteinaceous surface layer (S layer) external to the outer cell membrane. S layers are considered to play a protective role for microorganisms in hostile environments. The S layer protein (~150 kDa) was extracted from *Cr* 314 strain (a human clinical isolate) by acid extraction and purified by preparative isoelectric focusing and electrophoresis. The S layer proteins from 6 different *Cr* strains varied in molecular weight by SDS-PAGE analysis revealed slight but reproducible differences in the molecular weights of the S layers from 6 *Cr* isolates (150-170 kDa). Western blot analysis using monospecific rabbit anti-S layer antiserum confirmed these differences Quantitative reactivity of the antiserum using an ELISA demonstrated an antigenic relationship among the six S layer positive human strains of *Cr* (OD 0.86-1.30), while a nonhuman primate (Nhp) strain showed less reactivity (0.55) which approached the antibody recognition of the S layer negative strain 33238S⁻ (0.42). Amino acid composition of S layer protein of *Cr* 314 showed that asparagine, aspartic acid, alanine, valine, glycine and lysine were major components and >30% of the amino acids residues were hydrophobic. S layer proteins from three *Cr* strains (314, 33238S$^+$ and 6250) were cleaved by cyanogen bromide. The SDS-PAGE profiles of the digested products were similar between the human isolates, but dissimilar between human and Nhp isolate. A 17 kDa digested polypeptide was observed in these three strains and a 24 kDa polypeptide fragment appeared in only the human strains. Amino-terminal sequence analysis revealed that the 17 kDa polypeptide had a similar amino acid sequence to a *Cr* 51 kDa porin and was composed of nearly 50% hydrophobic residues. The 24 kDa polypeptide had no homology to current database sequences **We conclude that the S layer proteins from *C. rectus* have immunoheterogeneities among different human strains and were significantly different antigenically from the monkey strain. The hydrophobic nature of the S layer and sequence homology may indicate an association between the S layer and the porins of the cell membrane.** Supported DE-07267

1490 Binding of Fibrinogen to *Streptococcus gordonii*. SI YOUNG LEE, SON JIN CHOE* and KACK-KYUN KIM (Dept. of Oral Microbiology, School of Dentistry, Seoul National University, Korea)

Attachment of bacteria to host tissues is commonly regarded as the crucial initial step in the pathogenesis of bacterial infections. In many bacterial infections, binding to extracellular matrix proteins has been described. *Streptococcus gordonii*, one of the strains most often associated with endocarditis in humans, was chosen to study oral bacterial binding to fibrinogen (Fgn). To quantitate the binding, the wells of 24-well plate were coated with 75 μg of Fgn/ml of 0.05M carbonate buffer (pH 9.5) for 16 h at 37 °C. [^{3}H]-labeled bacteria (5 x 10^7 cells/ml) in Hank's Balanced Salt Solution containing 0.5% BSA were added to each washed well and incubated with gentle agitation for 1 h at 37 °C. The attached bacteria were lyzed with a solution containing 1% SDS, 8M urea, and 1M NaCl for 30 min at 37 °C. The radioactivity of lysates was counted to enumerate number of bound bacteria. Most, except mutans group, oral streptococci tested adhered to Fgn, but they showed no adherence to BSA or gelatin. With a constant number of bacteria (5 x 10^7 cells/ml), coating wells of plates with Fgn solutions of increasing concentrations resulted in an increase in the number of adherent bacteria and the attachment was saturable. Heat-treatment of bacteria for 1 h at 95 °C resulted in 90% inhibition of the binding. To further charactenze the binding, various substances were tested for the capacity to inhibit. Treatment of bacteria with protease or trypsin caused inhibition. Neither lipoteichoic acid or various sugars nor the presence in bacterial medium of 3% sucrose, the substrate for exopolysaccharide production had any effect on binding. The observation that Fgn is recognized by oral bacteria points to the possibility that this interaction mediates bacterial attachment to host tissue, and the results suggest the bacterial component responsible for Fgn-binding is a protein nature.

1491 Characterization of *Actinobacillus actinomycetemcomitans* Adhesive Structures with a Peptide Library. L A. Kaiser*, A. Seyedain, P. Ciborowski, and R.R. Koepsel. (School of Dental Medicine, University of Pittsburgh, Pittsburgh PA).

In order to understand the interaction between oral microorganisms and the cells in the oral cavity it is necessary to dissect the mechanisms by which these interactions occur. We are investigating the bacterial structures involved in the adherence of *Actinobacicillus actinomycetemcomitans* (*A.a.*) within the oral cavity. We have used a random hexamer peptide library, displayed as part of a surface protein of the filamentous bacteriophage f1 (an *E. coli* phage), to select for peptides with specific affinity for the surface of *A.a.* The complete library was incubated with *A. a.* cells, the mixture incubated on ice for 30 minutes and the unbound phage removed by several washings. The bound phage were eluted with 2M urea. These phage were used to infect *E. coli* cells and the resulting amplified population was again used to bind to *A.a.* After five rounds of binding and amplification individual phage were selected for DNA sequence analysis of the peptide insert site. We obtained sequence information for 33 independent isolates. One of the sequences appeared four times in the 33 isolates and another appeared twice. Additionally there are several short sequence motifs that appear in a number of the sequences. In order to test whether these sequences can specifically bind to the surface of *A.a.*, we amplified individual phage and tested their binding ability relative to the complete library. A phage carrying the sequence which was most frequently isolated and a phage carrying one of the conserved motifs bound to *A a.* two orders of magnitude more avidly than the complete library. These results demonstrate a specific interaction between the peptide sequences and structures on the surface of *A.a.* We will use peptides identified in this manner to isolate those *A.a.* specific proteins involved in binding interactions.

