SUMMARY AND CONCLUSION

In chapter I water samples were collected from three different sites at Puthalam salt pan located extreme south of Kanyakumari district. The collected samples were aseptically transported to the laboratory and subjected to serial dilution by sea water. From the dilutions $10^{-6}$ dilution was taken for further analysis.

Plating on Zobell marine agar plate, 3 kinds of colonies (red, yellow and white) were observed after 3 days of incubation at 37°C for 12 days. In this investigation, observed colonies were screened for the production of antioxidant enzymes such as SOD and catalase. The data obtained indicates that among the 3 colonies, red coloured colonies were found to produce more SOD and catalase compared to others, hence red colonies were used for further studies such as biochemical characterization, growth in the presence of various pH, temperature, agars, carbon sources, nitrogen sources, inorganic sources, organic solvents, generation of growth curve and antimicrobial analysis.

The physico chemical analysis of salt pan shows that the dissolved O2 content, free CO2, bicarbonate, chloride, salinity, magnesium, nitrogen, potassium, copper and manganese contents were found to be dominant in site 1 compared to site 2 and 3. Hardness of water is more in site 1, which is being dominant with iron and zinc.

Biochemical characterization of isolate indicates that the strain is gram negative rod with evenly spread colonies and is motile. It shows positive reactions in catalase, oxidase, gelatin liquefaction, starch hydrolysate, casein production, glucose, sucrose, dextrose, arabinose and mannitol tests. Established growth of isolate was
noticed at 29% NaCl, 42ºC and pH 8.8. Growth curve explains that the strain started to grow from the 3rd day of plating. Onset of log phase occurred between 3rd and 6th day and the growth rate maintains as such upto 9th day, beyond that growth declined. Maximum biomass production was noticed in Zobell marine agar plate followed by casein hydrolysate agar and starch agar. Among the carbon sources used, prominent growth was found to be observed in glucose, fructose, lactose, starch, maltose and sucrose along with Zobell marine agar.

Comparative analysis with other carbon sources, the isolate reaches its optimum growth at 10% starch (0.98±0.22), followed by sucrose at 5% (0.79±0.17), glucose at 9% (0.78±0.24), galactose at 6% (0.76±0.13), lactose at 4% (0.74±0.13), fructose at 5% (0.74±0.18) and maltose at 4% (0.68±0.14) respectively. Based on these findings, the isolate of present study is an alkaliphilic thermophile can utilize carbohydrates as energy source.

The strain acquires its profound growth in the presence of tryptophan, tyrosine, arginine, glycine and histidine. Biomass yield was high in plates supplemented with MnCl₂, calcium chloride, ferrous sulphate, potassium thiosulphate, sodium sulphate, lithium carbonate, sodium bicarbonate, sodium chloride, magnesium sulphate and KCl. Colonies were more visible and clear in the presence of methanol, diethyl ether, ethanol and chloroform respectively. Antimicrobial activity of the test strain indicates that zone of inhibition was found to be more against Enterobacter sp., Klebsiella sp., E.Coli and Proteus vulgaris. The isolate of present investigation was identified as Pseudomonas sp. SM1 by 16SrRNA sequencing. As the microorganism grows in extreme salinity, alkaline pH, and high temperature, it developed a mechanism to compact such harsh environments. Hence these organisms have
evolved to be of industrial use viz. textile industry, food industry and pharmaceutical industry. The strain is also a potential source of SOD and catalase, which further is of immense importance in cosmetics amongst others. Further studies are required in this direction to establish and develop safe supplements, therapeutics and cosmetic products.

In chapter II characterization of SOD and catalase in various pH, temperature, enzyme concentration, substrate concentration, effect of metal ions such as NaCl, MnCl₂, MgSO₄, and ZnSO₄, effect of various buffers such as bicarbonate, sodium citrate, glycine and acetate buffers as well as activities in the presence of inhibitors such as EDTA, TCA and SDS were carried out. The results indicate that production of these enzymes was found to be more in optimized medium than control. Protein was precipitated by ice cold ethanol followed by purification on Sephadex G-150 column using NaCl-sodium phosphate buffer of various ionic strength in case of SOD. Catalase was purified by Sephadex G-150 column equilibrated with 50mM potassium phosphate buffer (pH 7.0) containing 2M NaCl. SOD in crude sample shows highest activity at pH 9.0 and temperature 67°C where as purified SOD shows its optimum activity at pH 9.8 and temperature 77°C. At pH 9.0 and the temperature 65°C catalase in crude sample acquires its peak activity and purified catalase reaches its maximum activity at pH 10.0 and temperature 65°C. Moreover activity of SOD and catalase was markedly increased when increasing concentration of enzymes upto 2.5ml superior to that activity declined gradually. The Km value of SOD was calculated as 0.25mM and catalase was found to be as 40mM respectively.
Metal ions such as NaCl, MnSO₄ and MgSO₄ significantly increased the activity of antioxidant enzymes of present study when increasing the concentration of respective ions, but excess concentration of ZnSO₄ markedly decreased their activities. Among the three buffers used, enhanced activity was noticed in bicarbonate buffer followed by glycine and citrate buffer. These enzymes were more stable in bicarbonate buffer (pH upto 9.5) and glycine buffer (pH upto 10.2). Activity of SOD was not affected upto 244.8mM of TCA, 107.2mM of EDTA and 103.8mM of SDS, where as activity of catalase was found to be decreased noticeably at higher concentrations of TCA and EDTA, but SDS does not affect catalase activity upto 103.8mM. Triton X-100, PMSF and β-mercaptoethanol completely inhibit SOD and catalase activity at their minimal concentration. The catalase isolated and purified in this analysis shows stability at extreme temperature and optimum activity was found to be at alkaline pH compared to other reported industrially important catalases. Due to its thermostability, it can be used in textile industries for bleaching effluents. In addition, this catalase has a number of unusual characteristics compared to other catalases. The activity of catalase was found to be increased exponentially when increasing the concentration of NaCl, MnCl₂ and MgSO₄. But compared to other reported catalases the activity of this catalase was found to be decreased gradually as the concentration of ZnSO₄ was increased.

In chapter III purified enzymes were subjected to one dimentionl SDS-PAGE anlaysis according to Laemmli, 1970. The obtained protein bands were further subjected to Nano LC-MS/MS analysis for identification. Analysis of functional enrichment of differentially regulated protein was performed in David 6.7 software. The results describe that SOD and catalase are dimers composed of 2 identical
subunits of molecular weight 48.2 Kdal and 111.2 Kdal respectively. The calculated isoelectric point of each subunit of SOD was found to be 5.48 and each subunit of catalase was calculated as 6.61 and 6.70 respectively. The expression of SOD and catalase, even under anaerobic conditions, protects the cells from reactive oxygen species produced following accidental exposure to oxygen. SOD and catalase could prevent the damage potentially imposed by the ability of the bacterium to survive in an environment in which oxygen is present. As demonstrated, oxygen is able to induce the expression of SOD and NADH oxidases as a mechanism of survival under conditions of oxidative stress.

In chapter IV anticancerous activity of SOD against cultured HeLa, A-549, MCF-7, Ht-29 and Hep-G2 cell lines were carried out and the observations indicate that SOD acts against these cell lines, feasibility of cell lines was found to be decreased in dose dependent manner and no toxicity was observed against normal cell line. Similar effects were noticed in catalase also. Moreover SOD and catalase possess significant cardiac protective activity towards H9C2 cell lines.