SCIENCESPRINGDAY

FACULDADE DE CIÊNCIAS E TECNOLOGIA UNIVERSIDADE NOVA DE LISBOA

Department of Life Sciences (DCV-FCT/UNL)

Peptidoglycan amidation: an unexplored step of bacterial cell wall synthesis.

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Objectives

Peptidoglycan is a unique and essential stress-bearing and shape-maintaining polymer of the bacterial cell wall; its biosynthetic pathway is the target of the clinically most relevant antibiotics.

The identification and characterization of MurT and GatD enzymes involved in the amidation of the glutamic acid residues of the *Staphylococcus aureus* peptidoglycan (Fig.1), lead us to the finding that this secondary modification of the peptidoglycan synthesis is essential for bacterial growth, antibiotic resistance expression (Fig.2) and pathogenecity, namely the muramidase activity of lysozyme (Fig.3).

The major goal of this work is to decipher the detailed molecular mechanism of one of the few unexplored steps of bacterial cell wall biosynthesis, information that could validate peptidoglycan amidation as a target for novel antimicrobial agents.

Methodology

- Characterization of the physical interaction of the protein complex by Bacterial two-hybrid system and Surface plasmon resonance (SPR) using the His_6 tagged fusions of MurT and GatD; site-specific mutagenesis to identification the essential amino acids for physical interaction and for amidation activity.
- Determination of the virulence potential of the peptidoglycan amidation:
  - on the host inflammatory response by measuring the capacity of several bacterial strains to stimulate IL-1β and TNFα secretion in macrophages.
  - on the course of the processes of infection and colonization, using murine models.
- Determination of the structure of the MurT-GatD complex and the amino acid residues essential for the complex formation using crystallography and NMR approaches.

Expected Results

- Characterization of the MurT/GatD complex in *S. aureus*
- Identification of the amino acids essential for physical interaction and for amidation.
- Information of the nature of the physical association between MurT and GatD and of the structure of the complex .
- Establishment of the MurT/GatD complex as a new virulence factor – role in host evasion.
- Impact of peptidoglycan amidation in an animal model of infection and colonization

Team

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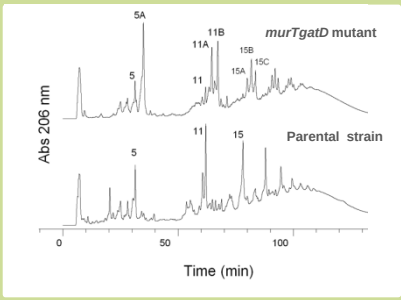

Fig. 1 – RP-HPLC profiles of cell Walls of the *murTgatD* mutant.

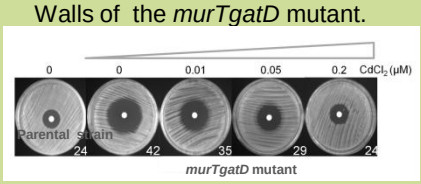

Fig. 2 – Reduced antibiotic Resistance of the *murT-gatD* mutant.

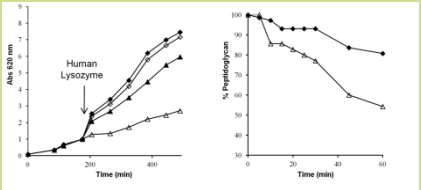

Fig. 3 - Increased sensitivity to lysozyme in *murT-gatD*l mutant.

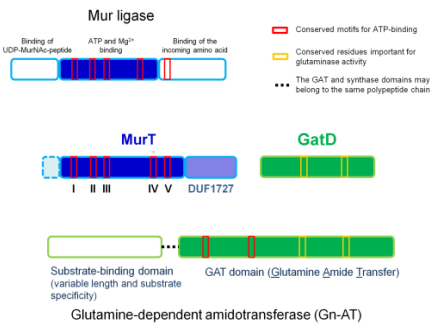

Fig. 4 - Proposed Model of MurT/GatD functional organization.

(1) Figueiredo, T. A., R. G. Sobral, A. M. Ludovice, J. M. de Almeida, N. K. Bui, W. Vollmer, H. de Lencastre, and A. Tomasz. 2012. Identification of Genetic Determinants and Enzymes Involved with the Amidation of Glutamic Acid Residues in the Peptidoglycan of *Staphylococcus aureus*. PLoS Pathog. 8:e1002508.

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