1492 Buccal Epithelial Cell Adherence by a Clinical *Actinobacillus actinomycetemcomitans* Isolate. D.H. FINE*, D. FURGANG, L. STEINBERG, I. KORIK (New Jersey Dental School, Newark, NJ)

Bacterial attachment to epithelial cells is a critical virulence trait in the infectious disease process. This study compared attachment of a recent clinical isolate of *Actinobacillus actinomycetemcomitans (Aa)* to it's derived laboratory variant. CU1010 is a stable rough, star positive, adherent Rifampicin resistant (70μg/ml) strain derived from the clinical isolate CU1000, which maintains all measurable phenotypic characteristics of the parent. CU1012 is a stable smooth non-adherent Rifampicin resistant variant, which phenotypically resembles common Aa lab strains like Y4. CU1010 and CU1012 were grown up in AaGM-BV broth in Falcon Tissue Culture Flasks (# 3028) for 3 days @ 37°C and adjusted stocks (A_{560}=0.8) collected as previously described (AADR'95 abstract # 1505), and filtered on AaGM-Rifampicin agar plates. Buccal epithelial cells (BEC) were scraped from the inside cheek mucosa with sterile tongue depressors, washed and collected in PBS. CU1010 and CU1012 stock were treated either with; PBS, 1% BSA or a polyclonal antisera specific to clinical strain surface antigens (Ab). 500 μl of BEC were mixed with 500μl of either; CU1010, CU1012, BSA-treated CU1010, BSA-treated CU1012, Ab-treated CU1010 or Ab-treated CU1012. Controls of BEC alone, CU1010 alone and CU1012 alone were used. The mixtures were incubated for 1.5 hours at 37°C while rotating. Unbound Aa was separated by a 5% Ficoll 400 gradient. Serial dilutions of BEC bound Aa were plated on AaGM-Rifampicin agar. Plates were incubated in 10% CO_2 at 37°C for 60-70 hours. CU1012 demonstrated little if any adherence to BEC while CU1010 showed substantial adherence (3.77x10^5±1.44 Aa recovered/ml) a ratio of 65 Aa /BEC. BSA treatment did not effect adherence (3.15x10^5±0.45 Aa recovered/ml). Ab treatment did reduce adherence by 85.9%, (5.3x10^4±0.97 Aa recovered/ml), a ratio of 9 Aa/BEC. These results suggest an adhesin interaction unique to clinical Aa surface proteins. Differences in adherence between CU1010, Ab treated CU1010, and CU1012 were statistically significant (ANOVA analysis p=0.001). In conclusion, CU1010 demonstrates an adhesin based adherence to BEC which appears to be lost upon conversion to CU1012. This work suggests that strains undergoing conversion in the laboratory may lose important virulence traits.

1493 Characterisation of adherence epitopes of ArgI of *P. gingivalis*. M.A. CURTIS*, J. ADUSE-OPOKU, J.M. SLANEY & P. SHEPHERD[1] (MRC Group, Dept. Oral Microbiology, LHMC, London and [1]Dept. Immunology, UMDS, London. UK)

The extracellular proteolytic activity of *Porphyromonas gingivalis* W50 with specificity for arg-x peptide bonds is composed of three inter-related forms : ArgI, ArgIA and ArgIB. ArgI is a heterodimer of an α catalytic component and a β component, which on the basis of its primary sequence resembles haemagglutinins/adhesins from other bacteria. The aim of the present work was to determine the identity of the binding determinant(s) on the β component of ArgI using a panel of monoclonal antibodies (mAbs) produced to whole cells of *P. gingivalis* DCR 2015. Four mAbs (1A1, 2B/H9, 7D5 and 3B1) were shown by Western blotting to recognise determinants on the ArgI β component. One of the mAbs (1A1) was found to inhibit the haemagglutination of human red blood cells by *P. gingivalis* culture supernatants. The binding site for 1A1 was mapped by immunochemical analysis of different regions of the ArgI gene (*prpRI*) expressed in *E. coli* XL-1 Blue which showed that the 1A1 epitope was present within the β component of PrpR1 of residues 865-949 in the deduced sequence. Finer mapping was achieved by expression of the β component as a gluthathione-S-transferase fusion protein and a series of 3' truncations of this construct which spanned the region defined previously. On the basis of the immunoreactivity of these constructs the binding site for mAb 1A1 was located to within a twenty-five residue sequence at PrpR1 907-931. These data support the proposal that the β component of ArgI has a role in adherence mechanisms and that a determinant present within this region plays a critical role in the haemagglutination process of the whole organism.

