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STUDIES ON MICROBIAL DIVERSITY AND THIER ANTIMICROBIAL ACTIVITY AGAINST UTI PATHOGENS

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1. INTRODUCTION

The soil is considered as the land surface of the earth which provides the substratum for plant and animal life. The soil represents a favourable habitat for microorganisms and is inhabited by a wide range of microorganisms, including bacteria, fungi, algae, viruses and protozoa. The physical structure, aeration, water holding capacity and availability of nutrients are determined by the mineral constituents of soil, which are formed by the weathering of rock and the degradative metabolic activities of the soil microorganisms. Cultivated soil has relatively more population of microorganisms than the fallow land and the soils rich in organic matter contain much more population than sandy and eroded soils. Microbes in the soil are important to us in maintaining soil fertility, cycling of nutrient elements in the biosphere and sources of industrial products such as enzymes, antibiotics, vitamins, hormones, organic acids etc. But certain microbes in the soil are the causal agents of various human and plant diseases.

The plant and animal remains deposited in the soil contribute organic substances. Soil microorganism's breakdown a variety of organic materials and use a portion of these breakdown products to generate or synthesize a series of compounds that make up humus, a dark coloured amorphous substance composed of residual organic matter not readily decomposed by microorganisms. The three major fractions of humus are humic substances, poly-saccharides and other non-humic substances, and humin. These materials impact on the physical, chemical and bio-chemical properties of soil in many ways. Humus improves the texture and structure of the soil, contributes to its buffering capacity and increase the water holding capacity of the soil.

The vast differences in the composition of soils, together with differences in their physical characteristics and the agricultural practices by which they are cultivated, result in

corresponding large differences in the microbial population both in total numbers and in kinds. The great diversity of the microbial flora makes it extremely difficult to determine accurately the total number of microorganisms present.

The bacterial population of the soil exceeds the population of all other groups of microorganisms in both number and variety. Bacterial population is one-half of the total microbial biomass in the soil ranging from 1,00,000 to several hundred millions per gram of soil, depending upon the physical, chemical and biological conditions of the soil. As per the system proposed in the Bergey's Manual of Systematic Bacteriology, most of the bacteria which are predominantly encountered in soil are taxonomically included in the three orders, Pseudomonadales, Eubacteriales and Actinomycetales of the class Schizomycetes. Bacteria are also classified on the basis of physiological activity or mode of nutrition, into two groups, i.e., autotrophs and heterotrophs. Autotrophic bacteria are capable of synthesizing their food from simple inorganic nutrients. They are of two types, i.e., photoautotrophs (*Chromatrum*, *Chlorobium*), which utilize CO_2 as carbon source and derive energy from sunlight and chemoautotrophs (*Nitrobacter*, *Nitrosomonas*), which utilize CO_2 as carbon source and derive energy from the oxidation of simple inorganic substances present in soil. Herterotrophic bacteria derive their carbon and energy from complex organic matter, decaying roots and plant residues. Most of the bacteria present in soil are heterotrophic in nature. Both aerobic and anaerobic bacteria are present in soil. The majority of the beneficial soil-dwelling bacteria need oxygen and therefore aerobic in nature, whilst those that do not require air are referred to as anaerobic and tend to cause putrefaction of dead organic matter. It is generally agreed that there are many species of bacteria in soil are yet to be discovered.

More than hundreds of different species of fungi inhabit the soil. They prefer to live in the soil in an aerobic condition. Fungi perform important functions within the soil in relation to nutrient cycling, disease suppression and water dynamics, all of which help plants become healthier and more vigorous. Fungi exist in both the mycelial and spore stage. Soil fungi are microscopic plant-like cells that grow in long threadlike structures or hyphae that make a mass called mycelium. The mycelium absorbs nutrients from the roots it has colonised, surface organic matter or the soil. From the mycelia the fungi is able to throw up its fruiting bodies, the visible part above the soil (e.g., mushrooms), which may contain millions of spores. When the fruiting body bursts, these spores are dispersed through the air to settle in fresh environments and are able to lie dormant for up to years until the right conditions for

their activation arise. The physical structure of soil is improved by the accumulation of mold mycelium within it.

Fungi are active in decomposing the major constituents of plant tissues namely cellulose, lignin and pectin. Saprophytic fungi convert dead organic matter into fungal biomass, carbon dioxide and organic acids. These fungi have enzymes that work to "rot" or "digest" the cellulose and lignin found in the organic matter, with the lignin being an important source of carbon for many organisms. Without their digestive activities, organic material would continue to accumulate until the forest became a huge rubbish dump of dead leaves and trees. By consuming the organic matter fungi play an important role in immobilising and retaining nutrients in the soil.

Actinomycetes represent a ubiquitous group of microbes widely distributed in natural ecosystems around the world and very significance on the recycling of organic matter (Srinivasan, *et al.*, 1991). *Actinomycetes* are soil organisms which have characteristics common to bacteria and fungi and yet possess sufficient distinctive features to delimit them into a distinct category. In the strict taxonomic sense, *Actinomycetes* are clubbed with bacteria in the same class of Schizomycetes but confined to the order Actinomycetales (Kumar, *et al.*, 2005).

The *Actinomycetes* are a group of bacteria which possess many important and interesting features. They are of considerable value as producers of antibiotics and of other therapeutically useful compounds. They exhibit a range of life cycles which are unique among the prokaryotes and appear to play a major role in the cycling of organic matter in the soil ecosystem (Veiga, *et al*., 1983).

Therefore, *Actinomycetes* hold a prominent position due to their diversity and proven ability to produce new compounds, because the discovery of novel antibiotic and non-antibiotic lead molecules through microbial secondary metabolite screening is becoming increasingly important.

Secondary metabolites are produced by some organisms such as bacteria, fungi, plants, actinomycetes and so forth. Among the various groups of organisms that have the capacity to produce such metabolites, the actinomycetes occupy a prominent place(Berdy, 2005; Ramasamy, *et al*., 2010 and Sundaramoorthi *et al*., 2011). Actinomycetes are prokaryotes of

Gram-positive bacteria but are distinguished from other bacteria by their morphology, DNA rich in guanine plus cytosine (G+C) and nucleic acid sequencing and pairing studies. They are characterized by having a high G+C content (>55%) in their DNA(Franco *et al*., 2009; Gurung *et al*., 2009 and Ogunmwonyi *et al*., 2010).

Recent days the discovery of known metabolites and actinomycetes are increasing due to the exploitation of natural ecosystems. Exploitation of less and unexplored ecosystems for actinomycetes is highly necessary for the discovery of novel bioactive metabolites. Actinomycetes are important sources of new bioactive compounds such as antibiotics and enzymes (Vining *et al.*,1992) which have diverse clinical effects and are active against many organisms (Bacteria, Fungi, Parasites etc.).

Urinary tract infections (UTIs) are common infectious diseases in clinical practice. An estimated 150 million people worldwide are diagnosed with a UTI each year, 1 and 40–50% of women present a UTI at least once in their lifetime.2–5 The results of a survey performed in the USA estimated that a UTI episode was associated with an average of 6.1 days with symptoms, 2.4 days of reduced activity and 0.4 days of bed rest, thus generating an estimated annual cost (direct and indirect) of 1.6 billion dollars.6–8 In China, UTIs account for 9.39–50% of nosocomial infections.9 10 Most cases of UTI are caused by Gram-negative bacilli, with Escherichia coli accounting for over 90% of uncomplicated UTIs.11 Uncomplicated infections can be treated with short courses of antibiotics, while complicated UTIs require longer and more intensive courses of antibiotics. However, resistance to the commonly used antibiotics is increasing and making treatment more difficult.

The problem of resistance against thepresent antibiotics in bacteria increases dayby day. So there is an urgent need to searchnew antibiotics or the sources of newantibiotics. A lot of work has been doneduring last few decades that has witnessedthe production of novel antibiotics fromdifferent microorganisms. Soil is a primarysource of microorganisms. Soil bacteria andfungi have played a significant and animportant role in antibiotic discovery. The numbers and species of microbes in soilis depend on environmental conditions likenutrient availability, soil texture, presence ofmoisture in soil and type of vegetationcover and their number varies according tothe type of environmental condition (Atlasand Bartha, 1998). From ancient times iswell understood that, natural products havea key role in the discovery and developmentof many antibiotics (Newman and Cragg, 2007).

Antibiotics are one of the important pillarsof modern medicines (Ball *et al.*, 2004), butold antibiotics lose their efficacy and theyare necessarily replaced with new ones formany species of pathogenic bacteria (Hancock, 2007). Microorganisms that areable to producing secondary metaboliteshave a diverse chemical structure andbiological activities and are produced onlyby some species of a genus *Bacillus* (Stachelhaus *et al*., 1995). Some of theimportant examples of these antibioticsused in medical treatments are bacitracin,Gramycidin S, polymyxin, and tyrotricidin(Drablos *et al*., 1999) produced by different*Bacillus* sp.

Biological interactions are the interactions between organisms in a community. In the natural world no organismexists in absolute isolation and thus every organism must interact with the environment and other organisms. Anorganism's interactions with its environment are fundamental to the survival of that organism and the functioning ofthe ecosystem as a whole. In antagonistic interactions, one species benefits at the expense of another. This is themost common phenomenon seen in the microbial world, where one species tries to remove the others by means ofproducing antagonistic compounds (Callaway, 1995).

The antagonism between bacteria and fungi is an important controlling factor for microbial colonization and growth(Romani *et al*., 2006). A good example of antagonism is the antagonism between *Aspergillus niger* and *Serratia marcescens* in soilwhere the spread of *Aspergillus niger* through soil was inhibited by *Serratia marcescens* when the organisms wereinoculated into separate sites in soil. Similar results were obtained with the inhibition of *Aspergillusniger* by *Agrobacterium radiobacter* and of *Penicillium vermiculatum* by either *S. marcescens* or *Nocardiaparaffinae* (Lindblom and Tranvik, 2003).

However, soil is also a rich medium for fungi as they offer dead organic matter or litter. Such fungi, may or may notbe helpful to plants; are supposed to interact with the soil natural microflora and if antagonistic interactions occur,the soil fertility may be compromised. This compromised soil fertility may result in either decreased crop yield or inincreased use of fertilizers (Verkaiek, 2001). In Central India, Jabalpur district and its surrounding villages are known for theagriculture due to its climate. The leguminous crops such as pulses (yellow lentil, Soybean etc.), gram and peas areamong the major crops grown in this region. The present work was aimed at identifying the soil microflora (bothbacteria and fungi) from a local leguminous soil fields and studying the biological interactions among them.

SCOPE OF THE STUDY

- Soil sample were collected from Thanjavur Districts
- Isolation and identification of bacteria from soil sample.
- Isolation and identification of fungi from soil sample
- Isolation and identification of actinomycetes from soil sample.
- Effect of antibiotics from UTI pathogens
- Antibacterialactivity (bacterial, fungal, actinomycetes) against UTI pathogens.

2. REVIEW OF LITERATURE

Several antibiotics were tested by against a range of *Actinomycetes* found in soil. From these tests four antibiotics, nystatin (50 pg. /ml.), actidione (50 pg./ml.), polymyxin B sulphate (5.0 pg./ml.) and sodium penicillin (1.0 ug./ml.), were selected for incorporation into a starch+casein medium to achieve selective growth of *Actinomycetes* on soil dilution plates. For selective development of *Actinomycete* colonies mixture of antibiotics was tested with a number of soils and its efficiency compared with several other methods. Among all the seven antibiotics tested against 10 a range of *Actinomycetes,* the antifungal ones (nystatin, actidione) did not inhibit any strains not even at the highest concentration of l00 ug/ml. Most appropriate mixture for the enumeration of soil *Actinomycetes* colonies was starch + casein medium with the two antifungal antibiotics (nystatin, actidione). And for isolation of *Actinomycetes* colonies the use of same medium with all four antibiotics was most satisfactory (Williams and Davies, 1964).

