6. SUMMARY AND CONCLUSION

Coastal waters support dynamic ecosystems, contain valuable natural resources, and have important environmental values. The study of physico-chemical properties of coastal environments is important, because the variations in the physico-chemical properties such as temperature, salinity, pH, dissolved oxygen and the dissolved nutrients influence the density and diversity of polychaetes in the marine environment. Polychaetes are one of the important reserves and play a vital role in aquaculture as food for the larval stages of crustaceans and fish in addition to serving as food for various cultivable organisms.

Environmental variability was assessed through physico-chemical parameters of water. The rainfall was recorded 650 mm for the sampling station. The maximum rainfall of 203.8 mm was recorded during the monsoon season (November, 2011) and minimum of 14.27 mm was observed during summer season (June, 2011). The water temperature was ranged from 24.5 to 32.5°C during the sampling period. Minimum temperature was recorded in the month of November (2011) and maximum was found in the month of May (2011). The salinity was ranged from 15.5 to 36.5 ppt during the sampling period. Minimum salinity 15.5 ppt was recorded in the month of November (2011) and 36.5 ppt maximum was found in the month of April (2011). The pH was ranged from 7.4 to 8.3 in the sampling station. Minimum pH (7.4) was recorded in the month of November and maximum (8.3) was observed in the month of April (2011). The turbidity was ranged from 45 to 164 NTU in the sampling station. Minimum turbidity (45 NTU) was recorded in the month of May (2011) and maximum (164 NTU) was observed in the month of November (2011). The turbidity was ranged from 684 to 1108 mg/l in the sampling station. Minimum turbidity (684 mg/l) was recorded in the month of September (2011) and maximum (1108 mg/l & 1040 mg/l) was observed in the months of November and May (2011). The DO was ranged from 3.7 to 5.2 mg/l in the sampling station.
The minimum DO (3.7 mg/l) was recorded during summer (2011) and maximum was observed during November (2011). The nitrite was ranged from 0.239 to 3.745 mg/l the sampling station. Minimum was recorded during the premonsoon season in July (2011) and the maximum during the monsoon season in the month November (2011). The nitrate value ranged from 3.86 to 12.54 mg/l in the sampling station. Minimum nitrate content was recorded in the month of August (2011) and maximum in the month of November (2011). Total nitrogen value ranged from 9.32 to 28.19 mg/l in the sampling station. The minimum total nitrogen was recorded in August (2011) and maximum in the month of November (2011). The inorganic phosphate estimated was ranged from 0.110 to 0.89 mg/l the sampling station. Minimum inorganic phosphate was observed during the pre monsoon season in July (2011) and maximum in the month of November (2011). The total phosphate value ranged from 1.19 to 32.62 mg/l in the sampling station. Minimum total phosphate was recorded in July (2011) and maximum in the month of November (2011). The TOC concentration in sediments was in range from 0.962 to 14.28 mg/g during the sampling period. Minimum TOC was recorded during premonsoon in the month of August (2011) and the maximum in the month of November (2011).

In the present study, totally thirty six macro faunal species of polychaetes such as *Arenicola marina*, *Ancistrotyliss parva*, *Arabella mutans*, *Armandia longicaudata*, *Armandia intermedia*, *Capitella capitata*, *Chaetopterus sp.*, *Cirratulus chrysoderma*, *Cossura costae*, *Chone sp.*, *Cirratulusesp.*, *Dodecaceria sp.*, *Dorvillea gardineri*, *Euchone rosea*, *Euchone tentaculata*, *Fabricia filamentosa*, *Goniada emeriti*, *Glycera alba*, *Glycera sp.*, *Lumbrineris heteropoda*, *Lumbrineris aberrans*, *Lumbrineris brevicirra*, *Maldane sarsi*, *Nephtys dibranchis*, *Nephtys hombergi*, *Nephtys sp.*, *Notocirrus australis*, *Nereis virens*, *Nereis capensis*, *Nereis sp.*, *Notomastus aberans*, *Perinereis cultrifera*, *Scolelepsis squamata*, *Notomastus sp.*, *Polydora ciliata*, *Omupis sp* and *Pisione indica* were identified. Maximum
number of species and their abundance were recorded during summer where as the *Vice versa* was true during monsoon. Among this species composition five species of polychaetes (*Capitella capitata, Nereis virens, Polydora ciliata, Perinereis cultrifera* and *Glycera alba*) were dominated in all the seasons.

