

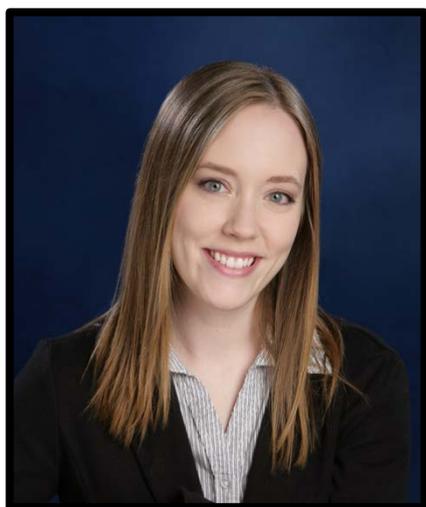
THE GRADUATE COLLEGE OF THE
UNIVERSITY OF OKLAHOMA HEALTH SCIENCES CENTER

ANNOUNCES THE FINAL EXAMINATION OF

Nyssa C. Cullin

FOR THE DEFENSE OF THE DOCTOR OF PHILOSOPHY DEGREE
GRADUATE COLLEGE

Department of Microbiology and Immunology



Wednesday, November 29, 2017, 1:30pm
Bird Library Auditorium, 299

CHARACTERIZATION OF THE MUREIN HYDROLASE LYTF OF *STREPTOCOCCUS SANGUINIS*

COMMITTEE IN CHARGE: Jens Kreth, PhD, Co-Chair;
David Dyer, PhD, Co-Chair; Darrin Akins, PhD; Eric Howard,
PhD; Rodney Tweten, PhD; Lauren Zenewicz, PhD

ABSTRACT: Dental caries, or tooth decay, is an incurable biofilm-associated disease that results from dysbiosis of the oral bacterial population and commonly affects both children and adults. Understanding how the oral biofilm develops and maintains itself is necessary to developing preventative measures. Extracellular DNA (eDNA) is essential to the biofilm and contributes physically through cell-cell and cell-tooth aggregation, nutritionally as a carbon and energy source, and genetically by increasing overall genetic diversity. *Streptococcus sanguinis*, a highly abundant, health-associated early colonizer, supports the uptake and release of eDNA via activity of the competence pathway, but the mechanisms for eDNA release are largely unknown. It was hypothesized that LytF, a competence-associated, murein hydrolase in *S. sanguinis*, plays a role in eDNA release and biofilm development; thus, the objective of this study was to investigate the

regulation and activity of LytF.

Using *lytF* mutant and complemented strains, the competence regulation of *lytF* and hydrolytic activities of its gene product were examined. While expression of competence related genes was not altered in the *lytF* mutant, transformation efficiency was decreased and bacterial chains became elongated. Zymogram analysis proved that LytF is indeed a murein hydrolase and is not species-specific with regard to its muralytic activity; it can also hydrolyze the peptidoglycan of other oral streptococci. Scanning electron microscopy showed that bacteria producing an excess of LytF presented with morphological abnormalities characterized by enlarged and engorged cells. Additionally, these bacteria showed a decrease in biofilm formation. When purified LytF was used as a treatment it was shown to induce the release of eDNA from *S. sanguinis*, *S. gordonii*, *S. mutans*, and *S. oralis*. Similar treatment conditions showed extreme morphological abnormalities in biofilms.

In all, LytF is a highly regulated murein hydrolase that plays a role in eDNA release, biofilm formation, and due to its overall conservation among other oral streptococci, a potentially significant role in oral biofilm ecology. This work demonstrates that proper regulation of this competence-controlled protein is essential to *S. sanguinis* biofilm development and furthers the need to understand how bacterial species maintain themselves to contribute to the overall health of the oral biofilm.