Title: Recognition of extracellular matrix molecules by anaerobic bacteria of medical interest

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Abstract:

Prevotella is a Gram-negative anaerobic bacteria found in oral, vaginal and gastrointestinal microbiota and has been associated with opportunist infections in such sites. Host tissue adhesion is considered the fundamental step to an infectious process and persistence. Bacteria may express on its surface, proteins that interact with components of the extracellular matrix (ECM) responsible for adhesion. *P. intermedia, P. melaninogenica* and *P. nigrescens* need 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 ECM components mediated by the expression of binding 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. Purified laminin was immobilized on glass slides and challenged with different concentrations of the bacterial strains. Adherence was quantified by fluorescence microscopy. Initial tests with *P. intermedia* showed no adhesion to laminin. Adherence 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 $5x10^7$ 10^8 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. melaninogenic*a. For identification of bacterial ligands, extraction of outer membrane proteins (OMPs) was performed. Enriched OMP fractions were visualized by SDS-PAGE and silver staining. It was observed different patterns of protein expression in strains analyzed. Eventually the enriched OMP extracts will be separated by affinity chromatography on a column containing immobilized laminin. Proteins eluted from this column will be identified by mass spectrometry. We concluded that the *P. intermedia* strain used does not have the ligands responsible for the adhesion to laminin, in contrast both the *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: *Prevotella*, Laminin, Adhesion, Outer membrane proteins

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