Summary of thesis

Marine microbial diversity is a harbor for innumerable nutrients, organic polymers and secondary metabolites that may serve as therapeutics in human pathological conditions. The myriad organisms that are constituents of this diversity produce various molecules that not only help the individual organism to sustain its population but also keep a check on the encroachment of other organisms that may threaten its own survival. The present is a quest for such molecules produced by the previously unknown organisms of the marine diversity that may inhibit microbial threats to human health.

In this pursuit, our study began with the exploration of the marine diversity from various samples such as water and sediments from seas, mangrove forests, salterns from different parts of India in search of previously unknown organisms. Studies on 218 marine samples yielded 385 isolates corresponding to 35 different genera, most of them belonging to the genus *Bacillus* (17%), *Pseudomonas* (16%), *Pseudoalteromonas* (11%) and *Halomonas* (10%). Subsequent investigation helped identify and isolate three strains designated as AK 49^T, AK 61^T, AK 62^T showing less than 97% 16S rRNA gene sequence similarity, indicating novel species of the respective genus.

Cells of strain AK 49^T was isolated from water sample of mangrove forest of Coringa village in Andhra Pradesh, India. It is a Gram negative, rod shaped, aerobic, motile with size 0.5–1.0 µm wide and 1.5–3.5 µm long. Strain AK 49^T grows optimally 30 °C, pH 7.0 and 2% NaCl (w/v). The type strain of *A. coringensis* sp. nov. is AK49^T (=MTCC 12003^T = JCM 19197^T). The GenBank/ EMBL/DDBJAccession Number for the 16S rRNAgene sequence of strain AK49^T is HG529994.

Cells of strain AK 61^T was isolated from a sediment sample collected from Coringa mangrove forest, Andhra Pradesh, India. It is a Gram stain positive, rod shaped, 0.8–1.0 µm

wide and 3.0–3.5 µm long, motile, divide by binary fission. Central endospores are observed. Cells grow facultatively anaerobically. Optimum temperature for growth is 25–37 °C, optimum pH 6.0-9.0 and salt requirement is 0–1% (w/v) NaCl. The type strain of *B. mangrovi* sp. nov. is AK61^T (= JCM 31087^T = MTCC 12015^T = KCTC 33872^T).

Cells of strain AK 62^{T}are Gram negative, rod shaped, 0.8-1.0 µm wide and 5.0-6.0 µm long, motile with singe polar flagellum. The strains AK 62^T grows aerobically at 20-42°C with an optimum temperature of 25-37°C, optimum pH 7.0-9.0 and tolerates up to 18% NaCl (w/v) with optimum growth at 2-8% NaCl (w/v). The type strain of *Marinbacterium* sp. is AK 62^T (= JCM 31159^T = MTCC 12102^T = KCTC 52667^T).

The explored bacterial diversity was screened for the antimicrobial producers. Three strains, namely AK90, TS18 (b), TS43 (a) were found to be potential antimicrobial producers and identified by 16S rRNA gene sequencing. In search of a novel antimicrobial we proceeded with strain AK90 as 16S rRNA gene sequencing revealed 99.93% sequence similarity with *Virgibacillus dokdonensis*. At the time of initiation of the present study, no report of antimicrobial peptide production from the *Virgibacillus* genus was available in the literature. Yet, previous findings did suggest that isolates of *Virgibacillus* have probiotic potential in the human gut. This prompted our curiosity whether an untapped reservoir of molecules having antimicrobial potential lies concealed within this taxonomic group.

The 16S rRNA gene sequence of AMP producing strain AK90 was submitted to the EMBL database and assigned accession number LT852420. The whole genome sequence was assigned the accession number NFZX00000000. The whole genome sequening followed by the genomic analysis revealed a lantibiotic gene cluster that contained five open reading frames (ORFs), designated as *vrgT, vrgM, vrgA* and *vrgR*.

The antimicrobial substance produced by AK90 was identified as a novel lantibiotic named as Virgicin. The preliminary growth inhibition studies showed the growth inhibition activity of virgicin against Gram positive indicator strains like (*L. monocytogenes* MTCC 839, *S. aureus* MTCC 1430, *B. subtilis* MTCC 121 and *M. luteus* MTCC 106) though there was no activity observed agains the Gram negative or yeast test strain (*C. albicans* MTCC 1637, *E. coli* MTCC 1610, *V. cholera* MTCC 3904, *P. aeruginosa* MTCC 1934). Purification studies unveiled a peptide of mass 2.4 kDa. Killing kinetics experiment revealed that virgicin acts both in concentration and time dependent manner. Further investigation also indicated that Virgicin is stable at broad ranges of temperature and pH as well as digestion by various proteases. Virgicin acts against pathogens by membrane selective mechanism of killing as observed in fluorescence and transmission electron microscopy. CD spectroscopy indicates that Virgicin is probably random coil in structure. Interstingly, virgicin has one disulfide bond. The studies on activity of virgicin under reducing conditions were performed. Reduction of virgicin with DTT doesnot affect its antimicrobial activity against Gram positive indicator strains whereas antimicrobial activity increases against Gram negative indicator strains and *C. albicans* as compared to the native virgicin. The ability of virgicin to kill bacteria under reduced conditions as well can make it useful against food spoilage bacteria to be used in preservation of processed foods.

In summary, we conclude that 385 bacteria were isolated from vast marine bacterial diversity and three novel species of bacteria were characterized. The present study identified a novel lantibiotic named as virgicin from a genus previously not reported to produce antimicrobials. The study can be taken forward for addressing the issues like stabilty of peptide in different environments and food products, high level production of virgicin and improved inhibition spectrum.