CHAPTER 7

7. SUMMARY AND CONCLUSION

The mangrove ecosystem is an unexplored source for actinomycetes with potential to produce biological active secondary metabolites. In the present study, totally 298 different actinomycetes were isolated. Of these primarily screened strains showed 25.05% inhibition on human pathogen *E.coli* and least inhibition (17.77%) against *P.vulgans.* Based on the performance during primary screening, only 6 strains were selected for secondary screening and antimicrobial properties. Those 6 strains were analysed for their morphological, physiological and biochemical characterizations.

.

There are eight different solvents were used for the extraction of antimicrobial compounds to study the antimicrobial properties of actinomycetes against 11 pathogenic bacteria. Among these, the maximum inhibition was observed with methanol extract from +17 strain with 30 mm against *C. glabrata* whereas the minimum (7 mm) against *S. typhi.* Similarly, methanol crude extract from +18 strain showed maximum inhibition zone (46 mm) against *E.coli* and minimum inhibition zone against *V. paraheamolyticus* (8 mm). The methanol crude extract from +50 strain showed maximum inhibitions of 42 mm against *E.coli* and moderate activity of 10 mm against *S.aureus* and *S. typhi* each. The methanol crude extract of +112 strain showed maximum (40 mm) against *E.coil* and minimum (10 mm) against *S. typhi*. The methanol crude extract from +118 strain showed maximum inhibition zone (21mm) against *E.coli* and minimum against *S.aureus, S.typhi* and *C.neoforman.* Finally, the strains +18 and +50 have been selected for characterization of antimicrobial compounds based on the antibacterial and antifungal activity.

The bioactive compounds from actinomycete +18 revealed that the maximum absorption of 263 and 312nm in methanol. IR Spectrum of compounds showed asymmetric (NH_2) amino group stretching which showed the strong absorption at 3427 cm-1, and the symmetric medium stretching at 1640 cm^{-1} showed a peak for NH_2 group along with 1566 cm^{-1} and this characteristic aromatic stretching at 771cm^{-1} for aromatic ring absorption. NMR spectrum resulted the strong broad peak at 1.58 ppm can indicated that amino group with two protons and a peak with little lower intensity at 2.6 ppm for

acetyl group in the aromatic ring or aliphatic linear chain. The bioactive compounds from actinomycetes +50 revealed that the maximum absorption of 263 and 329nm in methanol. IR Spectrum of showed that, the maximum absorption at 3436 cm^{-1} absorption peak showed the NH_2 asymmetric stretching containing with the aromatic ring and 1639 cm^{-1} for NH_2 medium symmetric stretching, 768 cm^{-1} aromatic stretching which contains NH_2 group in the ring carbon. NMR spectrum expressed only one strong peak at 1.5 ppm it might be due to the amino group in the aromatic ring. In our present observation, the strain +118 did not showed any compound level detection.

The purified bioactive principles were further analysed for their antimicrobial activity against selected *E.coli*. Of these, +118 strains showed very high inhibition (26 mm) followed by +18 strains (13mm) and +50 strains with (11mm).

The 16S rDNA genes of actinomycetes (+18, +50 and +118) isolated from the Muthupet sediment samples were partially sequenced. The sequence comparison of marine actinomycetes with other sequences available in the EMBL database was studied. The phylogentic analyses revealed that, 1416bp sequences on the +18 actinomycetes, whereas strain +50 actinomycetes showed 959bp and strain +118 showed 1403bp. The phylogenetic analyses of partial 16S rDNA sequences showed that, the +18 was closely similar (99 %) to the existing of *Nocardiopsis* species accession number AY 336502.1 , whereas +50 was 100% similarity with the existing *Streptomycetes* species accession number GU263865.1 and +118 was closely (99%) similar to the existing *Streptomycetes* species accession number HQ992769.1. The secondary structure of 16S rDNA of actinomycetes such as +18, +50 and +118 showed 66, 38 and 61 stems respectively in their structure. However, both the isolates were similar in energy threshold, cluster factor, conserved factor, compensated factor, conservativity, part of sequences, greedy parameter and treated sequence as indicated by genebee software. www.genebee.msu.ru.

