Reconstruction of lantibiotic gene cluster in methylotrophic yeasts P. pastoris

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The number of new cases of multidrug-resistant infections increases annually. Thereby, the discovery of new antibiotics becomes a high-priority challenge for biomedicine. Lantibiotics are ribosomally synthesized and post-translationally modified peptides that carry noncanonical thioether amino acids lanthionin and methyllanthionin. Lantibiotics mediate their antibacterial activity by blocking the cell wall synthesis or forming pores in a bacterial membrane. However, their clinical application is limited due to low stability and solubility, insufficient production levels and narrow activity spectra. Nevertheless, the genetically encoded architecture of lantibiotics allows the creation of various peptides that could be modified with lantibiotic biosynthesis machinery resulting in a more advanced antimicrobial drug. In this work, we focused on the development of a heterologous expression system for lantibiotic. The biosynthetic cluster composed of pre-lantibiotic sequence, methyltransferase and lantibiotic synthetase (LanL) was transferred into methylotrophic yeast *Pichia pastoris*. We found that particular pre-lantibiotic sequences were essential for efficient lantibiotic production. We substituted the original lantibiotic core sequence with a panel of natural lantibiotics to understand whether LanL is substrate-tolerant toward lantibiotics of different classes and architecture. We analyzed structures and antimicrobial activity of the purified lantibiotics comparing recombinant lantibiotics with native once. The recombinant lantibiotics displayed pronounced antibacterial activity against gram-positive bacteria including pathogenic *S. aureus* MRSA strain. We consider our yeast-based system for lantibiotic production combined with gene-editing techniques and high-throughput screening methods could serve as a useful tool in the discovery of the next-generation antimicrobial agents. This work is supported by RFBR grant 18-29-08054.