The identification and isolation of two new genera which are marine derived actinobacteria. It is revealed thatapproximately 90% of the microorganisms were cultured by using the presented method which is from the prospective new genera, it indicates as a result which is indicative of its high selectivity. From the Bismarck Sea and the Solomon Sea off the coast of Papua New Guinea 102 *Actinomycetes* were isolated from the sub-tidal 8 marine sediment. Biological activity testing of fermentation products from the new marine-derived *Actinomycetes* showed that is has activities against multidrug-resistant gram-positive pathogens, malignant cells and vaccinia virus replication (Nathan, *et al*., 2004).

A total of 94 *Actinomycete* strains were isolated from the marine sediments of a shrimp farm, 87.2% belonged to the genus *Streptomyces,* and others were *Micromonospora* spp. Among them fifty one percent of the *Actinomycetes* 12 strains showed activity against the pathogenic

Vibrio spp. strains. Thirty eight percent of marine *Streptomyces* strains produced siderophores on chrome azurol S (CAS) agar plates. From the total strains seven strains of *Streptomyces* were found to produce siderophores and they inhibit the growth of *Vibrio* spp. *in vitro*. Two strains are belonged to the *Cinerogriseus* group, which is the most frequently isolated group of *Streptomyces*. From the obtained results it can be assumed that in aquaculture it can be use as biocontrol agent (You,*et al.*, 2005).

Actinomycetes population from continental slope sediment of the Bay of Bengal. The range of Actinomycetes population is from 5.17 to 51.94 CFU/g and 9.38 to 45.22 CFU/g dry sediment weight. From stations in 1000 m depth no *Actinomycete* colony was isolated. Populations in stations in 500 m depth in both cruises were higher than that of 200 m depth stations. Three *Actinomycetes* genera were identified. Found *Streptomyces* was the dominating one in both the cruises, followed by *Micromonospora* and *Actinomyces*. Spiral spore chain showed the maximum abundance and the spore surface was smooth (Das, *et al.*, 2008).

The collection of 62 isolates of *Actinomycetes* isolated from 7 soil samples collected from Agriculture Research Center Semongok, Sarawak. All the 62 isolates exhibit dark grey, grey, dark brown, brownish, whitish and yellowish white colours. The selection of phytopathogens as test strains antimicrobial test was done and it was observed that 3, 25, 35 and 37 of the isolates showed antagonistic reaction with *Fusarium palmivora*, *Bacillus subtilis*, *Pantoae dipersa* and *Ralstonia solanacearum* respectively. All the six isolates were identified as *Streptomycetes* spp. To see the use of *Actinomycetes* in agriculture industry further study will be done to fully utilize these potential microbes for sustainable agriculture (Jeffrey, *et al*., 2008).

A total of 173 *Actinomycetes* colonies were isolated from near shore marine environment and mangrove ecosystem at 8 different locations of Kerala, West Coast of India. Among them, 64 isolates were morphologically distinct on the basis of spore mass colour, reverse side colour, aerial and substrate mycelia formation and production of diffusible pigment. The majority (47%; n=30) of these isolates were assigned to the genus *Streptomyces*. Antimicrobial activities of isolates were also tested against various bacterial and fungal pathogens. 64 isolates, 21 isolates had antimicrobial activity, with 2 isolates showing broad spectrum of antimicrobial effect (Remya and Vijayakumar, 2008).

Seventynine *Actinomycetes* were isolated from soils of Kalapatthar (5545m), Mount Everest region. Among all the isolates twenty seven (34.18%) of the isolates showed an antibacterial activity against at least one test bacteria among two Gram positive and nine Gram negative bacteria in primary screening by the technique of perpendicular streak method. In secondary screening thirteen (48.15%) showed antibacterial activity. After that the MIC test was done and the minimum inhibitory concentration (MIC) of antibacterial metabolites of the isolate K.6.3 was 1mg/ml, and that of isolates K.14.2 and K.58.5 was 2mg/ml. The active isolates from primary screening were heterogeneous in their overall macroscopic, biochemical, and physiological characteristics (Gurung, *et al*., 2009).

They collected sample from Chennai (Tamil Nadu) coastal area. 34 strains were isolated and among them 10 potential marine *Actinomycetes* strains were screened by cross streak method against five fish pathogenic bacteria. The extract was tested by disc diffusion method against bacterial pathogens and the ethyl acetate extract showed a good inhibition range of 6-15 mm in diameter. The most potential *Actinomycetes* strain was characterized and identified as *Streptomyces* spp (Pugazhvendan, *et al.*, 2010).

A total of 55 *Actinomycetes* isolates from soil sample of Karanjal region in Sundarbans were characterized for morphological identification and antimicrobial activity. The total numbers of isolates were 27, 14, 11 and 3 which belong to *Actinomyces, Nocardia*, *Streptomyces* and *Micromonospora* respectively, as they were identified from the sample. Against one or more gram11 negative pathogenic bacteria such as *Shigella boydii*, *Shigella flexneri*, *Shigella sonnei*, *Pseudomonas*, *Shigella dysenterriae* type-1, *Vibrio cholerae*, *Salmonella typhi*, *Plesiomonas*, *Hafnia* spp., *Vibrio cholerae*, and *Escherichia coli* twenty *Actinomycetes* isolates were found which can produces antibiotic. A diverse group of *Actinomycetes* were found in sundarbans soil and among them three of the tested isolates had a broader spectrum antibacterial activity that shows there potential as a source of antibiotics for pharmaceutical interest (Arifuzzaman, *et al.*, 2010).

Actinomycetes isolated from different locations of the Manakudi Estuary of Arabian Sea in Tamilnadu, India. All the isolated strains exhibits higher activity against the Gram positive bacteria; methicillin resistant and susceptible *Staphylococcus aureus*, *Enterobacter* sp, *Salmonella typhi, Bacillus subtilis, Klebsiella pneumoniae*, *Proteus vulgaris*. Intermediate activity was shown by them against Gram negative organism *Pseudomas auregionosa and* it shows no antagonistic effect towards yeast like *Candida albicans*. Pink *Actinomycetes* (PJS)

with white aerial mycelium and pink substrate mycelium and black colonies (BJS) of white aerial mycelium and yellowish white substrate mycelium shows potent inhibiting effect of other microorganisms (Satheeja Santhi, *et al.*, 2010).

The antifungal activity of the crude extract prepared from the strain *Streptomyces* spp. From the Puducherry coast of India 8 strains were isolated from the marine sediment. Primarily eight strains were screened for antifungal activity against three species of *A.fumigatus*, *A. niger* and *A. flavus*. The metabolites were extracted using ethyl acetate; it is than lyophilized and screened for antifungal activity against the three *Aspergillus* species by well diffusion method. Maximum zone of inhibition observed was 21mm for *A. fumigatus* in comparison with the standard antifungal antibiotic Nystatin which shows 20 mm (Thenmozhi and Kannabiran, 2010).

Actinobacteria were isolated by the serial dilution method from marine sediments collected from Bay of Bengal at a depth of 10-40m near pudimadaka coast of Andhra pradesh. During the study total 78 isolates were obtained and among all the isolates *Streptomycetes* is predominant. Among all the 78 isolates antibacterial and antifungal activity exhibited by 22 isolates exhibited antibacterial and antifungal activity, respectively. After this the strains were further characterized and identified to be belonging to the genus *Rhodococcus* and *Streptomyces* (Kumar, *et al.*, 2011).

Actinomycetes are the representative of terrestrial microorganisms and usually are isolated from soils. When compared to terrestrial *Actinomycetes*, however, a small portion of work has been conducted on marine *Actinomycetes*. The sediment samples were collected from different stations of the Muthupet mangrove ecosystem (10°15'-10°35'N and 79°20'–79°55'E), situated along the Southeast coast of India, for isolation of *Actinomycetes*, using Kuster agar medium. The isolated strains were identified using physiological and biochemical methods. Seven *Actinomycetes* sp were isolated and tested for their antagonistic activity against human pathogens like, *Escherichia coli*, *Pseudomonas* sp., *Klebsiella* sp. and *Bacillus* sp. (Sathiyaseelan and Stella, 2011).

Isolate and characterize the antimicrobial *Actinomycetes* from sediments of Mangrove ecosystems of Nizampatnam located in the south coastal region of Andhra Pradesh, India. The Mangrove soil samples were collected, pre-treated and plated on asparagines glucose agar medium. The isolation, characterization of the rare *Actinomycetes* from the mangrove

ecosystem will be useful for the discovery of the novel bioactive metabolites that are effective against wide-range of pathogens (Mangamuri, *et al.*, 2012).

The soil samples were collected from Coringa mangrove, Andhra Pradesh and the isolations were carried out using dilution-plate technique. 27 isolates were obtained and were characterized morphologically, biochemically, physiologically. Screening tests were performed to determine enzymatic activity and antimicrobial activity. StrainA exhibited antimicrobial activity against test microorganisms like *Staphylococcus aureus, Pseudomonas aureginosa, Pseudomonas fluroscence, Lactobacillus acidophilus, Lactobacillus casei, Candida albicans, Streptococcus mutans, Bacillus subtilis, Bacillus megaterium,* and *Xanthomonas.* Maximum activity was exhibited against *Xanthomonas* where as minimum against *Candida albicans.* 96 hr incubation resulted in optimum antimicrobial activity (Kavya Deepthi, *et al*., 2012).

Isolate *Actinomycete* colonies having antibacterial and antifungal activity from soil samples. A total of 27 *Actinomycete* colonies were isolated in pure culture from five soil samples using starch casein agar medium. Entire isolates were screened for their antimicrobial activity by agar plug method against five each of human pathogenic bacteria and fungi. Of this, 7 strains inhibits *B. substilis,* 3 strains inhibits *Klebseilla* sp, 6 strains inhibits *B. cerus,* 5 strains inhibits S. *aureus* and only 2 strains inhibits *E. coli.* In case of fungi all the actinobateria has moderate activity with less fungal strains, only 1 strain (RA 5) inhibits entire fungus except *Penicillium* sp. The metabolites from potent strain was produced by fermentation, separated by centrifugation, it was tested for their antimicrobial activity against the test bacterial and fungal strains by well diffusion and disc diffusion method. The metabolites from *Streptomyces* sp. have showed good antibacterial and antifungal activity (Silambarasan *et al*., 2012).

Four *Actinomycete* isolates, SAM2-1, SMP3-1, and J8-1 and J17-2 were isolated from mangrove soils collected in Samut Prakarn and Samut Songkram provinces, the inner gulf of Thailand. These isolates were identified as *Streptomyces* based on their phenotypic and chemotaxonomic characteristics. On the screening of antimicrobial activity, they could inhibit *Staphylococcus aureus* ATCC 6538P and *Bacillus subtilis* ATCC 6633 but showed weak inhibitory activity against *Kocuria rhizophila* ATCC 9341, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginos a* ATCC 27853 and no activity against *Candida albicans* ATCC

10231. Only isolate J8-1 showed strong inhibitory activity against *Bacillus subtilis* ATCC 6633 (Hunadanamra, *et al*., 2013).

The physico-chemical characteristics of sediments in two different mangrove areas located at the eastern mangroves region i.e Coringa Wild Life Sanctuary (CWS) and Seethanagaram Mangroves area (SMA) of Andhra Pradesh, South India. Salinity varied from 32.0 to 42 ‰ in both collection area and the pH in the soil sediment ranged between 4.32 to 4.84 mg/L. Concentrations of nutrients viz. nitrate is more in CWS (mean 0.321 mg/L) compared to SMA (0.286 mg/L). Organic carbon was recorded as 4.28 % in CWS and 3.12% in SMA. Phosphates are 0.06 mg/L in first collection area and 0.04 in the second collection area. The humic acid percentage was 2.32% in CWS and 1.1% in SMA. The nature of soil texture is characterized by the abundance of solty loam, silty clay and silty clay loam (Dogiparti, *et al*., 2013).

