## Characterization of MSlys, the endolysin of Streptococcus pneumoniae phage MS1

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## Abstract

Streptococcus pneumoniae is one of the most frequently isolated pathogens that colonize the upper and lower respiratory tract. This colonization can be responsible for diverse diseases, including otitis media, pneumonia, among others. Despite the use of pneumococcal conjugate vaccines, the number of isolated S. pneumoniae continues to be alarming. In this work, we identified and characterized a novel endolysin (MSlys) encoded in the pneumococcal phage MS1. We further performed antimicrobial assays with MSlys against planktonic and biofilm cells, evaluating their viability before and after treatment. Additionally, the activity of MSlys on cells was also analyzed using scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM). MSlys is a modular endolysin carrying a catalytic domain with amidase activity and a choline-binding domain, folding mostly in  $\beta$ -sheets. MSlys is active against clinical S. pneumoniae collected from children with otitis media and in conditions close to those found in the middle ear. Treatment for 2 h with MSlys (4  $\mu$ M) reduced planktonic cultures by 3.5 log10 CFU/mL, and 24- and 48-h-old biofilms by 1.5 and 1.8 log10 CFU/mL, respectively. Imaging of biofilms by SEM and CLSM after MSlys treatment showed damaged and thinner biofilm structures compared to the control samples. The recombinantly expressed MSlys may be a suitable candidate for the treatment of pneumococcal infections, including middle ear infections.

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