SUMMARY AND CONCLUSIONS

- The widespread problems of acquired drug resistance and emergence and re-emergence of new pathogens and infection by microorganisms leads to high rate of mortality. Therefore continuous search for new antimicrobial compounds is essential for development of lead compound and for treating infections caused by these microorganisms in human and veterinary medicines. Among prokaryotes, the genus Bacillus is diverse in their metabolic diversity and in production of various kinds of bioactive compounds which finds applications as pharmaceuticals. The extreme conditions might stimulate expression of novel genes coding for production of secondary metabolites in Bacillus species.

- The water and soil samples were collected from Arabic sea Mumbai, hot water spring Unkeshwar, soda water Lonar Lake and coal mine Wani enriches nutrient media viz. Sehgal and Gibbons media, Harikoshi media, Acidophilic media and Nutrient broth by extremophilic microorganisms after inoculation and incubation of nutrient media. The incubation conditions and nutrient composition of media selectively enriched extremophilic microorganisms from extreme environment.

- A total 40 Bacillus isolates were isolated from enriched media and inoculated into respective media broth up to stationary phase for production of bioactive secondary metabolites. as Bacillus isolates have ability to produce secondary metabolites in stationary phase.

- The cell free supernatant of total 40 Bacillus isolates were screened against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Aspergillus flavus* for antimicrobial activity. Among these 20 Bacillus isolates viz. halophilic (HaBI₁,
HaBI₂, HaBI₄, HaBI₆, HaBI₇ and HaBI₉), thermophilic (ThBI₁, ThBI₄, ThBI₅, ThBI₇ and ThBI₉), alkalophilic (AlBI₁, AlBI₃, AlBI₄, AlBI₅ and AlBI₇) and acidophilic (AcBI₁₁, AcBI₁₃, AcBI₁₄ and AcBI₁₅) showed inhibition of growth of test microorganism.

- The bioactivity showing Bacillus isolates were tentatively identified as *Bacillus pumilus*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus stercorothermophilus*, *Bacillus thermophilus*, *Bacillus halodurance*, *Bacillus alkalophilus*, *Bacillus polymixa*, *Bacillus megaterium*.

- Three potential *Bacillus pumilus* isolated from halophilic environment showing broad spectrum activity against bacterial and fungal pathogen were further confirmed its identification by using 16S rRNA sequencing as *Bacillus pumilus* JX912979, *Bacillus pumilus* JX912980 and *Bacillus pumilus* JX912981. The 16S rRNA sequence are more conserved even during evolution hence identification of *Bacillus pumilus* was confirmed.

- The most suitable media for production of bioactive secondary metabolites from *Bacillus pumilus* strains was selected by allowing *Bacillus pumilus* culture to grow up to stationary phase in Sehgal and Gibbons media, Nutrient broth, Luria Bertani broth, Minimal medium and Dunddas complex medium and screened cell free supernatant for bioactivity against test microorganism. The Sehgal and Gibbons media produce maximum bioactive secondary metabolites as media ingredient contains complex carbon and nitrogen source which induces expression of genes required for production of secondary metabolites during stationary phase.

- The optimum production of bioactive secondary metabolites from halophilic *Bacillus pumilus* strains were optimized by modifying
Sehgal and Gibbons media into 9 types by substituting different carbon and nitrogen source. As more preferred carbon and nitrogen source for growth, suppresses production of secondary metabolites during stationary phase. The modified Sehgal and Gibbons-II media having sucrose and ammonium chloride were observed best for qualitative improved production of secondary metabolites.

- The bioactive secondary metabolites classes viz. peptide, lipopeptide, polyketide and coumarin were extracted, from production medium, however extract of peptide and coumarin showed antimicrobial activity against test microorganism. The analytical chemistry technique used for extraction have ability to extract selectively these secondary metabolites.

- The bioactive peptide and coumarin extracted from *Bacillus pumilus* JX912980 strains was studied by fractionation guided bioactivity method. The fractionation was carried out by ammonium sulphate precipitation, column chromatography and purity was confirmed by polyacrylamide gel electrophoresis and Thin layer chromatography.

- The bioactive peptide of *Bacillus pumilus* JX912980 strains showing molecular mass 3.8 kDa and 3.9 kDa have N-H, C-H and C=C stretching, 711m/z, and 712m/z mass and presence of aromatic protons and N-H protons as confirmed by Infra red spectroscopy, Mass spectroscopy and NMR spectra. These data were used for partial characterization of peptide into Bacitracin derivatives.

- Similarly bioactive coumarin of *Bacillus pumilus* JX912980 strains having Rf value 0.57 as revealed by Thin layer chromatography also have IR peak at 1622-50 cm⁻¹ due to carboxyl stretch which was supported by α, β unsaturated C=O stretch and peak at 3415 indicated aliphatic C-H and N-H stretch and O-H stretch of acid group. The m/z determined is 158 by Mass spectrophotometer and NMR indicated
aliphatic chain containing N-H stretching. These structural analysis has used to classified the bioactive compound into amicoumacin family of bioactive compound.

- The bacitracin and amicoumacin derivatives obtained from *Bacillus pumilus* JX912980 strain were compared with Bacitracin and Novobiocin for antimicrobial activity against human infectious and food spoilage causing microorganism viz. *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Vibrio harveyi* and *Vibrio alginolyticus*. The antimicrobial activity shown by Bacitracin and coumarin as with Bacitracin of *Bacillus lichaniformis* and Novobiocin indicated the significance of these compounds as probable lead compound for therapeutic usage and food preservation.