The physicochemical study of soil is based on various parameters like total organic Carbon, Nitrogen (N), Phosphorus (P2O5), Potassium (K2O), pH and Conductivity. The nutrient's quantity present in soil of Bhusawal, District Jalgaon (Maharashtra). All the eight selected places of Bhusawal have medium or high minerals content. In order to study the effect of phosphate fertilizer, phosphorus and application of nitrogen to increase percentage yield of crops. This information will help farmers to solve the problems related to soil nutrients, amount of which fertilizers to be used to increase the yield of crops (Chaudhari, 2013).

Kousha and Vatankhah, (2015) studied that the*Pseudomonas* sp was isolated from Rhizosphere inNorth Iran. The antimicrobial activity of cell-freesupernatant and partially purified bacteriocin wasdetermined by well diffusion method. A total of 100 *S.aureus* isolated from clinical specimens. The bacteria wereprimarily identified by colony morphology, microscopy of Gram's stain and routine biochemical tests and antibioticdisk sensitivity tests (Cefoxitin, Ceftriaxon, Amoxicillin, Cefotaxime, Co-trimoxazole; Penicillin, Tetracyclin, Azitromycin). The isolate *Pseudomonas* sp.broadest antimicrobial spectrum against MRSA isolatedclinical samples. However, the spectrum of inhibitoryactivity of these bacteria suggests a potentially useful meansfor controlling the growth of food-borne pathogens bacteriasuch: *S. aereus* and MRSA isolated food samples.

Sagahon, *et al*., (2011) evaluated that the 160 strains were isolated from soil of corn crops of which 10 showed antifungal activity against these phytopathogens, which, were identified as:

Bacillus subtilis, Pseudomonas spp., *Pseudomonas fluorescens* and *Pantoea agglomerans* by sequencing of 16S rRNA gene and the phylogenetic analysis. From cultures of each strain, extracellular filtrates were obtained and assayed to determine antifungal activity. The best filtrates were obtained in the stationary phase of *B. subtilis* cultures that were stable to the temperature and extreme pH values; in addition they did not show a cytotoxicity effect against brine shrimp and inhibited germination of conidia. The bacteria described in this work have the potential to be used in the control of white ear rot disease.

Mashoria *et al*., (2014) studied that the a trial was done to find out a new antimicrobial agent producing bacteria from soil samples collected from different regions of Bhopal, Madhya Pradesh (India). Isolation of different bacterial colonies from soil sample was carried out. All the isolated bacterial colonies were then screened for their antimicrobial activity against the pathogenic bacteria *Salmonella typhi* (MPCST- 109), *Serratia ficaris*(MPCST-076), *Streptococcus facalis* (MPCST-072), *Pseudomonas vesicularis* (MPCST-088), *Staphylococcus cohni* (MPCST-121) *E-coli* and *Pseudomonas aeroginosa*. Among the total 28 bacterial isolates, only 12 of them (42.85%) were capable of biosynthesizing antimicrobial metabolites. One of the Bacterial colony that was obtained from Mandideep region of Bhopal, found to exhibit the highest antimicrobial activity against most of the used pathogenic bacteria in studies. The Physiochemical and biochemical characters of the isolated bacteria were matched with *Pseudomonas* sp. Thus, it was given the suggested name PBR-11. This study indicates that microorganisms isolated from Bhopal region (India) soil could be an interesting source of antimicrobial bioactive substances. Soil samples obtained from different locations of Bhopal and Guna (India) were analysed to determine the presence and types of antibiotic-producing bacteria, using nutrient agar media.

Five fungal strains and two bacterial strains were isolated from a leguminous crop field in Jabalpur area and were screened for antagonistic activities towards each other. All five fungal extracts showed inhibition towards test bacteria *Klebsiella* sp. and *Morganella morganii*. *Morganella* was more affected bacteria. Among fungi, *Penicillium* was least effective (Jaiswal *et al*., 2012).

The anti-bacterial activities of more than eighty soil inhabitant *Penicilliums*p isolates were evaluated. Isolates were cultured, simultaneously with bacteria: *Pseudomonas syringae, P.viridiflava, Xanthomonas translucens, Agrobacterium tumefascines, Rathayiibacter iranicus* and one isolate of *E. coli* in PDB and the results were evaluated after one week. All

tested isolates, had anti-bacterial activity against one or more bacterial strains tested. It seems that, most of the examined Penicillia, produce one or more antibacterial substances and are able to prevent growth and proliferation of bacteria in their habitat (Javadi *et al*., 2012).

Wild olive crude extracts were screened for antibacterial activity against five different bacterial human pathogens. Four different solvents were used for the extraction. Antibacterial activities of plant extracts were compared with those of standard antibiotics. The capacity of the extracts and antibiotics were evaluated on the basis of their capacity to inhibit the growth of pathogenic bacteria measured as zone of inhibition (ZOI, expressed in mm). Almost all bacteria showed to be sensible against the antibiotics with the value of ZOI ranging from 10-32 mm, while the effectiveness of olive extracts varied from one species to other with ZOI values of 7-12 mm. Extract obtained with methanol appeared to be the most effective against all pathogenic bacteria compared to those obtained with other solvents (Paudel *et al*, 2012).

Spirulina as many other cyanobacteria species have the potential to produce a large number of antimicrobial substances, so they are considered as suitable organisms for exploitation as biocontrol agents of plant pathogenic bacteria and fungi. In the present study, antimicrobial activity of Spirulina platensis solvent extracts was investigated against pathogenic bacteria and fungi. The antimicrobial activity of Spirulina platensis was determined against pathogenic bacterial and fungal isolates. The methanol extract of Spirulina platensis showed maximum zone of inhibition against all the bacterial and fungal isolates. The hexane extract of Spirulina platensis showed minimum inhibition zone against bacterial and fungal pathogens when compared to the other solvent extracts (Usharani *et al*., 2015).

Aslam and Sajid, (2016) studied that the isolates exhibited significant tolerance to alkaline conditions and grew well at pH 9-11. The taxonomic status of the isolated strains was determined by morphological, biochemical and physiological characterization and by 16s rRNA gene sequencing. The results revealed that majority of the isolates (90%) belong to the genus Streptomyces. Most of the isolates exhibited remarkable antimicrobial activity up to 20mm zone of inhibition against MDR ventilator associated pneumonia causing bacteria including *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumoniae, Escherichia coli, Enterobacter* and *Acinetobacter* sp.

3. MATERIALS AND METHODS

Collection of soil sample

The soil samples were collected form the two different site (Vallam and Mariyamman kovil) in Thanjavur Districts, Tamilnadu, India. Thanjavur is the headquarters of the Thanjavur District. The city is an important agricultural centre located in the Cauvery Deltaand is known as the Rice bowl of Tamil Nadu(Plate I).

Analysis of physicochemical parameters in soil samples

Determination of soil pH

In a clean conical flask 20 g of air-dried soil was taken and 100 ml distilled water was added for making 1:5 soil suspension. It was shaken for one hour at regular intervals. After shaking, the suspension was filtered through Whatmann no. 42 filter paper. The pH of the sample was determined using a pH meter (Sartorium brand pH meter).

Estimation of soil electron conductivity

To 1ml of water 1g of soil was mixed that is 1:1 suspension. The suspension was filtered using suction. A circular Whatman no. 42 filter paper was put in the Buchner funnel and the filter paper was moistened with distilled water and made sure that it was tightly attached to the bottom of the funnel that all holes are covered. The vacuum pump was started. The suction was opened and the suspension was added to Buchner funnel. The clear filtrate was transferred into 50 ml bottle and the conductivity cell was immersed into the solution and the readings were noted (Levine, 2001).

Estimation of organic carbon

The determination of soil organic carbon was based on the Allison - Black (1965) chromic acid wet oxidation method. 10 - 20 mg soil was weighed into a dry tared 250 ml conical flask to which 10 ml (1 N) $K_2Cr_2O_7$ was added and swirled gently to disperse the soil in the solution. Then 20 ml concentrated H2SO4 was added and immediately the flask was swirled until the soil and the reagents were mixed and heated while swirling the flask on a hot plate until the temperature reached 135°C (approximately ½ a minute). It was then set aside to cool slowly on an asbestos sheet in a fume cupboard. Two blanks (without soil) also were running in the same way to standardise the FeSO4 solution. When cooled (20–30 minutes), diluted to 200 ml with deionised water and proceeded with the FeSO4 titration using the "ferroin" indicator. Three or four drops of ferroin indicator was added and titrated with 0.4 N FeSO4. As the end point was approached, the solution took on a greenish colour and then changed to

a dark green. At this point, ferrous sulphate was added drop-by-drop until the colour changed sharply from blue-green to reddish-grey.

Calculations

From the equation: $2Cr_2O7_2$- + 3C + 16H+ → $4Cr_3$+ + $8H_2O$ + $3CO_2$ ↑ 1 ml of 1 N Dichromate solution is equivalent to 3 mg of carbon. Where the quality and normality of the acid / dichromate mixture used were as stated in the method, the percentage carbon was determined from the following formula:

$$\text{Organic carbon } (\%) = \frac{0.003 \text{ g x N x 10 ml x } (1 - T/S)}{ODW} \times 100$$

$$\frac{3(1 - T/S)}{W}$$

Where: N = Normality of K2Cr2O7 solution T = Volume of FeSO4 used in sample titration (ml) S = Volume of FeSO4 used in blank titration (ml) ODW = Oven-dry sample weight (g)

Estimation of organic matter

In a dry conical flask 0.2 g of soil sample was taken. 2 ml of $K_2Cr_2O_7$ followed by 4 ml concentration H_2SO_4 were added to it and the contents were gently mixed. The flask was kept aside for 30 min for the complete reaction to take place and the contents were diluted by adding 40 ml distilled water. 2 ml of phosphoric acid was added to it followed by the addition of 1 ml of diphenylamine indictor. The contents were titrated against 0.4 N FAS until the colour changed to brilliant green. The blank titration was carried out with the same amounts of reagents but without the soil sample (Stevenson and Cole, 1999).

Calculation

Percentage of carbon (%) = 3.951(1-T / S) W 2.

Percentage of organic matter (%) = % of C 1.724

Here, W = Weight of soil in g T = ml of FAS S = Ferrous solution with blank titration (ml)

Estimation of available nitrogen

About 0.2 ml to 1 ml of working standard solution was pipetted out in S1 to S5 test tubes respectively. 0.2 ml of sample was taken in U1 test tube. All the test tubes were made up to 9 ml using sterile distilled water. 1.5 ml of sodium hydroxide and 1ml Nessler‟s reagent was added to all tubes. The intensity of the colour development was read at 540 nm using green filter. The concentration of nitrogen of the solution was calculated using standard graph.

Calculation

Test OD / test OD conc. of Std. 100/ volume of sample taken.

Estimation of total phosphorous

In a china dish 0.5 g powdered soil was taken and it was moistened with distilled water to the consistency of a thin paste. 2 ml Conc. HNO3 followed by 2 ml Conc. Perchloric acid were added. The contents were heated slowly on a hot plate until they become nearly dry. The dish was cooled and 1 ml perchloric acid was added and the contents were treated again until they were dry. The dish was cooled and 20 ml of diluted H2SO4 was added. It was boiled slowly for 10 minutes and allowed to cool. The contents were filtered through Whatman no. 42 filter paper and the final volume was made up to 250 ml. 50 ml aliquot was taken in a beaker. 2 ml of ammonium molybdate and 5 drops of SnCl2 were added to it. After the development of colour, the absorbance was read in a spectrophotometer at 690 nm.

Estimation of total potassium

In a beaker 5 g of air-dried and sieved soil sample was taken. 10 ml of ammonium acetate was added to it and stirred. The supernatant was kept overnight. The supernatant was filtered through Whatman no. 42 filter paper. The sample was leached 4-5 minutes with equal amount of ammonium acetate and the final volume was made up to 100 ml. The solution was used for determination of potassium using flame photometer. Flame photometer was switched on and the flame was lit as per the instructions given in the use's manual. The standard solutions were aspirated into the flame and the instrument was calibrated accordingly first. Then the sample was aspirated into the flame and the concentration of potassium present in the sample was determined.