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1494 Molecular Biological Analysis of Adhesin Component Protein from *Eikenella corrodens*. H. YUMOTO*, H. AZAKAMI, H. NAKAE, and S. EBISU (Dept. of Conserv. Dent., Tokushima University School of Dentistry, Tokushima, JAPAN).

We previously found that *Eikenella corrodens* 1073 had a cell-associated *N*-acetyl-D-galactosamine -specific lectin-like substance (EcLS), which mediated the adherence of this bacteria to various host tissue cell surfaces. In this study, we cloned the gene encoding one of the component proteins of EcLS. EcLS was divided into approximately 300kDa and 45kDa proteins by SDS-PAGE under reducing condition. Based on the NH_2-terminal amino acid sequence of this 45kDa protein, we cloned the region for N-terminus of 45kDa protein. To clone a complete gene of 45kDa protein, we performed gene walking by PCR and the Southern hybridization technique. This cloned gene was inserted into the T7 expression vector pET22b(+) and transformed into *E.coli*. Analyzing the nucleotide sequence of cloned fragments, we found an open reading frame (ORF) encoding a polypeptide of 330 amino acids (Mr: 35,748). This ORF displayed high degrees of homology to the porins of *Neisserial* species. Using the T7 expression system, a 45kDa protein was produced in *E. coli* after induction.

These findings show that the 45kDa protein of *E. corrodens* 1073 is one component of the EcLS complex, and may act as a major antigen and periodontopathogen on the outer membrane as well as *Neisserial* porin proteins.

1495 Effect of Ginger (Zingiber officinale Rosc.) on Adherence of *C. albicans*. S. THAWEBOON*, B. THAWEBOON and W. BUAJEEB. (Faculty of Dentistry, Mahidol University, Bangkok, THAILAND)

Ginger (Zingiber officinale Rosc.) is a local plant growing in almost every part of Asia and Africa. It is as a component of foods and beverages, and as a tasty vehicle for many pharmaceutical preparations. The purpose of this investigation was to study the action of the hot watery extract of ginger on the adherence of 3 strains of *C. albicans* (*ATCC 10281A* and 2 strains isolated from active infections) to human buccal epithelial cells (BEC). The extract was prepared by boiling the dried ground rhizome with distilled water at 80°C for 30 min. and filtering through gauze. BEC collected from 7 healthy male volunteers with no systemic disease or candidosis and not taking drugs were washed and suspended in phosphate buffer saline (PBS), (7.5x10^5 cells/ml). Equal volumes (400 μl) of BEC and each strain of *C. albicans* (10 yeast/ml) were mixed and incubated with 100 μl of ginger extract at 37°C for 30 min.as the experimental groups. One hundred μl of PBS was used instead of the ginger extract in the control groups. Cells were harvested on 12 μ-polycarbonate filter (Nucleopore Corp.,U.S.A.) and gram stained. The results showed that, at a concentration of 50% (w/v), ginger extract was significantly reduced the adherence of all strains of *C. albicans* to BEC. ($p<0.05$ as tested by t-test) (70.82% for *ATCC 10281A*, 56.60% and 58.00% for the 2 clinical strains). These indicate that ginger extract decreases the adherence of *C. albicans* to BEC and may be beneficial in preventing candidal colonization to the oral mucosa.

1496 The Effect of Oral bacteria on Candidal Adhesion to Buccal epithelial cells (BEC) *in vitro*. R. G. NAIR*, L. P. SAMARANAYAKE (Oral Biology Unit, Faculty of Dentistry, The University of Hong Kong, Hong Kong).

The effect of four different species of oral commensal bacteria (*Streptococci* (x2), *Porphyromonas* and *Escherichia* spp.) on the adhesion of *Candida albicans* and *Candida krusei* to the human buccal epithelial cells (BEC) was investigated using a new modified membrane filter system. The filters (12μ diameter pores) acted as a support for BEC which were pre-exposed to three known concentrations (10^6, 10^5, 10^4 organisms/ml) of bacterial suspensions (for 45 mns to 1 h), and subsequent reincubation with known concentrations (10^7 org/ml) of yeast suspensions for varying periods. The adherent yeasts on BEC, transferred on to a glass slide were Gram stained and counted using light microscopy (x100). Compared with the control group, all the bacteria significantly suppressed ($p<0.0001$) subsequent adhesion of *C. albicans*, to BEC except for *Strep. sanguis*. Both streptococcal species, *Strep. sanguis* and *Strep. salivarius* suppressed *C. krusei* adhesion when pre-exposed to three different bacterial concentrations, though variable results were obtained with *P. gingivalis* and *E. coli*. There were also significant differences in the relative adhesion of *C. albicans* and *C. krusei*. These results taken together, imply that the adhesion of yeasts to buccal epithelial cells is modulated both by the quality and the quantity of pre-existing bacterial flora on buccal epithelial cells.

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