## Poster 151

# Biochemical characterization of highly stable endolysins with a powerful and broad anti-Gram-negative lytic activity in the presence of weak acids

Hugo Oliveira[a,*], Thiagarajan Viruthachalam[b], Maarten Walmagh[c], Sanna Sillankorva[a], Francesco Secundo[d], Rob Lavigne[c], Maria Neves-Petersen[e], Leon Kluskens[a], Joana Azeredo[a]

[a] *Centre of Biological Engineering, University of Minho, Braga, Portugal*
[b] *School of Chemistry, Bharathidasan University, Tiruchirappalli, India*
[c] *Laboratory of Gene Technology, Katholieke Universiteit Leuven, Belgium*
[d] *Istituto di Chimica del Riconoscimento Molecolare, Milan, Italy*
[e] *International Iberian Nanotechnology Laboratory, Braga, Portugal*

Endolysins are bacteriophage-encoded specialized peptidoglycan-degrading enzymes, individually evolved to cause bacteriolysis and thus enabling the release of bacteriophage's progeny. Since the discovery of its genetic identity, endolysins quickly sparked the interest of many researchers and companies to believe that they can become a new generation of antimicrobials, especially against Gram-positive bacterial pathogens. However, exogenous endolysin action on Gram-negative bacteria is still restricted, due to the presence of an impermeable outer membrane, thus representing one of the most important challenges in endolysin therapy, which are explored here.

In this work, three different novel Gram-negative-like endolysins were characterized, in terms of their muralytic activity and (thermo)stability, using turbidimetry, circular dichroism and fluorescence measurements. We showed that these endolysins can be stored for at least two months, are stable and active under various pH values, and can also be highly thermostable, withstanding temperatures up to 100°C. It was demonstrated that endolysins are able to refold into its original conformation upon thermal denaturation explaining their high thermostability. Antibacterial assays were performed to investigate the endolysins` anti-Gram-negative activity. Interestingly, some endolysins can intrinsically destabilize the bacterial outer membrane and thus are natural efficient antimicrobials. To boost the antibacterial activity, endolysins were combined with several types of outer membrane permeabilizers, such as EDTA and organic acids, against a wide panel of Gram-negative bacteria, comprehending Enterobacteriaceae, Pseudomonadaceae and Moraxellaceae species. This synergy demonstrated a powerful and broad antibacterial effect of endolysin/weak acid combinations in comparison with the well-studied EDTA, active against planktonic, stationary and bacterial biofilms. Weak acids diffuse inside the cells, compromising the bacterial lipopolysaccharide and thus permeabilizing it to endolysins that retain a relative high muralytic activity in an acidic environment. The type of lipopolysaccharide structure and composition explains the antibacterial differences observed when endolysins are combined with specific outer membrane permeabilizers. Altogether, this work enlarges the knowledge of Gram-negative-like endolysins and illustrates potential ways to prevent and/or control Gram-negative pathogens.