Estimation of total calcium

In a beaker 5g of air dried, crushed and sieved sediment sample was taken. The sample was diluted with 1:9 ammonium acetate solution. The solution was extracted by filtering in Whatman filter paper and the extract was used for determining the calcium concentration. The calcium concentration was determined using atomic absorption spectrophotometer at the wavelength of 422.7 nm (Brady and Weil, 1999).

Calculation

Percent of total calcium = –B Where V = Volume of EDTA titrated for the sample (ml) B = Blank titration volume (ml) R = Ratio between total volume of the extract and extract volume used for titration N = Normality of EDTA solution. Wt = Weight of air-dry soil (g).

Estimation of magnesium

About 10 - 20 ml of soil saturation extract was pipetted out, and diluted to 20 - 30 ml with distilled water. Then 3-5 ml of buffer solution was added. After that few drops of erichrome indicator was added. This solution was titrated with 0.01 N EDTA until the color changed from red to blue (Cresser *et al*., 1993).

Calculation

Percent of total magnesium (meq/L) = Ca + Mg (meq/L) - Ca (meq/L).

Estimation of zinc, copper, iron and manganese

These procedures are according to Whitney (2011). 1.97 g of diethylene triamine pentaacetic acid (DTPA) and 1.1 g calcium chloride (CaCl2) was weighed in a beaker. Dissolve with distilled water and then transfer to a 1-litre volumetric flask. In another beaker, 14.92 g of triethanolamine (TEA) is weighed and dissolved with distilled water and make up to 1 litre. The pH was adjusted to exactly 7.3 with 6 N hydrochloric acid (HCl), and make to 1-litre volume with distilled water. The final extractant solution is 0.005 M DTPA, 0.1 M TEA, 0.1 M CaCl2. A series of standard solutions for micronutrients in DTPA extraction solution was prepared. Fe: 0, 1, 2, 3, 4, 5 ppm; Zn: 0, 0.2, 0.4, 0.6, 0.8, 1.0 ppm; Cu: 0, 1, 2, 3, 4 ppm; Mn: 0, 1.0, 1.5, 2.0, 2.5 ppm. 10 g of air-dried sieved soil (2-mm) was weighed into a 125-ml Erlenmeyer flask. 20 ml of extractant solution was added. It was shaken for 2 hrs on a reciprocal shaker. The suspension was filtered through a Whatman no. 42 filter paper. Zn, Fe, Cu and Mn were measured directly in the filtrate by an Atomic Absorption Spectrophotometer.

Calculation

Percent of total Zn, Cu, Fe, Mn (ppm) = (ppm in extract – Blank) Where: A = Total volume of the extract (ml) Wt = Weight of air-dry soil (g).

Dilution plating method

The samples were diluted serially as congo red by Waksman, (1922). 1gm of soil samples were diluted in 10 ml distilled water and further diluted in 10 fold distilled water blank as serial dilution. Then 0.1 ml of the10^{-3} diluted sample was poured and spread on Petri plates containing sterilized Nutrient agar medium. The inoculated plates were incubated in the room; temperature was maintained at 37°C for 24 hours. Replicate plates were maintained for each sample.

Isolation of *Actinomycetes* from the soil sample

Actinomycetes were isolated by serial dilution method from sediments (Hayakawa, 2008). Stock solution was prepared by diluting 1 g of sediment in 9 ml of sterile saline water and shaking well by using a vortex mixer (IKA). From the stock solution, 1 ml was used to prepare the final volume of 10^{-2} and 10-^{3} by serial dilution method.

Samples were inoculated on Starch Casein Agar (SCA) (composition: soluble starch: 10 g, K2HPO4: 2 g, KNO3: 2 g, casein: 0.3 g, MgSO4.7H2O: 0.05 g, CaCO3: 0.02 g, FeSO4.7H2O: 0.01 g, agar: 15 g, filtered sea water: 1000 ml and pH: 7.0±0.1). The medium was supplemented with 25 µg ml−1 nystatin or griseofluvin to minimize contamination with fungi and 10 µg ml−1 nalidixic acid or rifampicin to minimize contaminant growth of bacteria. (Hayakawan 2008 and Ravel, *et al*., 1998). Plates were incubated for 7 to 20 days at28 °C. Then the colonies with a tough or powdery texture, dry or folded appearance and branching filaments with or without aerial mycelia were subcultured on slants SCA (Mincer, *et al*., 2002). Until further use, the slants were kept in cold room at 4°C (Das, *et al*., 2010). The identification of *Actinomycetes* sp. from Bergey's Manual 9th edition and Selman, *et al.*, 1953.

Isolation of Bacteria from the soil sample

Nutrient agar media was used for bacterial cultures revival. Nutrient agar medium was prepared and pH of the media was adjusted to 6.5. The media was then autoclaved for 121^{0}C for15 lbs pressure at 15 minutes. The medium was poured in to petridishes and allowed to solidify. The griseofluvin(20mg/lit) was added for the purpose of avoid fungal growth in the medium. The agar was allowed to solidification.

The collected wound samples were spread on petriplates containing sterilized nutrient agar medium. The inoculated plates were incubated in the room temperature was maintained at

37°C for 24 hours. Replicate plates were maintained for each sample. The colonies growing on nutrient agar plates with different morphology were counted separately.

Characterization of Actinomycetes and Bacteria:

The potent isolates of Actinomycetes and bacteria were selected from screening methods were characterized by morphological, biochemical and physiological methods. The morphological method consists of macroscopic and microscopic characterization. Macroscopically, the Actinomycetes and bacteria isolates were differentiated by their colony characters, e.g. size, shape, colour, consistency, etc. For the microscopy, the isolates were grown by cover slip culture method (Kawato and Sinobu 1959). They were then observed for their mycelial structure, conidiospore and arthrospore arrangements on the mycelia under microscope (1000X). The observed morphology of the isolates was compared with the Actinomycetes and bacteriamorphology provided in Bergey's Manual for the presumptive identification of the isolates.

Gram Staining

Smear was prepared by spreading the broth culture on a glass slide followed by heat drying. The smear was covered with crystal violet for 30-60 s and washed off with water. The smear was covered with Gram's iodine for 30-60 s, decolorized with alcohol and washed with water. Finally the smear was stained with safranin counter stain for 2 min. After washing and drying, the slides were viewed at 100× under phase-contrast microscope (Pepper and Garba., 2004).

Motility test

A loop full of 48 h old bacterial culture grown in starch casein media was transferred to the center of a clean cover slip with petroleum jelly applied to the edges. A cavity slide was placed over the cover slip with the cavity facing downwards, covering the bacterial culture. Slide was gently pressed to seal-up with the cover slip and turned over quickly. The slide was observed under Eclipse E600 Microscope using low power (10×) and high power (40×) objectives. The bacteria moving swiftly across the microscope field were considered positive for motility.

Biochemical test for *Actinomycetes* sp.

Indole Test Tryptone broth medium was prepared. The medium was poured in to the test tubes. The isolates were inoculated separately to the broth and incubated at 30±2°C for two

days. The uninoculated broth was maintained as control. After the period of incubation, one ml of Kovac's reagent was added to each tube, including control. The tubes were gently shaken at an interval of 10 to 15 min, and allowed to stand until the reagent reaches the top. The formation of red color ring is indicating the positive results; whereas yellow color ring indicates negative result.

Methyl red test

The MR-VP broth was prepared. 5 ml of the broth was poured into sterile test tubes. The isolates were inoculated separately into the 30±2°C for two days. After the incubation 5 ml of methyl red indicator was added to the each tube. Red coloration of the broth indicates the positive result, while turning of methyl red to yellow is a negative result.

Voges Proskauer test

The MR-VP broth was prepared. 5 ml of the broth was poured into sterile test tubes. The isolates were inoculated separately into the 30±2°C for two days. After the incubation period5 ml of Barrit's reagent A and B was added. Development of red colour indicates the negative results.

Citrate utilization test

Simmon's citrate agar medium was prepared. The medium was poured into the sterile test tubes. The isolates were inoculated separately into the test tubes and incubated at 30±2°C for 4 days. After the incubation period, green colour turned to blue indicates the positive results.

Catalase test

A clean glass slide was taken and a drop of culture suspension was placed. Few drops of hydrogen peroxide were added to the culture. The evolution of air bubbles form the suspension is indicates the positive results.

Carbohydrate test

The utilization of different carbon sources by the was studied viz. Sucrose, lactose, (hexoses), and Mannitol (sugar alcohol). The carbon sources were added to the sterilized basal medium (carbohydrate fermentation medium) at the rate of 1%.

Starch hydrolysis test

The test organism was inoculated on the starch agar plates (agar containing starch as substrate). The plates were incubated at 37ºC for 24 h. The plates were then flooded with

Gram's or Lugol's iodine. The presence of clear zone around the colonies indicated secretion of amylase and hydrolysis of starch. A purple or blue zone colour indicated the absence of starch degradation. An un-inoculated starch agar plates was maintained as a control.

Isolation of fungi from soil sample

Potato Dextrose Agar (PDA), were used to isolate fungi from the collected samples. After sampling, within 24 hrs the soil samples from each station were subjected to appropriate dilutions (10^{-3} to 10^{-5}) to reduce the variability of the numbers of fungi on the plate and thus obtain a low probable error and 0.1 ml of sample was aseptically transferred into the plates containing PDA, with the addition of chloramphenical (20 mg /L) (Spread plate method) so as to have 30 to 100 fungal colonies developing on the plate. The plates were incubated at room temperature (28^{o}C) for 4-5 days. For each sample triplicates of petriplates were made. Control plates were also maintained.

Identification of fungi

The identification of fungal taxa was based on illustrated Genera of imperfect fungi (Barnett, 1965), Hyphomycetes (Subramanian, 1971), Dematiaceous Hyphomycetes and More Dematiaceous Hyphomycetes (Ellis, 1971, 1976), Micro fungi on land plants (Ellis and Ellis, 1985) and Manual of soil fungi (Gilman, 1957, 1998). The fungi growing from tree barks were identified on the basis of colony characteristics and conidial morphology using keys of Barnett and Hunter (1972), Booth (1977), Neergaard (1979) and Hawksworth *et al.*, (1995). The emerging fungal colonies were counted for numbers and identified to the species level based on morphological characteristics.

Antibiotic susceptibility test

Antibiotic susceptibility test were performed in disc diffusion method. Antibiotics are choosing based on mode of action. The isolates were determined sensitivity to intrinsic antibiotics like Penicillin G (P^{10}) cell wall synthesis inhibitors, Polymixin B (PB^{300}) membrane permeability alternatives, Gentamycin (GEN^{10}) protein synthesis inhibitors, Rifampicin (RIF^{5}) nucleic acid synthesis inhibitors, and Sulphamethizole (SM^{300}) metabolic process inhibitors to decide the antibiotics based upon the mechanism of physiological action. The overnight culture isolates was spread over Nutrient agar plate. It was incubated aerobically at 37°C for 48 hours along with control (sterile disc) and checked for the presence or absence of growth.

Determination of Antibacterial Activity: (Perez *et al.*, 1990)

Preparation of culture inoculums

The stock cultures of bacteria isolated from urine samples of diabetic patients were used in this study was maintained on nutrient agar slants at 4°C. Inoculums was prepared by suspending a loop full of bacterial cultures into 10 ml of nutrient broth and was incubated at 37°C ± 2°C for 24 to 48 hours.

Agar well diffusion method

The secondary screening of Antibacterial activity is purified extracellular crude extracts; was determined by agar well diffusion method. Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with 24 hours cultures of respective bacterial and fungal culture. Agar wells (5mm diameter) were made in each of these plates using sterile cork borer. About 100µl of different crude extracts of Actinomycetes, Bacterial and fungal culture were added using sterilized dropping pipettes into the wells and plates were left for 1 hour to allow a period of pre incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions. The plates were incubated in an upright position at 37°C±2°C for 24 hrs for bacteria and 28^{o}C ±2^{o}C for 24 to 48 hrs in fungi. Results were recorded, as the presence or absence of inhibition zone. The inhibitory zone around the well indicated absence of tested organism and it was reported as positive and absence of zone is negative. The diameters of the zones were measured using diameter measurement scale. Triplicates were maintained and the average values were recorded for antibacterial activit**y.**

3. RESULTS

Analysis of physico chemical properties of soil sample

Soil samples collected from Vallam (VM) and Mariyamman kovil (MK), Thanjavur (Dt). These samples were subjected to analysis of physico-chemical parameters such as pH, electrical conductivity (dsm^{-1}), organic carbon (%), organic matter (%), Avaiable nitrogen, Available phosphorus (mg/kg), Available potassium (mg/kg), Available zinc (ppm), Available copper (ppm), Available iron (ppm), Available manganese (ppm) and cation exchange capacity (C.mole proton^{+}/kg), calcium, magnesium, sodium and potassium were analysed and tabulated in Table 1.

