Actinomycetes are one of the most economically and biotechnologically valuable prokaryotes. Recent findings have revealed a tremendous diversity among actinomycetes in marine environment. Actinomycetes endow unique metabolic and physiological capabilities to survive in the hostile marine environment. It is surmised that marine actinomycetes might produce novel bioactive compounds (than the terrestrial counterparts) with the potential to be developed as therapeutic agents. Further, it is believed that sea water which is similar to the human blood plasma in its chemical nature could provide microbial products, that could be safer having less or no toxicity or side effects when used for therapeutic applications.

It is pragmatic that physiochemical status of an ecosystem not only influences the occurrence but also the diversity of microbial community. Therefore, understanding the physicochemical status of an ecosystem is inevitable for determining the structural and functional status of the biotic community. In the present study, no wide variation was observed in the physicochemical characteristic of the surface water samples collected from three different locations of Gulf of Mannar (sea shore, half a nautical mile and one nautical mile away from sea shore).

Results on the concentration of inorganic salts and trace elements in surface water samples reflected the healthy status of the habitat. However, heterogeneity in the concentration of inorganic salts and mean number of actinomycetes colonies in the three different sampling sites was observed. It is approximated that $8.7-10.2 \times 10^2$ actinobacterial colonies exist in a millilitre of sea water in the three sampling areas. Samples diluted with natural seawater followed by heat treatment allowed isolation of maximum number of actinomycetes colonies in ISP4 medium than that of the samples without heat treatment. The genus *Streptomyces* constituted a majority of actinomycetes isolated.
Five of the nine actinobacteria isolated in this study belonged to the genera *Streptomyces*. The isolates were designed as *Streptomyces* species based on cultural, morphological and physiological studies. Later, molecular analyses of the partial gene sequence of 16S rRNA substantiate that the taxonomic conclusions are in concert with the results of conventional methods.

Metal ion concentration in the environment has an important role in the functioning of life systems. Physicochemical analysis of the water samples revealed iron scarcity and it was found to be much lower than the concentration (0.04$^{-1}$ nM) required by microorganisms for growth. To scavenge the available iron, actinomycetes isolated (few but not all) were found to produce secondary metabolites called siderophores. This exceptional secondary metabolic capability is considered as a contingency mechanism to survive competition from other bacteria.

Of the five *Streptomyces* species screened only two were found to produce siderophores. Siderophore producing ability of isolates was confirmed by FeCl$_3$, CAS assay and CAS agar plate method. This study is one of the few reports available on siderophore mediated iron acquisition in *Streptomyces* species. 16S rRNA gene sequence comparison revealed that the two isolates have close sequence relationship with *Streptomyces variabilis* and *Streptomyces coelicolor*.

Nature of siderophores produced by the isolates was ascertained by a series of chemical assays. *Streptomyces variabilis* was found to produce hydroxamate siderophores. Mixed (hydroxamate and catecholate) type of siderophore was produced by *Streptomyces coelicolor*. Hydroxamate siderophore produced by the isolates was further distinguished into dihydroxamates based on the pH dependent absorption maxima on their reaction with ferric ions. Intensity of binding of siderophore with iron was revealed by color reaction at different pH. Interestingly, the hydroxamate siderophore produced by *Streptomyces coelicolor* was found to be hexadentate whereas the siderophore produced by *Streptomyces variabilis* was bidentate in nature. Siderophore production is one of the mechanisms by which *Streptomyces* can exert beneficial effects on their host.
Siderophore producing isolates were subjected to further analyses a) to understand the probiotic nature of the isolates, b) to reveal the influence of different media and nutrients on siderophoregenesis and c) to optimize the medium constituents for enhancement of siderophoregenesis. Finally, purified siderophores were structurally characterized by spectral analysis and subjected to therapeutic analysis.

Survival of *Streptomyces* strains under acidic conditions, resistance to high bile salt concentration and synthetic gastric juice ensures the probiotic qualities. Results of auto-aggregation and cell surface hydrophobicity revealed not only the colonization efficiency but also its sustenance during the intestinal transit. Through adhesion ability and colonization on tissues, the two isolates are supposed to prevent pathogen access by steric interactions or specific blockage on cell receptors. Absence of haemolytic activity and resistance to different antibiotics exhibited by the isolates are considered as primary requisite for an ideal probiotic. Based on the above results, it is evident that the isolates could be efficiently exploited as probiotic candidates bearing siderophoregenic attributes.

