ABSTRACT

Antimicrobial era is threatened by high levels of antibiotic resistance among pathogenic bacteria principally, multi drug resistant bacteria. Discovering novel antimicrobials with desired potency can solve the problem of antibiotic resistance strains. Therefore, present study was carried out to discover a new actinobacterial isolate, producing novel and potent antimicrobial compounds, active against drug resistant bacteria especially Methicillin Resistant *Staphylococcus aureus* (MRSA) and Vancomycin Resistant Enterococci (VRE). *Streptomyces* isolate 2A, the most promising strain, isolated from a soil sample collected from Guru Nanak Dev University, Amritsar, Punjab (India) was characterized using polyphasic taxonomic approach. The 16S rRNA gene sequence of the strain showed 99.5- 99.9% similarity with *Streptomyces flavotricini* DSM 40152\(^T\), *Streptomyces toxytricini* DSM 40178\(^T\) and *Streptomyces globosus* DSM 40815\(^T\), but shared DNA-DNA homology of 37.6± (0.6) %, 34.4± (0.5) and 33.1± (0.4) with them, respectively. Based on the genotypic and phenotypic characteristics, it was identified as a novel species in the genus *Streptomyces*, for which the name *Streptomyces amritsarensis* sp. nov. was proposed, with the type strain 2A\(^T\) (=MTCC 11845\(^T\) = JCM 19660\(^T\)).

By using traditional one-variable-at-a-time optimization strategy maximum antimicrobial production was achieved over a pH range of 6.0-8.0 by incubating *S. amritsarensis* at 28°C under shaking conditions (180 rpm) using 2.0% inoculum. Among the various carbon and nitrogen sources studied, starch (1.5-2.0%, w/v) and KNO\(_3\) (0.2%, w/v) were found to be the best carbon and nitrogen sources, respectively. Statistical analysis using Placket-Burman design (PBD) demonstrated that KNO\(_3\), K\(_2\)HPO\(_4\) and NaCl had significant positive influences on production. Further response surface methodology (RSM) was applied to determine the optimal combination of the above three variables. In optimized medium, antimicrobial production was increased by 1.5-fold as compared to the basal production medium.

Two antimicrobial compounds were purified from culture supernatant of *S. amritsarensis* using a combination of silica gel, size exclusion and reversed phase chromatography techniques, and were identified as peptide and lipopeptide. The molecular mass of the peptide as determined by MALDI-TOF-MS was found to be 5.6 kDa. This bacteriocin like peptide showed activity against all the tested microorganisms.
including drug resistant bacteria, MRSA, VRE, multidrug resistant *Escherichia coli*, *Pseudomonas aeruginosa* (resistant to cefepime and imipenum) and *Klebsiella* sp. (resistant to cefepime), fungi, yeasts and *Streptomyces* spp. The MS/MS analysis of the purified lipopeptide revealed that it had amino acid sequence as Ala-Thr-Gly-Ser-His-Gln and a long chain fatty acid tail with six times repeated the molecular mass of 161 Da. Based on the molecular mass (878.5 Da) and amino acid composition, the lipopeptide was identified as a novel lipopeptide. The purified lipopeptide showed activity against a variety of Gram-positive bacteria. The MIC values of the peptide against various test organisms varied from 2-32 µg ml\(^{-1}\) and of lipopeptide varied from 10-45 µg ml\(^{-1}\). Both the peptides were thermo stable and active over a broad pH range.

Safety evaluation by Ames test revealed that both peptides were non-mutagenic. However, peptide and lipopeptide showed highest antimutagenic response of 97.84 and 97.93%, respectively against 2-AF (indirect-acting mutagen) at concentration of 50 µg 0.1 ml\(^{-1}\). Fenton’s reagent assay demonstrated DNA damage protecting properties of both the peptides. Cytotoxicity of the peptides was determined by sulforhodamine B (SRB) assay. The peptide showed significant cytotoxicity i.e. 81.3 and 82.1% against Prostrate Cell (PC3) and Chinese Hamster Ovary (CHO) cell lines, respectively. Lipopeptide was found to be non-cytotoxic, showing insignificant cytotoxicity i.e. 42.3 and 30.1% inhibition against PC3 and CHO cell lines, respectively. The emulgel formulation of peptides was prepared, and skin irritancy of the formulation was tested by Draize test in male albino rats. The test revealed that emulgel formulation of peptides and 0.5% peptides solution did not cause any type of skin irritation. Thus, emulgel formulation of peptides could be safely applied for treatment of skin infections.

Protoplasts were prepared from *S. amritsarensis* and regenerated, and the effect of regeneration on antimicrobial activity was studied. The maximum number of protoplasts (63.1x 10\(^4\) ml\(^{-1}\)) was achieved when 30 h old mycelium (taken from early logarithmic phase of growth), grown in S-medium containing 0.4% glycine, was incubated for 45 min in the presence of 10 mg ml\(^{-1}\) lysozyme. Regenerants showed considerable cultural and morphological variations when grown on various ISP media and starch casein nitrate agar (SCNA). The protoplast regenerated strains demonstrated significant enhancement in antimicrobial production (P≤ 0.05) and maximum i.e. 2.0 fold increase in activity in terms of the inhibition zone (mm) was observed in regenerant, R1 b.