According to the results, Vallam soil samples showed 7.0 pH and 6.8 pH in Mariyamman koil sample. 0.56 dsm^{-1} electrical conductivity were showed in Mariyamman koil soil sample when 0.48 dsm^{-1} in vallam soil sample. Perentage of organic carbon in Mariyamman koil soil

sample is 0.32 and 0.26% in vallam soil sample. Organic matter were presented in vallam soil sample were 0.52 and 0.64% in Mariyamman koil soil sample. 120.3% of available nitrogen (mg/kg) were presented in vallam soil sample when 118.2% of available nitrogen (mg/kg) in Mariyamman koil soil sample.

4.25 mg/kg of available phosphorous were obtained in Mariyamman koil soil sample when 3.75 mg/kg of available phosphorous in vallam soil sample. 124 mg/kg of available potassium were presented inMariyamman koil soil sample when 115mg/kg of available potassium in the vallam soilsample.

In Mariyamman koil soil sample 1.028ppm available zinc were presented while vallam sample showed 0.89ppm of available zinc 0.844ppm available copper were obtained from Mariyamman koil soil sample when 0.69 ppm available copper in vallam soil sample.4.56ppm available iron were showed in vallam soil sample when 4.23ppm available iron showed in Mariyamman koil soil sample. In Mariyamman koil soil sample soil sample 2.54ppm available manganses were obtained when vallam soil sample showed 2.19ppm of available manganses. 20.5 C.mole proton^{+}/kg cation exchange capacity were showed in Mariyamman koil soil sample when vallam soikl sample showed 19.8 C.mole proton^{+}/kg.

Mariyamman koil soil sample showed 14.5% of calcium when compared 16.3% were obtained in vallam soil sample. Magnesium 7.9% were showed in vallam soil sample where 7.4% recored in Mariyamman koil soil sample. 1.45% of sodium were showed in vallam soil sample when 1.03% of sodium presented in Mariyamman koil soil sample. 0.22% of potassium were showed in Mariyamman koil soil sample and its 0.19% potassium were showed in vallam soil sample.

Isolation and identification of Actinomycetes from the soil sample

Mariyamman koil soil sample soil sample and vallam soil sample were collected for analysis of actinomycetes and results were presented in table 2. Totally eight different morphological actinomycetes were observed in isolation plates. The Actinomycetes were isolated vallam soil sample showed 15 CFU of A1 in 10^{-2} when 6 CFUof A1 is 10^{-3} dilution. Mariyamman kovil soil sample showed 6 CFU of A1 in 10^{-2} and3 CFU of A1 in 10^{-3} dilution. In vallam soil sample A2 showed 12 CFU of A1 in 10^{-2} dilution when A2 were absent in 10^{-3} dilution. 18 CFU of A3 were showed in $10^{-2,}$ when 6 CFU of A3 showed in 10^{-3} dilution. 10^{-2} dilution showed 14 CFU of A4 in vallam soil sample when colonies were absent in 10^{-3} dilution. In

both the dilution 10^{-2} and 10^{-3} colonies are absent, but 4 CFU A8 were presented only in 10^{-3} dilution.

Mariyamman kovil soil sample showed that in 10^{-3} stetion 4 CFU of A2 when 3 CFU of A2 in 10^{-2}, 6 CFU of A3 were presented in both dilution 10^{-2} and 10^{-3}. 6 CFU of A4 were presented in 10^{-2} dilution where 6 CFU of A4 were presented in 10^{-2} dilution, when 6CFU of A4 in 10^{-3} dilution. In 10^{-2} dilution A5 were absent but 12 CFU of A5 were in 10^{-3} dilution. In 10^{-2} dilution 11 CFU of A6,10 CFU of A7 and 8 CFU of A8 were presented when colonies were not obtained A6, A7 and A8 in 10^{-3} dilution.

Isolated Actinomycetes sp were subjected to biochemical analysis such as gram's staining, motility, indole, MR, VP, citrate, catalase, carbohydrate and starch results were showed in Table 3. Isolates A1, A2, A3, A4, A5, A6, A7 and A8 were showed gram positive. In motility test A1, A7 and A8 showed motile when A2, A3, A4, A5 and A6 are showed non-motile.

Indole test showed that A3, A5 and A8 are indole positive when A1, A2, A4, A6 and A7 were showed indole negative. Methyl red test showed that positive results in A2, A3, A5, A6, A7 and A8 but absent in A1 and A4 strain.

Voges prokeaur test showed positive results in A1, A3, A4, A5 and A7 when A2, A6 and A8 were showed negative results. A1, A2, A4, A5 and A6 were showed citrate positive when citrate negative results were showed in A3 and A7.

A1 and A8 showed catalase positive when A2, A3, A4, A5, A6 and A7 were showed catalase negative. In carbohydrate analysis sucrose shoed that negative results in A6 when A1, A2, A3, A4, A5, A7 and A8 were showed positive result. In lactose test A6 showed showed invalid results, A2, A3, A7 and A8 were showed lactose negative.

In mannitol analysis A3 and A6 showed invalid result when A5 showed negative results of mannitol when A1, A2, A4, A7 and A8 were showed positive results of mannitol. A4 showed that negative results in starch test when A1, A2, A3, A5, A6, A7 and A8 were showed starch positive.

Isolation and identification of Bacteria from the soil sample

Mariyamman kovil soil sample and vallam soil sample were selected to analysis of bacterial diversity. In 10^{-4} dilution of vallam soil sampleB5 showed that absence of colonies when B1

23 CFU, B2 11 CFU, B3 10 CFU, B4 19 CFU and B6 13 CFU were showed. In 10^{-5} dilution also B5 showed tha absence of colonies when B1-13 CFU, B2-4 CFU, B3- 6 CFU, B4- 7 CFU and B6- 7 CFU were showed.

In 10^{-4} dilution of mariyamman kovil soil sample B1- 22 CFU, B2, 8 CFU, B3- 3CDU, B4- 16 CFU, B5- 18 FU and B6- 11 CFU were observed when 10^{-5} dilution showed that absence of colonies in B2 and B3. But colonies were presented in B1- 12 CFU, B4- 8 CFU, B5- 9 CFU and B6- 2 CFU.

Isolated bacteria were subjected to analysis to biochemical test such as gram staining, shape, motility, indole, methyl red, voges prosleaur, citrate and catalase results were tabulated in Table 5.

In gram staining test, B3 and B5 showed gram positive results when B1,B2, B4 and B5 showed gram positive result when B1, B2, B4 and B6 showed gram negative result. In shape all bacterial colonies B1, B2, B3, B4, B5 and B6 showed rod shaped. B1 shpwed motile when B2, B3, B4, B5 ad B6 showed non motile.

In indole test B3 and B4 showed positive results when B1, B2, B5 and B6 showed negative result. B3 and B4 showed positive methyl red when B1, B2, B5 and B6 showed negative methyl red. VP test showed that B2 and B4 VP poisitive, but B1, B2, B5 and B6 showed negative VP test. Citrate utilization were performed in B3 and B4 when B1, B2, B5 and B6 were showed citrate negative results. Positive catalase were showed in B1, B3 and B4 when negative catalase were showed in B2, B2 and B6.

Bacterial isolates were identified as B1- *E.coli*, B2-*Micrococcus* sp, B3- *B.substilis*, B4- *B.cereus*, B5- *P.fluroscence* and B6- *Enterobacter* sp.

Isolation and identification of fungi from the soil sample

Mariyamman kovil and vallam soil sample were subjected to analysis fungal diversity. The results were noted in table 6.

In 10^{-3} dilution, vallam soil sample F1- 6 CFU, F2- 5 CFU, F3- 8 CFU, F4- 3CFU, F5- 1CFU nd F6-2 CFU were observed, when 10^{-4} dilution showed F1- 2 CFU, F2- 2CFU, F3- 4 CFU, F4- 1 CFU but F5- and F6 showed absence of colonies.

In mariyamman kovil soil sample 10^{-3} dilution showed that F1- 4 CFU, F2- 8 CFU,F3- 9 CFU and F6- 3 CFU. But colonies were absent in F4 and F5. In 10^{-3} dilution of mariyaman kovil soil sample showed F1- 4 CFU, F2-8 CFU, F3- 9CFu and F6- 3CFU when F4 and F5 showed absence of colonies. In 10^{-4} dilution F4 and F5 showed that absence of fungal colonies when F1- 3CFU, F2- 3 CFU, F3- 2 CFU and F6- 1 CFU were observed.

Isolates were identified and named fungi by lactophenol cotton blue tecnhiques. F1- *Aspergillus flavus*, F2- *A. terreus*, F3- *A.niger*, F4- *A.awamori*, F5- *Fusarium semitectum* and F6- *Trichoderma viride*.

ANTIBIOTIC SUSCEPTILITY TEST

The isolated probiotic was tested to standard antibiotic as penicillin, polymixin, gentamycin, rifampicin, sulphamethizole and sterile disc. In all the organisms are *E.coli*, *S.typhi*, *Psedomonas* sp and *B.cereus* were observed in the zone of inhibition of 5, 10, 5 and 10 mm in polymixin and 10, 5, 5 and 10 mm in gentamycin, its compared to others penicillin, rifampicin and sterile disc was detect no zone of inhibition. Whereas sulphamethizole was showed 5 mm zone in *Lactobacillus* sp and others are not shows zone formation (Table 8 and Plate -).

Screening of antibacterial activity of *Actinomycetes* sp.

The antibacterial activities of *Actinomycetes* (A 1, A 2, A 3, A 4, A 5, A 6, A 7and A8) were determined against four pathogenic bacteria such as *Escherichia coli, Salmonella typhi, Pseudomonas sp.* and*Bacillus cereus*, cultures were given in Table 8. The antibacterial activity against *E.coli* in maximum zone measured inA2 (22 mm) and minimum A7 (12 mm) measured. *S.typhi* shows in maximum A2 (23 mm) and minimum in A8 (14 mm), whereas *Psueudomonas* sp. in maximum A7 (23 mm) and minimum A6 (15 mm). So that as *B.cereus* observed maximum in A1 (23 mm) and minimum in A8 (12 mm). (Table 9).

Antibacterial activity of bacterial sp

The antibacterial activities of Bacterial sp (B 1, B 2, B 3, B 4, B 5 and B 6) were determined against four pathogenic bacteria such as *Escherichia coli, Salmonella typhi, Pseudomonas sp.* and *Bacillus cereus*, cultures were given in Table 9. The antibacterial activity against *E.coli* in maximum zone measured in *K.pneumoniae* (4 mm) and minimum *E.coli* and *S.typhi* (2 mm) measured, but *B.substilis* and *P.fluorescens*observed in no zone of inhibition. *S.typhi* shows in maximum *P.fluorescens* (15 mm) and minimum in *K.pneumoniae* and *S.typhi* (5

mm), whereas *Psueudomonas* sp. in maximum *K.pneumoniae*(17 mm) and minimum *P.fluorescens*(11 mm). Whereas no zone of inhibition were observed in *B.cereus*. the results were recorded and tabulated in table 10.

