ABSTRACT

Since 1928, different types of antibiotics have been isolated and purified from the commonly available microbial sources like bacteria and fungi. Such popular antibiotic drugs include penicillin, streptomycin, chloramphenicol, vancomycin, erythromycin, polymyxin and bacitracin, etc. Similarly, various anti-microbial, antibacterial proteins and immuno-modulatory compounds routinely used in chemotherapy have been isolated from the variety of medicinal plants, mammals and seaweeds. Even though there are number of antibiotics being produced and marketed globally; the thrust for identification and screening of new antibiotics becomes extensive and unquenchable. These attempts have become unavoidable because of the evolution of drug resistant microbes from the originally susceptible target of the existing drugs and origin of new diseases for the human kind and animals.

The study began with isolation of bacteria from different natural habitats. The cells free culture supernatants of those isolates were tested for antibiotic activity against various pathogenic organisms set up to find out the bioactivity of microbial cell pellet and their culture supernatants. As a result of repeated experiments a new gram positive bacterial strain was isolated from fermented tomato fruit that secreted two new peptide antibiotics. Based on its 99% 16S rDNA sequence similarity with
Paenibacillus alvei, the isolate was designated as Paenibacillus alvei NP75. Among these two peptides, one is active against G-Gram-positive pathogens (Streptococcus pyogenes, Staphylococcus aureus, and Corynebacterium diphtheriae) while the other against G-Gram-negative organisms (Enteroxopathogenic E. coli. (EPEC), Rabbit Enteroxopathogenic E. coli. (REPEC), Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Klebsiella pneumoniae, Shigella flexineri, Citrobacter freundii and Pseudomonas aeruginosa) thus these peptides were named as paenibacillin P and paenibacillin N, respectively.

Both the peptides were purified from the cell free culture supernatant (CFCS) of the NP75 strain using sequential ultra-filtration, ammonium sulphate precipitation and reversed phase high performance liquid chromatography (RP-HPLC). The stability of the purified peptides at different temperatures, hemolytic properties and their susceptibility for proteases has been analyzed. Both paenibacillins were non-hemolytic whereas the strain Paenibacillus alvei NP75 displayed α—hemolytic properties. Higher-temperature tolerant paenibacillin N was easily degraded by proteinase K, while the temperature sensitive paenibacillin P was not affected by any of those proteases used in this study other than a specific protease that was secreted by the NP75 strain itself. This has given an interesting clue about how the producer strain manages to escape from the self killing mechanism.
(immunity against paenibacillin P). The specific protease produced by P. alvei NP75 was easily inhibited by phenyl methane sulfonyl fluorides (PMSF).

Partial purification of protease was completed using hydrophobic interaction chromatography, and the molecular size of that protease in SDS-PAGE was found to be 45 kDa.

*In situ* activity assay is one of the promising techniques for the characterization of peptide antibiotics. Towards this, the routine and new protocols were tried. Interestingly, the unexpected result of these experiments - the “switch over activity” - has led us to have further investigations. Here, we have addressed the potential problem in the methodology of in situ assay and we strongly recommend parallel running of plain sample solubilizing buffer SSB along with the peptide antibiotic in SDS–PAGE as well as to check the in situ activity of the plain sample solubilizing buffer SSB gel over Gram-positive organisms. This strategy would ensure thorough washing of the SDS from the gel and, hence, would evade false-positive results in the *in situ* analysis of peptide antibiotics.

The molecular mass of the paenibacillin N (1.157 kDa) and paenibacillin P (1.910 kDa) were identified in mass spectrometry analysis. MALDI-TOF Mass spectrometry analysis of the above peptide antibiotics further showed that they are different from one another as well as from other
functionally related known peptide antibiotics. The mode of synthesis of the peptides was traced by protein synthesis inhibition study on the NP75 strain which opened up an interesting phenomenon of co-production of the paenibacillin N and P through non-ribosomal and ribosomal protein synthesis machinery respectively. The mode of action of paenibacillin N against the target bacteria by **lyses** the bacterial cell wall by binding with lipopolysaccharide (LPS) on the outer leaflet of the bacterial outer membrane (OM). The plasmid curing studies in NP75 strains with SDS treatment reveals that the secretion of paenibacillin P encoded in the plasmid but paenibacillin N was not defined in the plasmid.