A new phage endolysin as a powerful tool to detect and kill Paenibacillus larvae

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American foulbrood (AFB) is an infection caused by *Paenibacillus larvae* (*P. larvae*), a Gram-positive spore forming bacteria. This disease occurs in honeybee larvae, when spores germinate and proliferate in their midgut and subsequently penetrate into the hemolymph, causing sepsis and larval death. This work was motivated by the need of finding alternatives to antibiotics, that will leave residues in honey if used to treat this infection.

Bacteriophages (phages) and/ or their endolysins might represent valuable tools to use in AFB control as have already proved to be powerful biological antimicrobials. We have previously isolated and reported the first known *P. larvae* phage genome and by its in silico analysis we further identified, expressed and characterized the first *P. larvae* endolysin, PlyPl23. This enzyme has two functional domains: a catalytic domain (Amidase_2) and a totally new cell wall binding domain (CBD). The latter confers specificity to the enzyme, targeting specific bonds of the cell wall surface.

The antimicrobial activity of PlyPl23 was tested in vitro against a panel of *P. larvae* strains and *in vivo* in bee larvae. PlyPl23 was effective in decreasing *P. larvae* infection yields in bee larvae experimentally infected with spores and no toxicity effects were encountered.

In a complementary study, the cell wall binding domain (CBD) of PlyPl23, fused to a green fluorescence probe was heterologously expressed. The specificity of the PlyPl23 CBD was assessed through flow cytometry and epifluorescence microscopy. Overall the results demonstrate the potential of a phage endolysin for detection and control of *P. larvae*.