

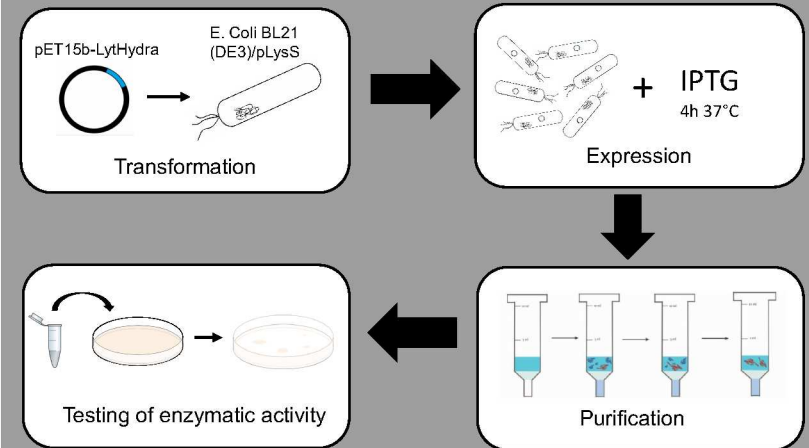
INTRODUCTION

According to the World Health Organization (WHO), the problem of antimicrobial resistance (AMR) is alarming. The growing number of multidrug-resistant bacteria draws researchers' attention to finding an alternative to antibiotics. One of these may be endolysins in the form of enzybiotics, specific enzymes degrading peptidoglycans of bacteriophage. Enzybiotics are antimicrobial substances based on enzymes and their biggest advantage unlike antibiotics is their specificity.

LytHydra is endolysin from actinophage Hydra infecting *Streptomyces griseus* with amidase activity. It is N-acetylmuramoyl-L-alanine amidase, which means LytHydra cleaves the bond between N-acetylmuramoyl acid residue and L-alanine. This bond is present in every peptidoglycan chemotype, which makes LytHydra very good candidate for enzybiotic. LytHydra has modular structure typical for gram-positive infecting phage endolysins:



MATERIALS AND METHODS



RESULTS

Genetic material:

LytHydra synthetic gene with optimised codon usage for *E. coli*
E. coli BL21 (DE3)/pLysS

Simple expression conditions and high purification efficiency:

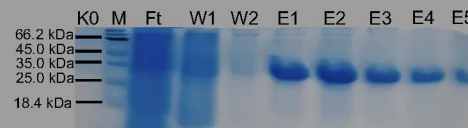
expression – 4 h, 37°C, LB medium
 purification – sonification
 Ni-NTA agarose (+cut of columns if necessary)
 purification efficiency – about 1.6 mg from 100 ml culture medium

LytHydra enzyme properties:

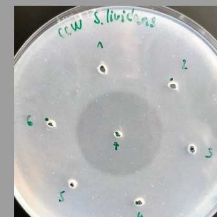
MW = 36758.90 Da
 pI = 9.91
 Ext. coefficient = 54110

High enzyme activity:

S. coelicolor – after 24 hours 5 µl of solution (about 5 µg of enzyme)
S. aureus – after 48 hours 5 µl of solution (about 5 µg of enzyme)
 cell wall suspension – after 24 hour 5 µl of solution (about 5 µg of enzyme)



Enzymatic activity testing on *S. coelicolor* after 24 hours.



Enzymatic activity testing on *S. lividans* cell walls after 24 hours (sample 7).

CONCLUSION

- Recombinant protein LytHydra was expressed, purified and its enzymatic activity was proven and confirmed.
- The most advantages are simple production conditions, effective purification and high enzymatic activity.
- LytHydra, as the predicted N-acetylmuramoyl-L-alanine amidase, cleaves the bond between N-acetylmuramic acid and L-alanine, which is found across all peptidoglycan chemotypes.
- All of these properties make Lythydra a suitable candidate for use as an enzybiotic, either unchanged or modified by bioengineering due to its domain composition.

ACKNOWLEDGEMENT

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