TITLE: Recognition of laminin by pathogenic oral *Prevotella* ssp

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ABSTRACT:

Prevotella is a Gram-negative anaerobic bacteria associated with opportunist infections in oral, vaginal and gastrointestinal tract. Host tissue adhesion is considered the fundamental step to an infectious process and persistence. P. *intermedia*, *P. melaninogenica* and *P. nigrescens* require a strong adhesion capacity in the gingival sulcus to initiate colonization and induce an oral disease. Previous studies show that some strains of *P. intermedia* and *P. nigrescens* have a strong affinity for extracellular matrix (ECM) components mediated by the expression of surface proteins, which so far are poorly characterized. This study aims to evaluate the interaction between *Prevotella* spp. with ECM components and identify the bacterial ligands responsible for this adherence. Initially, we immobilized laminin on glass slides and challenged with different concentrations of bacteria. Adherence was quantified by counting the number of adhered cells per field of view in fluorescence microscopy. Binding of *P. nigrescens* increased with inoculum concentration on a dose-dependent manner. An average of 317.4 bacterial per microscope field of view was observed with an inoculum concentration of 10^8 CFU/ml whereas, 316.6 for $5x10^7$ CFU/ml and 155.4 for 10^7 CFU/ml. Adhesion to the negative control (BSA) at a concentration of 10^8 CFU/ml was 98.1. Similar results were observed in *P. melaninogenica*. Tests with *P. intermedia* showed lesser adhesion to laminin. For identification of bacterial ligands, extraction of outer membrane proteins (OMPs) was performed. Enriched OMP fractions were visualized by SDS-PAGE and different patterns of OMP were observed among the strains analyzed. These proteins were subjected to an affinity chromatography column consisting of laminin immobilized on NHS-activated Sepharose. The proteins eluted from the column were subjected to SDS-PAGE and stained with silver nitrate. The eluted samples were digested with trypsin and sent to mass spectrometry analysis. Our results indicate that *P. nigrescens* and *P. melaninogenica* strains are capable of adhesion to laminin and are suitable for future studies on ligand identification. Our study will allow us to understand the mechanisms involved in bacterial adhesion to host tissues and may help the development of new strategies to prevent this colonization.

Keywords: Laminin, Adhesion, Proteins

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