Microbial agents are capable of causing various infectious diseases. Due to continuous and indiscriminate use of antibiotics, various pathogens have acquired resistance to them. Management of drug resistant infection is tiresome process. Curing of multi-drug resistant pathogenic infections require new drug of choice or by the enhancement of immune responses of the patient. Hence the elimination of pathogen from the host using different strategies are need of the hour. The present study is aimed at screening some of the potential antimicrobial and immunomodulatory agents against selected pathogens thereby directly inhibiting microbial propagation and also stimulating both the innate and acquired immune mechanisms of the host. Western Ghats, which is one of the largest biodiversity centers of the world, forms an ideal ground for searching new species with potential for novel antimicrobial agents. Soil sample was chosen as the source of antimicrobial producing agents, as they contain rich biodiversity of various microorganisms than any other natural media. For the present study, the soil samples were collected from Courtallam hills lying in the region of Western Ghats. It is well known that more than 70% of all known microbial drugs have been isolated from Actinomycetes. The potential nature of Actinobacteria makes scientists to select Actinomycete as a source of new drug of choice to tackle the pathogens causing infections. Based on the above considerations, it was planned to screen Western Ghats soil sample for Actinomycetes which act against selected microbes and enhance the immune response against the infectious diseases. The work was carried out in two phases.

- The first phase of work mainly focused on the isolation of Actinobacteria from Western Ghats soil sample, assay of their antmycobacterial and antibacterial activities, detection of type of antibiotic produced, enhancement of antibiotic production level by physical and chemical treatment of selected Actinomycetes and finally the identification of Actinomycetes isolates by morphological, biochemical, cell wall chemistry, sequencing of 16s rRNA gene and BLAST analysis of sequences. The effective isolates with potential for production of novel antimicrobial agents were selected for second phase of work.
The second phase of our present study was carried out on experimental model-tilapia *Oreochromis mossambicus*. The work was focused on the investigation of selected Actinomycetes on the growth performance, haematological parameters, humoral immunity, cell mediate immune response and disease resistance of *Oreochromis mossambicus*.

During our investigation, 23 soil samples were collected from undisturbed forest area of Courtallam hills and serially diluted samples were plated on SCN agar. About 118 different Actinomycetes were isolated from 23 soil samples. From the isolated Actinomycetes, thirty two isolates were selected at random and based on the morphological variations, the isolates selected from each sample were designated as AM 1, AM 2, AM 3…and AM 32.

The Actinomycete isolates were tested for their Antimycobacterial and antibacterial activity by giant colony method. From 32 isolated strains, 19 were selected randomly and their antimicrobial potential (water extract of mycelium and EtOAc extract of spent medium) was assayed by well plate method against *Aeromonas hydrophila*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Mycobacterium smegmatis*, *Mycobacterium vaccae* and *Staphylococcus aureus*.

Based on the antimicrobial activity, five isolates (AM 4, AM 6, AM 13, AM 16 and AM 32) were taken for further analysis. The extracts of five isolates were subjected to various analyses. Water extract of mycelium and EtOAc extract of spent medium were used to find out the type of antibiotic present in the samples. The type of antibiotic produced includes MLS class, glycopeptides / cell-wall binding antibiotics, β-lactam antibiotics and antifungal antibiotics. UV-VIS and FT-IR spectroscopic analysis of extracts support the presence of above classes of antibiotics in the samples.

It was found that among five isolates, AM 4 was highly active than other isolates. So it was planned to improve the antimicrobial production level of AM 4 by physical (UV- radiation) and chemical (NTG) treatment. It was cleared that UV
radiation was found to be more lethal than NTG. The antibiotic production level was assayed by agar plugs/agar gel disc method against *S. aureus* lawn. The experiment also revealed that UV irradiation influenced the higher level of antimicrobial production than NTG.

✓ The selected isolates were identified by both traditional as well as molecular method (16s rRNA gene sequencing). Actinomycetes AM 3, 4, 6, 13, 16, 28 and AM 32 were identified as *Streptomyces bikiniensis*, *S. flavofungini*, *Rhodococcus ruber*, *S. tendae*, *S. albofaciens*, *S. venezuelae* and *S. rochei* respectively. All the sequences were submitted in NCBI database with the following accession numbers - Actinomycetes AM 3 (JQ819728.1), AM 4 (JQ511979.1), AM 6 (JQ819733.1), AM 13 (JQ819729.1), AM 16 (JQ819730.1), AM 28 (JQ819731.1) and AM 32 (JQ819732.1). The sequences were analyzed to find out their relationships among themselves by bioinformatics tools to reveal their ORF, CpG Islands, Multiple Sequence Alignment and phylogenetic relationship.

