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Sarah Brusko
Virginia Commonwealth University

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Characterization of Metal Transport by the Streptococcus sanguinis Endocarditis Virulence Factor SsaB

Sarah Brusko and Todd Kitten

Phillis Institute for Oral Health Research, Virginia Commonwealth University, Richmond, VA

Streptococcus sanguinis and Infective Endocarditis

- Streptococcus sanguinis, present in the biofilm colonizing human tooth surfaces, may be beneficial in the oral cavity, though it serves as a causative agent of an extra-oral disease, infective endocarditis (Beila-Feer et al., 2012; Bor et al., 2013).
- Infective endocarditis, infection and inflammation of the heart valves or endocardium, diminishes proper functioning capabilities of the heart. Complications include congestive heart failure, aneurysm, and stroke (Bashore et al., 2006).
- S. sanguinis enters the bloodstream through lacerations of the oral cavity and may attach to pre-existing vegetations composed of platelets and fibrin formed previously in response to cardiac injury.
- Dental procedures, routine oral hygiene maintenance, and chewing can lead to this transient bacteremia (Wilson et al., 2007).

Determination of Metal Content Using Inductively Coupled Plasma Optical Emission Spectrometry

- For experiments involving growth in brain-heart infusion (BHI) broth (Difco), cells were cultured overnight in 80% BHI/20% pooled rabbit serum in 6% O2. Three ml of each culture was then added to 36 ml BHI broth that had been pre-incubated under the same conditions. Incubation was continued at 37°C for 5 hours.
- For growth in all purpose tween (APT) broth (Difco), cells were cultured anaerobically overnight in APT broth, then diluted as above in pre-warmed APT and incubated at 37°C for 6 hours. E. coli cells were incubated with shaking (225 rpm), while the other strains were incubated statically in tightly-sealed tubes containing anaerobically-preincubated APT.
- Cells were harvested by centrifugation at 4°C for 10 min at 3,743 x g and washed twice in 10 ml cold phosphate-buffered saline (PBS) that had been pre-treated with Chelex prior to use, and all glass and plastic vessels used for metal analysis were soaked overnight in 1 M HNO3 prior to use.

Next, SK36 and the ssaB mutant were analyzed following growth in APT broth, rich in both metal ions, to determine whether this was due entirely to the higher concentration of iron relative to manganese in BHI broth.

Analysis of Metal Content by ICP-OES in Cells Grown in APT Broth

- Higher accumulation of manganese than iron in APT broth compared to BHI broth for both SK36 and the ssaB mutant suggested that manganese is accumulated through both SsaB-dependent and SsaB-independent mechanisms.
- Relative cellular abundance of iron and manganese in S. sanguinis varies dramatically depending on relative abundance in the growth medium, highlighting the importance of using physiologically relevant media in future studies.

- This data also implies that S. sanguinis is flexible in its metal requirements and is rather efficient in sequestering iron, which would otherwise react with cellular hydrogen peroxide to produce DNA-damaging hydroxyl radicals via the Fenton reaction.

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