

Role of Hemin and Growth Media on the Autofluorescence of *Streptococcus mutans*

Grace F. Gomez¹, George J. Eckert², Richard L. Gregory¹; ¹ Indiana University School of Dentistry, Indiana, Indianapolis, Indiana; ²Department of Biostatistics, Indiana University, Indianapolis, IN.

Abstract

Carious lesions fluoresce under blue light. The primary cariogenic bacterium *Streptococcus mutans* has been shown previously to fluoresce within blue light wavelengths. In this study we wanted to determine the role of hemin and various growth media on the fluorescing properties of *S. mutans* under planktonic (total biomass) and biofilm (biofilm mass) growth conditions. UA159 was grown for 24 h at 37°C in Tryptic Soy Broth (TSB), Brain Heart Infusion (BHI) and Todd Hewitt broth (THB) with and without hemin in 5% CO₂. Biofilm was grown for 24 h in a 96 well sterile microplate in the above described media with and without hemin. A stock solution of Protoporphyrin –IX was prepared and diluted to concentrations ranging from 1.6-3.1×10⁻⁴ g/ml. A SpectraMax (M3) was used to determine the fluorescence from UA159. RFU of total biomass and biofilm mass was assessed by exciting at fixed wavelengths of 385 and 405 nm at a spectral band width of 10 nm. Emission spectra at 770 nm were observed with 385 nm and an emission of 800 and 810 nm with 405 nm. ANOVA on the ranks of the measurements was used, with four different factors including wavelength (770, 800 and 810 nm); total biomass or biofilm mass; various growth media (TSB, BHI, THB) and the presence/absence of hemin and interactions among the factors. The analysis allowed each media-hemin combination to have different variances. Hemin decreased the amount of fluorescence; regardless of the levels of the other factors (p≤0.0001). Without hemin, BHI had more fluorescence than THB (p≤0.0003) and TSB (p≤0.0001). However with hemin, THB had more fluorescence than BHI (p≤0.0001) and TSB (p≤0.0001). The role of hemin and porphyrin-related compounds in the metabolism of *S. mutans* should be elucidated.

Keywords: *Streptococcus mutans*, fluorescence, biofilm

