Development of a qPCR Assay for Quantification of Saccharibacteria

Lin, John (School: Boston Latin School)

Saccharibacteria is an extremely small (~200nm) uncultivable microbe that lives an unique parasitic lifestyle on various host bacteria, including P. propionicum. Saccharibacteria was recently found in abundance in plaque samples of patients with periodontal disease. There is limited knowledge about the behavior of this intriguing microbe because of its evasiveness from traditional, bacterial quantification methods due to its minuscule size and inability to be cultured independently. This study aims to develop a unique method to quantify this bacteria. Primers were designed and optimized to target conserved regions specific to the Saccharibacteria genome. A plasmid was constructed using amplified regions of Saccharibacteria DNA and transformed in E. coli. The plasmid was extracted and diluted to a known number of plasmid copies. Serial dilutions were performed and run on a qPCR. A calibration curve was developed based on the qPCR data. Afterward, the assay was applied to compare Saccharibacteria growth with that of its hosts. A highly accurate assay was developed with 91.38% efficiency for quantifying Saccharibacteria. In addition, analysis of the growth curves reveals that Saccharibacteria enters stationary phase earlier than its host possibly to conserve resources before the growth of its host slows. These findings open the door to various applications. Clinically, the assay can be used as a tool to diagnose and assess the risk for periodontitis based on the number of Saccharibacteria present. In the research setting, the assay can be used to further examine the behaviors of Saccharibacteria, its interactions with its host, and its pathogenicity.

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