Antibacterial activity of fungal sp

The antibacterial activities of fungal sp (*A.flavus, A.niger, A.terreus, A.awamori, F.semitectum* and *T.viride*) were determined against four pathogenic bacteria such as *Escherichia coli, Salmonella typhi, Pseudomonas sp.* And *Bacillus cereus,* cultures were given in Table 11. The antibacterial activity against *E.coli* in maximum zone measured in *A.awamori* and *T.viride* (5 mm) and minimum *A.terreus* (1 mm)measured.*S.typhi* shows in maximum *T.viride* (13 mm) and minimum in *A.flavus* (4 mm), whereas *Psueudomonas* sp. in maximum *T.viride* (20 mm) and minimum *A.awamori* (2 mm). Whereas no zone of inhibition were observed in *B.cereus*.

Table 1 Physico chemical properties of two different soil samples from Thanjavur district

S.No	Name of the parameter	SAMPLE DETAILS	
		VM	MK
1.	pH	7.0	6.8
2.	Electrical conductivity (dsm^{-1})	0.48	0.56
3.	Organic Carbon (%)	0.26	0.32
4.	Organic Matter (%)	0.52	0.64
5.	Available Nitrogen (mg/kg)	120.3	118.2
6.	Available Phosphorus (mg/kg)	3.75	4.25
7.	Available Potassium(mg/kg)	115	124
8.	Available Zinc (ppm)	0.89	1.028
9.	Available Copper (ppm)	0.69	0.84
10.	Available Iron (ppm)	4.56	4.23
11.	Available Manganese (ppm)	2.19	2.54
12.	**Cat ion Exchange Capacity**(C. Mole $Proton^{+}$/kg)	19.8	20.5
Ex changeable Bases (C. Mole $Proton^{+}$/kg)			
13.	Calcium	16.3	14.5
14.	Magnesium	7.9	7.4
15.	Sodium	1.45	1.03
16.	Potassium	0.19	0.22

VM- Vallam; MK Mariyamman kovil

Table 2 Diversity of *Actinomycetes*sp from two different soil samples

S.No	Isolates	Vallam		Mariyamman kovil	
		10^{-2}	10^{-3}	10^{-2}	10^{-3}
1	A_1	15	6	6	3

2	A_2	12	-	3	4
3	A_3	18	6	6	6
4	A_4	14	-	12	6
5	A_5	-	-	-	12
6	A_6	-	-	11	-
7	A_7	-	-	10	-
8	A_8	-	4	8	-

Table 3 Identification and biochemical characterization of*Actinomycetes*sp

S.No	Isolates	Gram's stain	Motility	Indole	MR	VP	Citrate	Catalase	Carbohydrae			St
									S	L	M	
1	A_1	+	+	-	-	+	+	+	+	+	+	+
2	A_2	-	-	-	+	-	+	-	+	-	+	+
3	A_3	+	-	+	+	+	-	-	+	-	+/-	+
4	A_4	-	-	-	-	+	+	-	+	+	+	-
5	A_5	+	-	+	+	+	+	-	+	+	-	+
6	A_6	+	-	-	+	-	+	-	-	+-	+/-	+
7	A_7	+	+	-	+	+	-	-	+	-	+	+
8	A_8	+	+	+	+	-	+	+	+	-	+	+

MR- Methyl red, VP- Voges proskeaur, TSI- Triple Sugar Iron, S- Sucrose, L- Lactose, M- Mannitol, St- Starch

(+) - Positive, (-) - negative, (+/-)– invalid

Table 4: Bacterial diversity from two different soil samples

S.No	Isolates	Vallam		Mariyamman kovil	
		10^{-4}	10^{-5}	10^{-4}	10^{-5}
1	B_1	23	13	22	12
2	B_2	11	4	8	-
3	B_3	10	6	3	-
4	B_4	19	7	16	8
5	B_5	-	-	18	9
6	B_6	13	7	11	2

Table: 5 Morphologicaland biochemical characteristic of bacteria

S.No	Isolates	Gram staining	Shape	M	I	MR	VP	Ci	Ca	Identified as
1	B_1	-	rod	+	-	-		-	+	*E.coli*
2	B_2	-	rod	-	-	-	-	-	-	*Micrococcus* sp
3	B_3	+	rod	-	+	+	+	+	+	*B.substilis*
4	B_4	-	rod	-	+	+	+	+	+	*B.cereus*
5	B_5	+	rod	-	-	-	-	-	-	*P.fluorescens*
6	B_6	-	rod	-	-	-	-	-	-	*Enterobacter* sp

M- Motility, I- Indole, MR- Methyl red, VP- Voges proskeaur, Ci- Citrate, Ca-Catalase

Table 6: Diversity of fungi from two different soil samples

S.No	Isolates	Vallam		Mariyamman kovil	
		10^{-3}	10^{-4}	10^{-3}	10^{-4}
1	F_1	6	2	4	3
2	F_2	5	2	8	3
3	F_3	8	4	9	2
4	F_4	3	1	-	-
5	F_5	1	-	-	-
6	F_6	2	-	3	1

Table 7: Identification of fungi by Lactophenol cotton blue Method

S.No	Isolates	Name of the isolated fungi
1	F_1	*Aspergillus flavus*
2	F_2	*A. terreus*
3	F_3	*A. niger*
4	F_4	*A.awamori*
5	F_5	*Fusarium semitectum*
6	F_6	*Trichoderma viride*

Table 8 Antibacterial activity of *Actinomycetes*sp against UTI pathogen

S.No	Isolates	Zone of inhibition (mm)			
		E.coli	***S.typhi***	***Pseudomonas* sp.**	***B. cereus***
1	N 1	20	19	22	23
2	N 2	22	23	21	20
3	N 3	15	20	17	19
4	N 4	20	16	20	21
5	N 5	17	15	20	18
6	N 6	14	18	15	13
7	N 7	12	18	23	13
8	N 8	16	14	18	12

Table: 9 Antibacterial activities of soil bacteria against UTI pathogen

S.No	Name of the bacteria	Zone of inhibition (mm)			
		E.coli	***S.typhi***	***Pseudomonas* sp.**	***B. cereus***
1	*Escherichia coli*	2	7	12	-
2	*Klebsiella pneumoniae*	4	5	17	-
3	*Bacillus subtilis*	-	6	13	-
4	*Bacillus cereus*	3	12	12	-
5	*Salmonella typhi*	2	5	16	-
6	*Pseudomonasfluorescens*	-	15	11	-

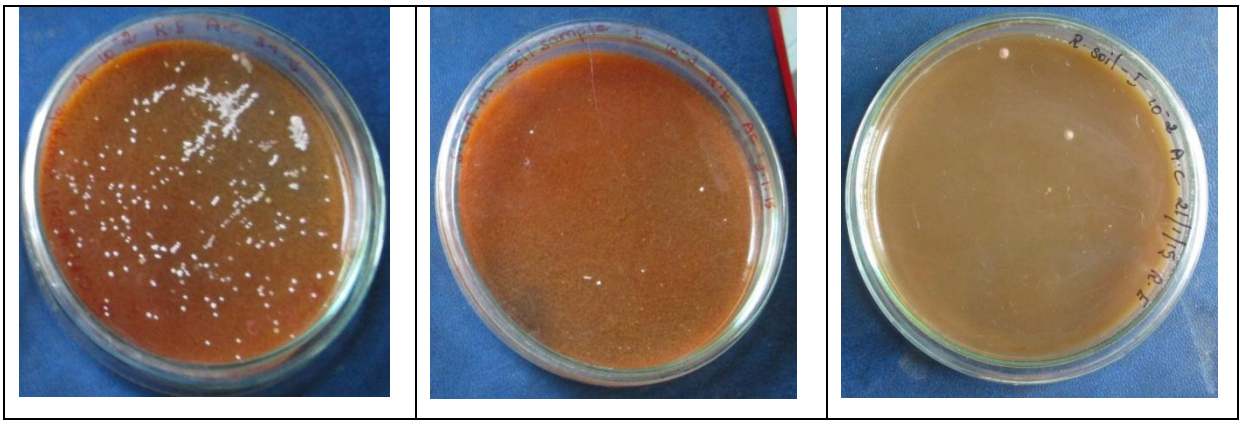
(-)-No zone of inhibition

Table: 10 Antifungal activities of soil fungi against UTI pathogen

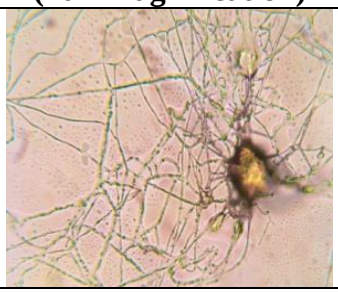
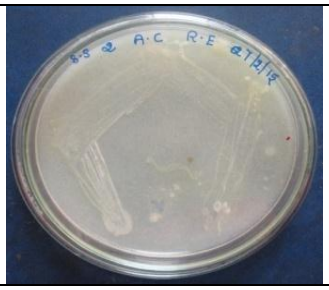
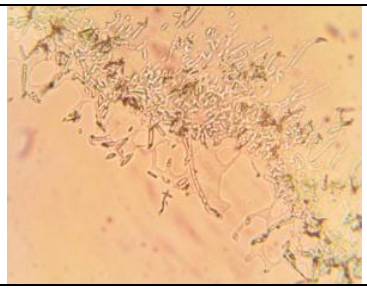
S.No	Name of the fungi	Zone of Inhibition (mm)			
		E.coli	***S.typhi***	***Pseudomonas* sp.**	***B. cereus***
1	*Aspergillus awamori*	5	7	2	-

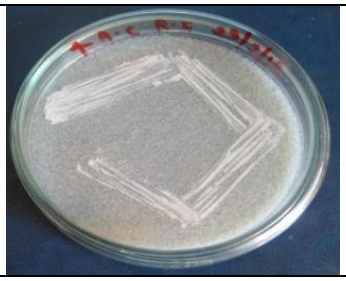
2	*A.flavus*	3	4.	11	-
3	*A.niger*	2	12	15	-
4	*A. terreus*	1	5	10	-
5	*Fusarium semitectum*	2	10	16	-
6	*Trichoderma viride*	5	13	20	-

PLATE I: **Isolation of *Actinomycetes* from soil samples**



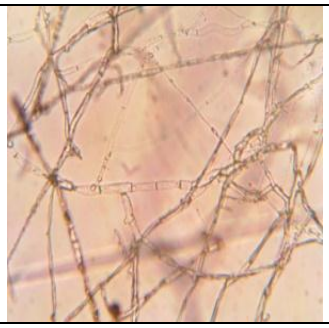
Identification of *Actinomycetes* isolates

Streak plate	(40X magnification)
	
EA1	
	
EA2	
	
EA3	

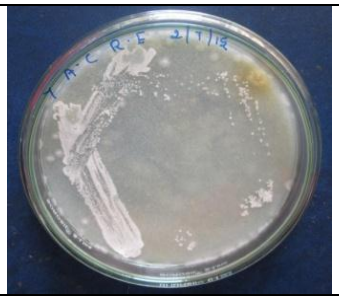
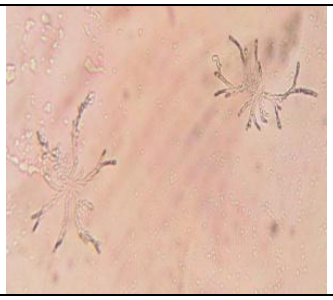



EA4

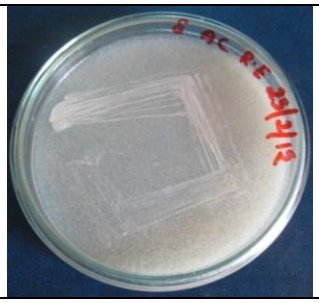
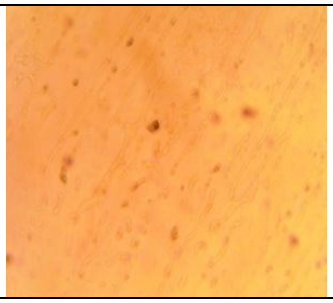

