

Talk

Expanding the enzybiotic toolbox by switching catalytic and cell wall binding domains



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ABSTRACT

Motivation: Bacterial resistance to common antimicrobial therapies (i.e., antibiotics) is reaching alarming levels worldwide [1]. This pushes for the development of alternative antimicrobials, such as bacteriophage-encoded endolysins, also called enzybiotics. This antibacterial approach, rooted in phage therapy, could pose a new and valuable adding to the antimicrobial arsenal, with increased specificity and much lower chances of enabling bacterial escape and resistance mechanisms when compared to antibiotics [2]. Endolysins are usually comprised of a cell wall binding domain (CWBD) and one or several catalytic domains that cleave the bacterial cell wall. Both rational design and domain shuffling have proved to be effective approaches towards the improvement of phage endolysins for therapeutic uses [3]. Thus, the aim of this work is to construct new endolysins active against a range of pathogenic bacteria by switching protein domains.

Methods: A new lysin, termed Csl2 was designed by fusing the catalytic lysozyme domain of Cpl-7, encoded by pneumococcal phage Cp-7 [3], and the CWBD from LySMP, encoded by *Streptococcus suis* phage SMP [4]. The gene encoding the chimeric enzyme was cloned in the expression vector pT7-7, overexpressed in the *Escherichia coli* strain BL21(DE3) and the protein Csl2 purified following a two-step chromatographic procedure. Relevant biochemical properties of Csl2 were studied, and its bacteriolytic activity was tested against several Gram-positive pathogens.

Results: Protein Csl2 was efficiently overexpressed in *E. coli* BL21(DE3) and purified to >90%. Its secondary structure was similar to that of Cpl-7 according to preliminary circular dichroism experiments. Csl2 was found to be active against several streptococci, mainly from mitis group (namely: *S. mitis*, *S. oralis*, *S. pseudopneumoniae*) and *Streptococcus suis* from serotypes 2 and 9. The bacteriolytic host range of Csl2 differed from that of parental enzymes, with higher activity against *S. suis* strains.

Conclusions: 1) A new bacteriolytic enzyme, Csl2, was constructed by combining Cpl-7 catalytic domain and LySMP putative Cpl-7-like CWBD.

2) Csl2 resembles Cpl-7 biochemically and structurally, but has a different bactericidal spectrum.

3) Csl2 is specially active against several *S. suis* strains, so it might be a candidate for therapy in *S. suis* infections.

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