Effect of different cloning strategies in pET-28a on solubility and functionality of a staphylococcal phage endolysin

ABSTRACT

Endolysins have been studied intensively as an alternative to antibiotics. In this study, endolysin derived from a phage which infects methicillin-resistant Staphylococcus aureus (MRSA) was cloned and expressed in Escherichia coli pET28a. Initially, the endolysin was cloned using BamHI/XhoI, resulting in expression of a recombinant endolysin which was expressed in inclusion bodies. While solubilization was successful, the protein remained nonfunctional. Recloning the endolysin using NcoI/XhoI resulted in expression of soluble and functional proteins at 18°C. The endolysin was able to form halo zones on MRSA plates and showed a reduction in turbidity of MRSA growth. Therefore, cloning strategies should be chosen carefully even in an established expression system as they could greatly affect the functionality of the expressed protein.

Keyword: Antimicrobial activity; Endolysin; Inclusion body; MRSA; pET28; Protein expression