INSTITUTO DE TECNOLOGIA QUÍMICA E BIOLÓGICA /UNL
Knowledge Creation

Master Dissertation Project

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Lab/Institution: Bacterial Cell Surfaces and Pathogenesis Laboratory / ITQB
TITLE: Role of PGN hydrolases in the ability of *S. aureus* to evade the host innate immune system

BACKGROUND

Peptidoglycan (PGN) is a macromolecule composed of long glycan strands, cross-linked by short peptides, which surrounds most bacterial cells and forms a load-bearing mesh that sustains their shape. Bacterial PGN is telltale molecule that betrays the presence of bacteria. It is concealed by an outer membrane in Gram-negative bacteria, or by layers of proteins and glycopolymers in Gram-positive bacteria. It was assumed that an infected organism could only recognize PGN in the form of fragments released into the surrounding medium by the activity of different bacterial PGN hydrolases or specific host enzymes.

We have recently changed this view by showing that PGRP-SA, a PGN receptor protein produced by the Drosophila innate immune system to detect PGN and give away the presence of bacteria, can directly bind PGN at the bacterial surface in conditions such as the absence of wall teichoic acids (WTAs) (***Atilano et al., PLoS Pathogens 2011***). We have also shown that Atl and LytA, the major PGN hydrolases produced, respectively, by *S. aureus* and *S. pneumoniae*, important Gram-positive bacterial pathogens, trim the outermost peptidoglycan fragments to prevent PGRPs from recognizing leftover peptidoglycan molecules extending beyond the external layers of bacterial proteins and polysaccharides (***Atilano, Pereira et al., eLife 2014***). With this project we want to address the role of other autolysins in the ability of *S. aureus* to evade detection by the host innate immune system.

OBJECTIVES

1. To determine the role of different *S. aureus* PGN hydrolases (autolysins) in the concealment of bacteria to host PGN receptors.
2. To determine the sub-cellular sites of the bacterial cell surface targeted by these *S. aureus* autolysins.
3. To compare the processes that a bacterial PGN hydrolase and a host PGN receptor use to bind PGN within the bacterial cell surface.

PROJECT DESCRIPTION

Task 1

Construction of plasmids that permit the expression of a *S. aureus* PGN hydrolase, and its derivative linked to GFP fluorescent protein, in *Escherichia coli*. The availability of PGN hydrolases linked to GFP will permit the co-visualization with mCherry-PGRP-SA, a fluorescent PGN host receptor (***Atilano et al., PLoS Pathogens 2011***).

Task 2

Purification of the *S. aureus* PGN hydrolase and determination of its ability to digest purified PGN. The enzymatic activity of the purified PGN hydrolase will be compared to that previously observed with the staphylococcal Atl (***Atilano, Pereira et al., eLife 2014***).

Task 3

Determination of the sub-cellular sites of the bacterial cell surface of *S. aureus* that are targeted by the staphylococcal PGN hydrolase using fluorescence microscopy. We will compare the localization and intensity of the fluorescent signal observed with that reported for mCherry_PGRP-SA (***Atilano et al., PLoS Pathogens 2011***).

TIMELINE (use fill tool for the cells)

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10
Task 1	■	■	■	■						
Task 2				■	■	■	■			
Task 3							■	■	■	
Thesis									■	■