EA5 (On

EA6

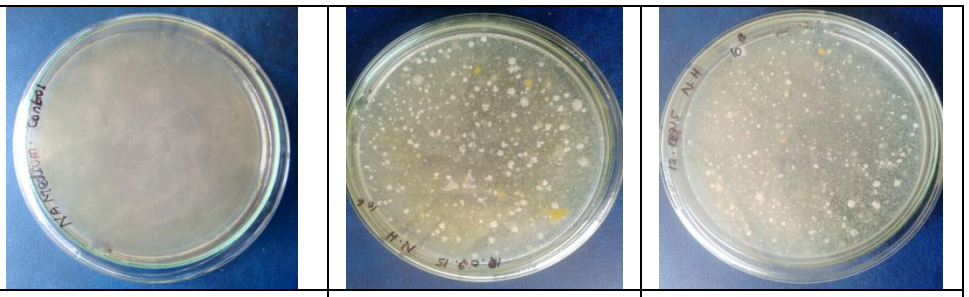



EA7

EA8

Plate II: Isolation of bacteria from soil samples



Control	Soil I	Soil II

Purification of bacterial isolates

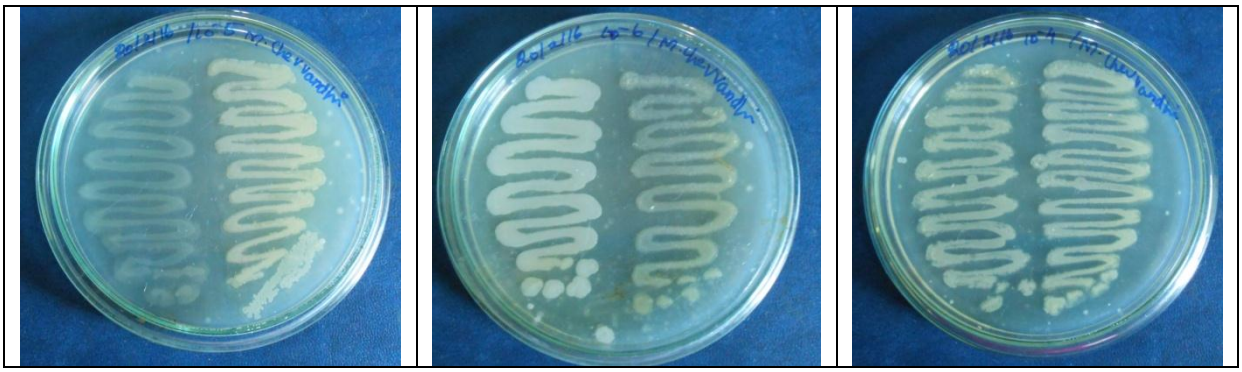
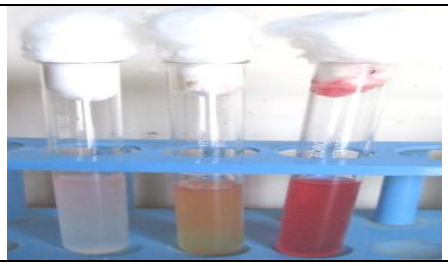
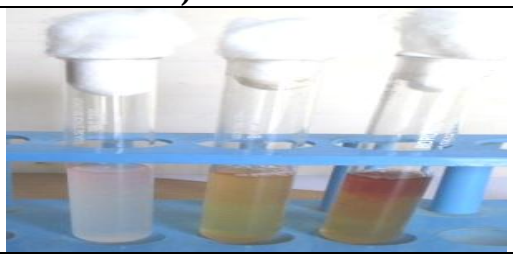


Plate III: Biochemical characterization of *Actinomycetes* sp and Bacterial sp

a) Indole

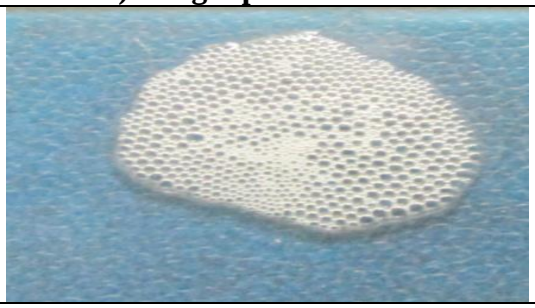


b) Methylred



c) Voges proskeaur

d) Citrate



e) Catalase

f) Sucrose

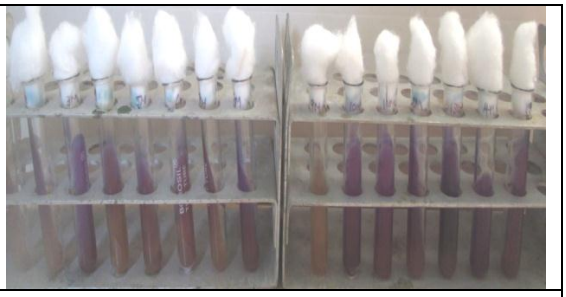
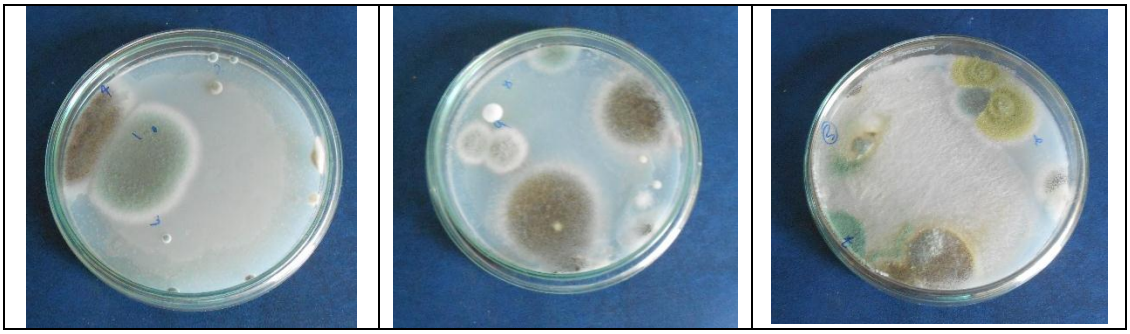
	
g) Lactose	**h) mannitol**

Plate IV: Isolation of fungi from soil samples



Micrographical observation of isolated fungal species

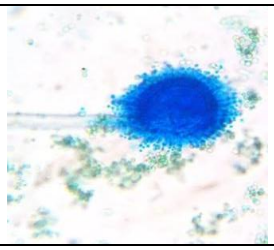
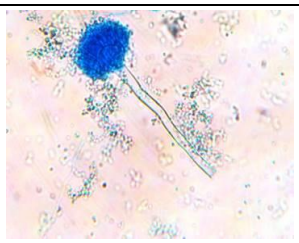
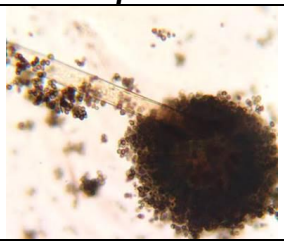
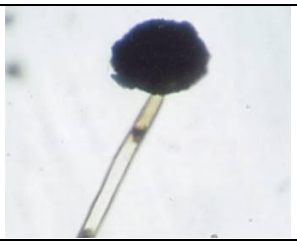
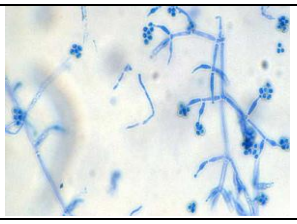
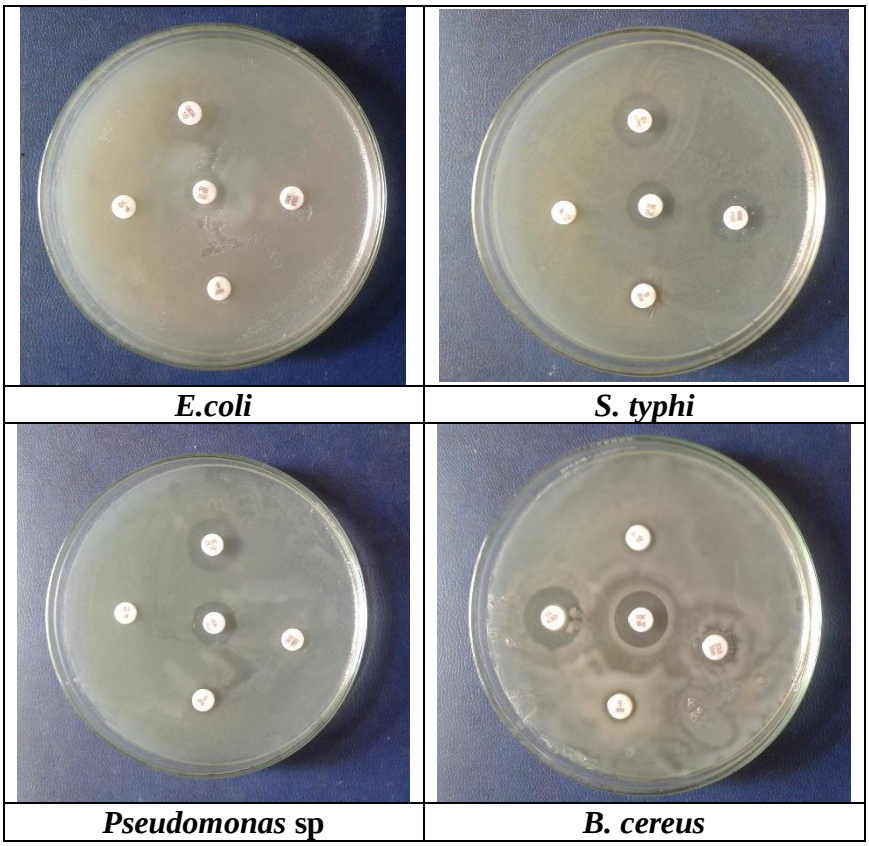
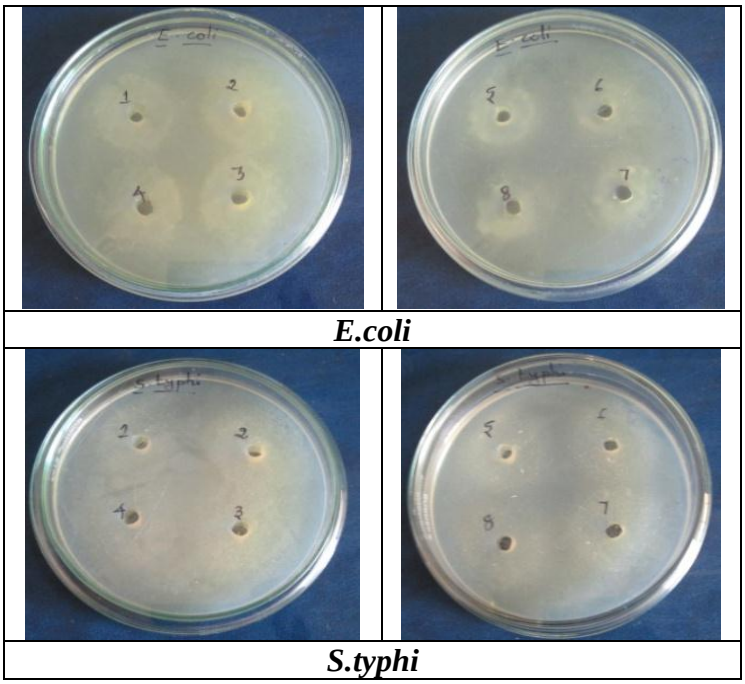
	
