ABSTRACT

Title: ***Linking LC-MS Identified S. mutans Proteases to Collagen Type I and Gelatin Degradation Mechanisms***
Abstract

Dental caries remains one of the most prevalent oral diseases in the United States. If left untreated, these lesions can not only compromise oral health but also lead to pain, infection, systemic complications, and decline overall health. When involving dentin, as seen in root caries and moderate caries lesions, the process will also involve dentin breakdown. It has been reported that the mechanisms responsible for the destruction of the dentinal collagenous matrix are attributed to endogenous enzymes (MMPs) present in dentin and in the oral cavity. Conversely, bacterial enzyme involvement has been reported, though its role remains controversial. In my previous research, I confirmed the presence of proteases in the intracellular fraction of *S. mutans*, and they demonstrated both gelatinolytic and collagenolytic activity. Moreover, the genes encoding these proteases, have been previously documented in the literature. Confirming their ability to degrade type I collagen is essential to later define their role in dentin breakdown during caries progression. This insight will identify key enzymes involved in the process and enable innovative strategies to arrest caries. This research proposal aims to determine whether SMU_759 and SMU_761 are the *S. mutans* proteases involved in collagen and gelatin degradation using gene disruption and protein overexpression. The study will employ the cloning-free gene disruption method to delete the *S. mutans* protease genes and later clone the target genes into a His-tagged pET21 vector for recombinant expression in *E. coli*. The proteins will be purified and confirmed by SDS-PAGE, providing material for future assays on gelatin and collagen activity and interactions with endogenous MMPs. The findings will clarify the mechanisms of these proteases and support future research proposal (NIH R21) in their role in caries progression, their interactions with endogenous MMPs, and the development of innovative strategies to target both bacterial and endogenous proteases.