SUMMARY OF RESULTS:

A soil isolate showing antimicrobial activity has been isolated. Based on the biochemical tests, colony morphology it was identified as genus *Bacillus*. Vitek 2 compact identification have shown it to be one among *Bacillus subtilis/ amyloliquefaciens/ atrophaeus* with a confidence level of good identification, 91% probability. However, MALDI-TOF Bio typing identified the culture as *Bacillus subtilis* with a high-level confidence score of 1.905. 16S rDNA amplification identified the isolate as *Bacillus atrophaeus* and assigned the GenBank accession number as JX156420.1 by the NCBI. The phylogenetic tree analysis using Mega 5.0 has shown the URID 12.1 more closely related to *Bacillus subtilis*. The optimum antimicrobial peptide production was observed at the end of logarithmic and early stationary phase. The antimicrobial activity of the cell free supernatant was tested against Gram positive, negative bacteria and *Candida* strains by cut well agar assay. However, the peptide was active against only Gram-positive bacteria.

The antimicrobial peptide, ASP-1 was stable over a range of pH values ranging from 1-14 with a reduction in the activity at high alkaline pH. It was also stable at high temperatures with more than 50% activity after autoclaving for 20 mins at 121ºC and 15 lbs pressure. However, there is complete loss of activity at pH-12 after autoclaving. The activity of ASP-1 was not affected by organic solvents (50% v/v) and surfactants (1% v/v) indicating the hydrophobic nature of the peptide. The antimicrobial compound was resistant to the action of proteolytic enzymes, Trypsin 10 mg/ml and Proteinase K 5 mg/ml. Gel overlay assay after SDS PAGE has shown activity in the region of a band less than 3 kDa indicating the low molecular weight of ASP-1. Whereas, in native PAGE the activity was observed at the top of the resolving gel indicating may be the net positive charge or aggregation of the peptide in its native form. Ninhydrin test after TLC have shown negative results.

ASP-1 was purified to homogeneity by a series of steps involving acid precipitation of the cell free supernatant, followed by solvent extraction, adsorption chromatography and RP-HPLC. The purity of the peptide was confirmed by analytical HPLC and MALDI-TOF. The molecular weight of ASP-1 was identified by MALDI-TOF as 804.4 Da along with it its sodium and potassium adduct at 826.4 Da and 842.4 Da

respectively. Secondary structure analysis using circular dichroism spectroscopy has shown that ASP-1 exists as random coils and β-sheets in the aqueous solution with an increase in the β-sheets in methanol, however, the helix structure is completely absent. The sequence of ASP-1 by MALDI-TOF/MS and ESI-FTICR MS revealed it as acetylated Phe-Thr-Ala-Val-Dhb-Phe-Ile/Leu. The peptide was further analyzed by alkaline hydrolysis, ESI-Q-TOF-MS and ion mobility assay which detected the presence of lactone ring and its cyclic nature in the intact peptide, subsequently revealing the sequence as acetylated-Phe-Thr-Val-Ala-Dhb-Phe-Ile/Leu. Based on the molecular mass (804.5 Da), peptide sequence and amino acid composition, the ASP-1 was identified as lactone-ring containing peptide similar to TL-119, a poorly studied cyclic depsipeptide.

Genetic analysis of the clinical isolates has shown that they have *mec*A gene, responsible for methicillin resistance and IS256 transposon which plays an important role in antibiotic resistance and biofilm formation. After screening 16 strains for biofilm formation against the negative strain *S. epidermidis* ATCC12228, 8 strains were identified as biofilm forming and tested for the antibiofilm forming activity of ASP-1. All the biofilm forming strains were tested positive for the presence of *icaA* and *icaD* genes and have shown black to dark black colonies when grown on Congo red agar plates. ASP-1 has completely inhibited the biofilm formation at 1X and 0.5X MIC concentrations and reduction in the biofilm formation was observed at 0.25X concentration.

MIC of the ASP-1 was determined against 29 Gram positive bacteria with a geometric mean MIC of 16.7 µg/ml. ASP-1 has shown a geometric mean MIC of 26.5 µg/ml against methicillin-resistant *S. aureus* strains. The MICs and MBCs are generally in the ratio of 1:4. Time-kill kinetics against *S. aureus* 29213 at 1X and 5X concentration of MIC have shown 1.88 $\log_{10}$ and 2.01 $\log_{10}$ reduction respectively after 12 h. There was a reduction of 98.68% and 99.02% of initial inoculum at 1X and 5X MICs respectively. Negligible hemolysis (6%) of human erythrocytes was observed at 128 µg/ml concentration of ASP-1 and 31.5% at 256 µg/ml. ASP-1 was not toxic to the mammalian cell line MCF 7 and HepG2 up to concentrations of 128 µg/ml with an IC_{50} of 192 µg/ml against the HepG2 and 280 µg/ml against MCF-7. The peptide also has a high (>10) therapeutic index.