**ABSTRACT**

Isolation, Heterologous expression, Purification, and Characterization of Plantaricins derived from Soil Metagenome

In the present study, metagenomic screening was employed to search for antimicrobial peptide antibiotic genes, or their variants and characterize them further to understand their function. Soil was chosen from dairy-based area as it is likely to contain lactic acid bacteria which are well known source of one such class of antibiotics, bacteriocins. A metagenomic soil DNA library was constructed using fosmid vector (pCC2FOS) and screening of clones was done using *Micrococcus luteus* and *Bacillus subtilis* as indicator organisms. Since the functional analysis did not show any positive result, clones were probed through colony PCR, with the help of gene-specific primers belonging to different classes of bacteriocins. Different amplicons obtained were sequenced and identified, which belong to nisin, plantaricin and enterocin. Further work was focused on plantaricins, belong to 2-peptide classIb bacteriocin. Gene-specific primers were designed to amplify *pln* E, F, J, and K genes from metagenomic DNA. These amplicons cloned in pGEM-T were sequenced and analyzed. The sequence of *plnE*, *F*, and *J* were identical to those reported in the database, but some difference was observed in *plnJ* sequence. These PCR amplicons were expressed in *E. coli* BL21(DE3) as TRX-(His)$_6$-fusion peptides and Pln E, F, J and K peptides were purified using Ni-NTA affinity chromatography, and finally retrieved after enterokinase treatment. All four peptides were active at nanomolar to micromolar range and their effects were dose- and host-dependent. PlnE/PlnF as well as PlnJ/PlnK showed synergistic activity against all the indicator organisms tested. Hybrid peptides were constructed by swapping the N-terminal and C-terminal region of of PlnE and PlnF and their effect was checked against same indicator organisms. Both the hybrid peptides retained antimicrobial activity, but were found to be less active than individual PlnE and PlnF, indicating the requirement of both the components of the two peptides together. Fused PlnJ-K peptide derived from gene fusion also did not show any additive effect, and its activity was comparable to PlnJ. Comparative analysis of growth of some *pln* recombinant clones in LB and Terrific broth (TB) showed similar response. Expression and production of plantaricin (PlnE) was higher in LB compared to TB. Using conditions optimized in batch culture, large scale production was carried out in a fermenter, where yield was maintained, which indicated the high stability of recombinant plasmids. Purification of recombinant PlnE pre-peptide, ABC transporter (PlnG), and accessory protein (PlnH) was carried out and their role in cleavage of PlnE precursor peptide was illustrated with the help of an in vitro assay. Activity of plantaricins was checked against Gram-negative organism, *E. coli* as well. It was found that with the help of some pre-treatments *E. coli* can be made susceptible to plantaricins. Finally, the present investigation has demonstrated that by increasing the yield of purified bacteriocins their strong antimicrobial activity can be tested against many bacteria, such that their application as a therapeutic molecule as well as a food-biopreservative can be realized.