Totally 60, 56 and 49 restriction enzymes sites were observed in actinomycetes +18, +50 and +118 respectively. However, the cleavage sites and the nature of the restriction enzymes of actinomycetes +18, +50 and +118 were differed. The GC content

of actinomycetes +18, +50 and +118 was found to reported as 58%, 59% and 60% respectively using NEB cutter programme V2.0 in www.neb.com/nebcutter2/index.php.

Biosorption mechanism of metal ions by microorganisms includes ion exchange, precipitation and complexation. Reduction and surface accumulation of metals may be a process by which microorganisms protect themselves from the toxic effects of metallic ions. The present study showed that the biosorption of silver in the form of nanoparticles using the culture supernatant of actinomycetes (+18, +50 and +118). The nanoparticles were primarily characterized by UV–Visible spectroscopy, a strong, broad peak, observed at 420 nm, was confirmed the presence of silver nanoparticles in the culture supernatant of +18, +50 and +118. A SEM micrograph of the dry mass was found nanoparticles. The X ray diffraction was confirmed the crystalline nature of the particles. The +18 strain AgNPs size was recorded as 16.89nm, +50 strain has the size of 20.55 and 10.65nm and the +118 strains was recorded at 31.15, 11.15 and 17.86nm. FTIR spectroscopy measurement showed the biomolecules that bound specifically on the silver surface. The antimicrobial activity of AgNPs was investigated against gram positive and gram negative pathogenic organisms such as *Staphylococcus aureus* and *E.coli* using disc diffusion methods.

The actinomycetes such as +18, +50 and +118 have been used as probiotic for the larviculture of tiger shrimp *P. monodon*. The 25 days of feeding trials with probiotic and control feeds resulted that the increment of length, weight, survival and biochemical composition such as protein, carbohydrate and lipid in test feed compared to control feed. Furthermore, increased actinomycetes cell concentration can increases the length and weight in *P.monodon* larvae.

In case of shrimp survival, in +18 strain the high survival was recorded by 95.7% at 7.5g/kg concentration of feed supplement with +18 strain and the low survival was observed at 5.0g/kg of actinomycetes. In case of, +50 strain, the 100% survival rate was recorded at 5.0g/kg concentration whereas the low survival was noticed at 7.5g/kg concentrations. Among the 3 strains studied, the +118 has resulted favourable survival

rate where the maximum survival of 98.57% was reported at 2.5g/kg concentration and low survival rate of 94.28% was obtained at 7.5g/kg. Control feed showed less survival.

Total Heterotrophic Bacteria (THB) was increased in high concentration of actinomycetes. Those THB were used as beneficial to shrimps. The TVC concentration was comparatively low in experimental feed than in control which is beneficial to shrimps. The biochemical composition, protein, carbohydrate, lipid, ash and moisture content was higher in actinomycetes feed than in control.

Very limited reports are available on the application of marine actinomycetes as a probiotics, especially *Streptomycetes* sp. In the present study, application of actinomycetes as probiotic for larval rearing of *P.monodon* can increase the growth (length and weight), survival and biochemical composition of *P.monodon* larvae.

The present study inferred that the methanol extract of +17, +18 +50, +112 and +118 mangrove actinomycetes were potentially control the growth of *E.coli* with very rich inhibition zone. The strains such as +18, +50 and +118 revealed the asymmetric (NH_2) amino group, NH_2 asymmetric stretching and no compound level stretch respectively. The purified bioactive chemical classes from +118 strains showed very high inhibition with 26 mm against *E.coli*. From the present findings, it is understood that +18, +50 and +118 strains were capabale of producing the silver nanoparticles and even minimum concentration of actinomycetes showed marked inhibition against *S.aureus* and *E.coli*. Further, it is also confirmed that the actinomycetes +18, +50 and +118 were potentially increased the growth and survival of shrimp larvae besides maintaining water quality in the culture system. Despite the fact that above, the mangrove actinomycetes such as +18, +50 and +118 can be considered as good candidate for discovery of drug to control the pathogenic bacteria and it can be utilized as probiotic to enhance the shrimp growth and survival for successful aquaculture production.