Siderophore production was found to be positively related to the growth of the isolates. Variation in siderophore production with media, incubation time, nutrients composition and pH was also observed. Of the five different media analyzed maximum siderophore production in both the isolates was observed with ISP4 medium. Resemblance of the ISP4 medium in its composition with marine environment may be the reason for maximum siderophore production. pH plays an important role in the solubility of iron and thereby its availability to the growing organisms. Maximum siderophore yield was obtained with alkaline conditions (pH 8). This may be due to the fact that marine isolate grows better at alkaline conditions. Further the redox state of Fe depends on the environmental pH. Growth and siderophore production was found to be repressed with increasing concentration of iron. Though higher concentrations of iron (20 μM) retard siderophoregenesis, it does not have any influence on the growth of the isolates. In this study an attempt has been made to modify the medium ingredients qualitatively and quantitatively with an aim to enhance the siderophore production in the isolates. ISP4 medium was considered as base medium and the ingredients were substituted with different additives.
Supplementation of ISP4 medium with different carbon, nitrogen, and amino acid sources attributed a notable difference in growth and siderophore production of the isolates. ISP4 medium supplemented with starch, casein and KNO$_3$ as carbon, amino acid and nitrogen source was suitable for siderophore production in marine Streptomyces. Variation in siderophore production in the isolates with different carbon, nitrogen and amino acid sources indicate that siderophoregenesis is substrate dependent process.

In the present study, response surface methodology (RSM) was used in combination with modeling and simulation to design, optimize and enhance siderophore production. Optimization of medium composition revealed the importance of various nutrient factors at different levels. A high similarity was observed between the predicted and experimental results which reflected the accuracy and applicability of RSM to optimize the process for siderophore production. Contour plots were generated to illustrate the interactive effect of the three factors (starch, casein and KNO$_3$) on siderophore production. Further, the optimum value of each factor affecting the response was also identified. Significance of the results was evaluated by analysis of variance (ANOVA). In the present study, interactive effect of different concentration of starch and casein enhanced siderophore production to a greater extent rather than with that of KNO$_3$ combination.

FT-IR, NMR and GC-MS analyses reveal the structure and relative configuration of siderophore produced by Streptomyces variabilis and Streptomyces coelicolor. Mass spectroscopy analysis revealed the sequence and the chirality of amino acids. The backbone linkages were determined by $^{13}$C and $^1$H NMR analysis. The structure of siderophore produced by Streptomyces variabilis resembles nocardomine whereas the siderophore produced by Streptomyces coelicolor resembles ferricoelicelin. Structure elucidation helps to understand the fate of iron after recognition by specific importers and subsequent channeling of iron to intracellular targets.
In this study, binding nature of siderophore with ten different divalent metal ions was analyzed by CAS agar plate test and microtitre plate assay. Metal complexation potential of the sidereophore produced by the two isolates found to vary. Of the two isolates siderophore produced by *Streptomyces variabilis* fails to bind with most of the metals analysed except Fe. In contrast, sidrophore produced by *Streptomyces coelicolor* bind with most of metals analysed except Cu and Al.

Apart from metal chelation siderophore produced by *Streptomyces* species attributed antagonistic activity against selected bacterial pathogens. Results indicate that gram positive bacteria are more susceptible than gram negative bacteria. The cell free supernatant of the *Streptomyces* species was found to be more effective in inhibiting the pathogens than the purified siderophores. The presence of different secondary metabolites in the cell free supernatant owe for a greater inhibitory activity.

This study reports the *in vitro* cytotoxic effect of siderophores produced by *S. variabilis*, *S. coelicolor* and the commercially available siderophore (desferrioxamine) against human breast cancer cells (MCF-7). Following 48 hr of incubation with the iron chelators, conspicuous changes in cellular morphology were observed with severity depending on the concentration used, the loss of the characteristic shape progressed into the disintegration to apoptotic particles and ultimately formation of cellular debris. The result substantiates that iron chelation therapy can been exploited as a possible treatment for cancer.

The outcome of this study proves that siderophores is not only an iron carrier but also found to be efficient in the suppression of microbial pathogens. This provides a basic ‘proof-of-concept’ that antagonistic bacteria and its bioactive compounds can act as probiotics. In this study, secondary metabolite produced by *Streptomyces variabilis* and *Streptomyces coelicolor* were found to be nocardamine and ferricoelicelin which resembles desferrioxamine (the only available therapeutic agent for acute iron intoxication). Further research on the secondary metabolites is required because the structures of natural products are beyond our thoughts.