✓ The second phase of work was carried out with a best performing isolate AM4, selected from the first phase of study. The selected isolate *S. flavofungini* AM 4 was found to be more potent strain than other isolates. The current study has been designed with the objective of finding the probiotic effect of *S. flavofungini* AM 4 on the overall health status of *Oreochromis mossambicus* (Mossambique Tilapia), a common fresh and brackish water cichlid fish.

✓ The feasibility of using *S. flavofungini* AM 4 as a probiotics was experimented by safety test and survival efficacy of streptomycetes in the experimental feed. Streptomycetes supplementation was given through feed. Four types of fish feeds were prepared which includes control feed containing standard basal balanced diet only, experimental fish feed I contained $1 \times 10^7$ CFU *S. flavofungini* AM 4 /g of feed, experimental fish feed II contained $1 \times 10^8$ CFU *S. flavofungini* AM 4 /g of feed, fish feed III contained $1 \times 10^9$ CFU *S. flavofungini* AM 4 /g of feed. In the present investigation fish were fed orally with the probiotic feed.
The effect of Streptomycetes on the growth parameters of *Oreochromis mossambicus* was studied in fingerlings weighing about 5 ± 0.5 g. Feeding experiment was carried out for a period of 90 days. Parameters like total weight, average daily weight gain, relative growth rate and specific growth rate of fishes were recorded. It showed that all experimental feed enhanced the growth performance of Tilapia than the control.

During feeding with *S. flavofungini* AM 4 supplemented diet, the health status was monitored by measuring haematological parameters like classical primary hematological indices such as Haemoglobin (Hb) content, RBC count, Packed Cell Volume (PCV), Total WBC count and calculated secondary blood indices like MCHC, MCH, and MCV. We also evaluated the probiotic effect of *S. flavofungini* AM 4 by measuring the somatic indices like spleen-somatic and hepatosomatic index in an experimental model fish. *S. flavofungini* supplemented diets improved the haematological levels and the somatic indices showed good health status of the fish.

A battery of assays for both specific and nonspecific arms of immunity has been performed to ascertain the status of the immune system when feeding fish with *S. flavofungini* AM 4 supplemented diet. The specific immune response was assessed in terms of antibody production to SRBC using agglutination assay and scale allograft rejection. The nonspecific immune response was assessed in terms of serum lysozyme activity and differential WBC count. The status of overall functional immunity was tested using disease resistance test against *Aeromonas hydrophila* challenging and wound healing efficacy test.

It was found that, in case of antibody production, the secondary response was quicker than primary response and the level of antibody production is more in
the fish group fed with 1x 10^9 CFU of *S. flavofungini* AM 4 /g of feed. It was also documented that, scale allograft was rejected during fifth day of experiment in case of fish received 1x 10^9 CFU *S. flavofungini* AM 4 /g of feed. Our results showed that, the specific immunity was enhanced by oral administration of *S. flavofungini* AM 4 and the serum lysozyme level was increased when compared with control groups. In the case of differential cell count, lymphocyte count was increased in all experimental groups and monocyte and neutrophil were decreased proportionately. With respect to disease resistance test, relative percent survival (RPS) was increased proportionately when the concentration of *S. flavofungini* AM 4 increased in the fish feed. In case of wound healing efficacy test, the wound closure took place on 5th day onwards and maximum of closure occurred on 6th day of observation in the group which received 1x 10^9 CFU *S. flavofungini* AM 4 /g of feed. Other experimental group showed closure on 7th day of observation. These findings showed that the immune status was enhanced by the effect of streptomycetes supplemented diet.

✓ It is clear from our present study that, Actinomycetes are the potent source of antimicrobial agents and has the ability to enhance the immunity of the host. The synergistic effect of Actinomycete activity as an immunomodulator and as an antimicrobial agent would be useful in the process of elimination of pathogens.