The bacterial density estimated in different polychaetes were ranged from $1.8 \times 10^5$ and $6.0 \times 10^5$ CFU/ml/g. Slight seasonal variation in bacterial density was noted with the maximum in summer season in *Perinereis cultrifera*, and the minimum was noted in monsoon season in *Nephtys sp.* Isolation of polychetes associated bacteria was also studied. During the 12 months collection period (January 2011 to December 2011), 15 different bacterial strains were screened for antibacterial activity. Among the strains BSJ2, which showed desirable results in the screening tests was taken for further studies and it was identified as *Lysinibacillus sphaericus* based on biochemical tests done as per Bergy’s manual of determinative bacteriology and also was confirmed with the help of 16S rRNA partial sequencing (NCBI Accession number: KF781636).

The present study mainly focused on the bacteriocin produced by *Lysinibacillus sphaericus* strain isolated from polychetae. Antagonistic activity was tested against ten human pathogens, except *Salmonella typhimurium* all the others were found to be sensitive to the bacteriocin. Two media (i.e) MRS and GP broth were tested for bacteriocin production. Surprisingly in both, maximum production was observed at stationary phase around 13h. Comparatively bacteriocin activity was higher in MRS broth (3,400AU/ml). Among various temperatures tested, maximum bacteriocin activity was observed at 50°C (25,400AU/ml). Regarding pH, the maximum inhibitory activity was recorded at pH 4.0 (12,800AU/ml). Regarding various NaCl concentrations tested, the maximum activity was recorded at 0.9% NaCl (25,400AU/ml).
When the bacteriocin in supernatant was precipitated with various saturation level of ammonium sulphate, the fraction separated by 50% showed maximum activity. Dialysis was done using molecular of KDa. The molecular weight of the purified bacteriocin was determined as 2.5 KDa. When the purified bacteriocin compound was treated with different enzymes, such as catalase, protease K and α amylase, its inhibitory activity was affected only by protease K.

Thus the present study revealed that the bacteriocin producing *Lysinibacillus sphaericus* strain has potential against various human bacterial pathogens. Apart from this, the possibility of using this strain as probiotic is to be explored.

This work also included the isolation, optimization, partial purification and applications of cellulase enzyme. The strain *Lysinibacillus sphaericus* was examined using cellulolytic agar with the Congo red as a preliminary potential screening method for identifying cellulase production. It produced 12mm zone in cellulose agar.

Medium optimization was carried out for the maximum growth of cellulase production. The isolated strain showed growth and enzyme activity at a broad range of temperature from 25°C to 45°C with an optimal cellulase production at 35°C. The bacteria survived in media of pH values from 3 to 11. At pH 7 the optimal growth and cellulase production was observed. Optimum salinity was found to be at 3% for the bacterial growth and enzyme activity.

The corn cob with 3% concentration was found to be the suitable substrate for the maximum cellulase production. The maximum enzyme activity of 43U/ml/min was found at 3% substrate concentration. The concentration of protein was determined by using Lowry’s method.

The protein was found to be 0.33U/mg in the period. SDS–PAGE was carried out to determine the molecular weight of the partially purified cellulase enzyme from *Lysinibacillus*
*sphaericus*. The results exhibited that the purified cellulase enzyme had a molecular weight range of 22-35 kDa.

The study thus delineated complete details on distribution of polychaets in Uppanar estuary their microbial density, seasonal variation and bioprospecting the associated bacterium.