A.flavus	***A.terreus***
	
A.niger	***A.awamori***
	
F.semitectum	***T.viride***

Plate V: Antibiotic susceptibility test for probiotic isolates

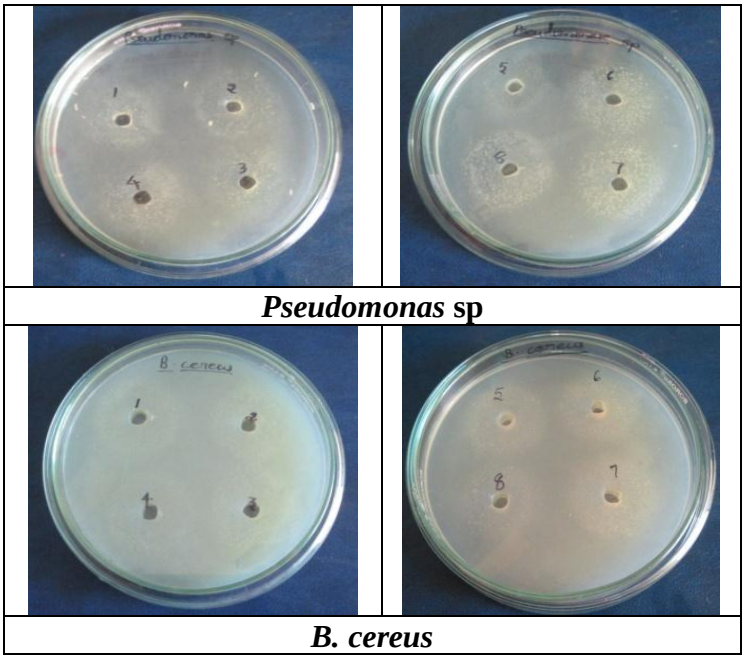


E.coli	***S. typhi***
***Pseudomonas* sp**	***B. cereus***

PLATE VI: Antibacterial activity of *Actinomycetes* sp. against UTI pathogen



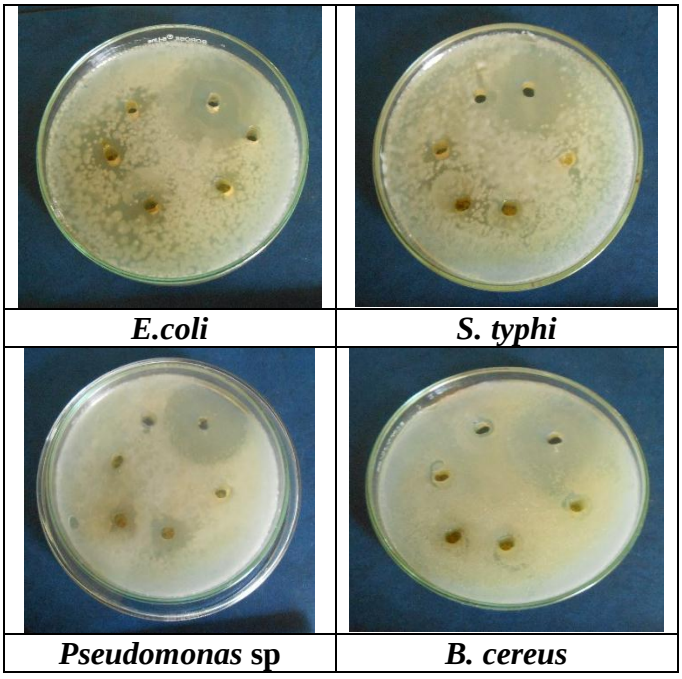
E.coli
S.typhi



***Pseudomonas* sp**

B. cereus

Plate VII Antibacterial activity of bacterial sp. against UTI pathogen



E.coli ***S. typhi***

Pseudomonas* sp** ***B. cereus

4. DISCUSSION

Soil samples were collected from Vallam (VM) and Mariyammankovil (MK), Thanjavur (Dt). These samples were subjected to analysis of physico-chemical parameters such as pH, electrical conductivity (dsm^{-1}), organic carbon (%), organic matter (%), Avaiable nitrogen, Available phosphorus (mg/kg), Available potassium (mg/kg), Available zinc (ppm), Available copper (ppm), Available iron (ppm), Available manganese (ppm) and cation

exchange capacity (C.mole proton^{+}/kg), calcium, magnesium, sodium and potassium were analysed.

According to the results, Vallam soil samples showed maximum pH value 7.0 pH when Mariyammankoil sample showed minimum 6.8 pH. Maximum Electrical conductivity were showed 0.56 dsm^{-1} in Mariyammankoil soil sample when 0.48 dsm^{-1} Electrical conductivity in vallam soil sample. High percentage of organic carbon 0.32% were showed in Mariyammankoil soil sample but 26% organic carbon were presented in vallam soil sample. In Mariyammankoil soil sample 0.64% of Organic matter were presented it showed maximum level when compare to vallam soil sample it showed 0.52%. Maximum available nitrogen (mg/kg) 120.3% was presented in vallam soil sample when Mariyammankoil soil sample showed 118.2% of available nitrogen (mg/kg).

4.25 mg/kg of available phosphorous were obtained in Mariyammankoil soil sample it showed high value when 3.75 mg/kg of available phosphorous in vallam soil sample. In Mariyammankoil soil sample high level of available potassium 124 mg/kg were obtained when 115mg/kg of available potassium were obtained in the vallam soil sample.

In Mariyammankoil soil sample 1.028ppm available zinc were presented while vallam sample showed minimum 0.89ppm of available zinc. Available copper were obtained from Mariyammankoil soil sample 0.844ppm is high when 0.69 ppm of available copper were showed in vallam soil sample. 4.56ppm available iron was showed in vallam soil sample it indicates the maximum when available iron showed minimum 4.23ppm in Mariyammankoil soil sample. In Mariyammankoil soil sample maximum available manganse 2.54ppm were obtained when vallam soil sample showed minimum 2.19ppm of available manganse. Maximum level 20.5 C.mole proton^{+}/kg cation exchange capacity were obtained in Mariyammankoil soil sample when vallam soil sample showed minimum 19.8 C.mole proton^{+}/kg cation exchange.

Mariyammankoil soil sample showed 14.5% of calcium when 16.3% of calcium was obtained in vallam soil sample it showed minimum level when compared to mariyammankoil soil sample. Maximum magnesium 7.9% were showed in vallam soil sample where 7.4% of magnesium were presented in Mariyammankoil soil sample. Sodium levels 1.45% were showed maximum in vallam soil sample when 1.03% of sodium presented showed minimum in Mariyammankoil soil sample. 0.22% of potassium showed maximum level in

Mariyammankoil soil sample when 0.19% of potassium showed minimum level in vallam soil sample.

Karthikeyan, *et al.*, (2013) studied the soil characteristics such as pH 7.22 to 7.69, electrical conductivity 0.41 to 0.54 dSm-1, cation exchange capacity16.3 to 23.8 c.mol proton+/kg, organic carbon 0.36 to 0.60%, nitrogen 2.74 to 3.29 (mg / kg), phosphorus 1.1 to 1.28(mg / kg), potassium 4.13 to 5.34 (mg / kg), zinc 0.53 to 0.89 ppm, copper 0.53 to 1.97 ppm, iron 2.46 to 8.54 ppm, manganese 3.10 to 3.69 ppm, calcium 10.5 to 12.8 (C. Mole Proton+ / kg), magnesium 6.7 to 9.2 (C. Mole Proton+/ kg), sodium 1.33 to 2.98 (C. Mole Proton+ / kg) and potassium 0.14 to 0.28 (C. Mole Proton+ / kg), showedvariation during different seasons. Relationship between load of *Actinomycetes*and soil physicochemical properties like soil temperature, pH, organic carbon, available nitrogen, phosphorous and potassium etc. were calculated. Thedistribution of soil microbial population is determined by a number of environmental factors like pH, moisturecontent and soil organic matter etc.

Ashok, *et al.*, (2014) reported that the physico-chemical parameters of the soil samples from the two different regions of Maravakadu, Thanjavur District, Tamil Nadu, were analyzed. In the present study the physico-chemical analysis of the mangrove soilsamples from three different regions of Muthupettai, Thiruvarur (Dt).

Mariyammankoil soil sample and vallam soil sample were collected for isolation of actinomycetes and results were presented. According to the results totally eight different morphological actinomycetes strains were observed. In vallam soil samples the actinomycetes strain counts were high in 10^{-2} dilution when compared to 10^{-3} dilution. In A5, A6, A_7 andA_8. actinomycetes strain counts were not presented in 10^{-2} but in 10^{-3}4cfu/ml were observed.

In Mariyammankoil soil sample actinomycetes strain counts were high in 10^{-2} dilution when compared to 10^{-3} dilution. In A6, A_7 and A_8. Actinomycetes strain counts were not presented in 10^{-3}. Mariyammankoil soil samples observed CFU/ml in all strains when compared to vallam soil sample.

Isolated actinomycetes sp were subjected to biochemical analysis such as gram's staining, motility, indole, MR, VP, citrate, catalase, carbohydrate and starch results were tabulated. Except A2 and A4 All other actinomycetes strains are gram positive. The strain of A1, A7

and A8 was motile and others are non-motile. A3, A5 and A8 are showed that Indole positive when others are indole negative. In Methyl red test A2 and A4 showed negative when all other stains are showed positive. In Vogesproskeaur test A2, A6 and A8 showed negative when all other stains are showed positive. In citrate utilization A3 and A7 showed negative when all other stains are showed positive. Catalase test showed positive in A3 and A7 when all other stains are showed negative. In carbohydrate analysis sucrose were absent in A6 but present in all other strains. Lactose were invalid in A6 but present in A1, A4 and A5 while absent in A2, A3 and A7. Maltose was invalid in A3 and A6 but absent in A5, while present in A1, A2, A4, A7 and A8. Starch were absent in A4 when present in all other strains.

Phongsopitanun, *et al*., (2014) reported that the two *Actinomycete* strains, D2-1 and D2-2, were isolated from the sediment sample. The morphology of isolate D2-1 exhibited the spiral spore type with rough surface while isolate D2-2 exhibited long spore chain with smooth surface. The upper colonial appearance of D2-1 on ISP 2 was dark grayish yellowish brown and D2-2 was yellowish white. The cultural characteristics on ISP 3, ISP 4 and ISP 6 of both isolates.

Ashok*et al.,* (2014) investigated that the total of seven different *Actinomycetes*isolates were screened from two soil samples of mangrove region. The cultural and microscopic characterization of *Actinomycetes*were identified as *Actinomadura livida, Nocardiopsis*sp. *Thermomonospora* sp. *Saccharoplatyspora hirsute, Streptomyces albus, S. cyaneus* and *S. griseoflavus,* werefrequently isolated from the soil samples.

Huang, *et al*., (2002) observed themangrove ecosystem of Nizampatnam for novel *Actinomycetes* and their antimicrobial properties. Duringour continuous search for novel antimicrobial metabolitesof Nizampatnam mangrove region led to the isolation ofmorphologically distinct *Actinomycetes* isolate VUK-10 onasparagine glucose agar media by employing soil dilutionplate technique. The strain VUK -10 exhibited typicalmorphological characteristics of the genus *Pseudonocardia.*

Karthikeyan, *et al.*, (2013) reported that the total of 21Actinomycetes were isolated from Ennoor. Morphological studies indicated that the strains belonged to the genera, *Actinokineospora, Actinopolyspora, Amycolata, Glycomyces, Microbispora, Microtetraspora, Micropolyspora, Nocardia, Nocardiopsis*(2), *Promicromonospora,*

Saccharothrix(2), *Saccharopolyspora*, *Streptomyces microflavus*, *Streptomyces*(4), *Streptoverticillium*, *Spirillospora* and *Thermomonospora.*

Mariyammankoil soil sample and vallam soil sample were collected for isolation of bacteria and results were presented. According to the results totally six different morphological bacterial strains were observed. In vallam soil samples the bacterial strain counts were high in 10^{-4} dilution when compared to 10^{-5} dilution. B5 strains were showed nil colonies in both 10^{-4} and10^{-5}dilution were observed.

In Mariyammankoil soil sample bacterial strain counts were high in 10^{-4} dilution when compared to 10^{-5} dilution. In B2andB3 bacterial strain counts were not presented in 10^{-3}. 10^{-4} dilution of mariyammankoil soil samples observed colonies when compared to vallam soil sample.

Isolated bacterial sp were subjected to biochemical analysis such as gram's staining, shape, motility, indole, MR, VP, citrate and catalase results were tabulated. B3 and B5 showed gram positive where bacterial strains are gram negative. All bacterial strains are showed rod shaped. B1strain showed motile when others are non-motile. B2, B5 and B6 are showed that Indole negative when others are indole positive. In Methyl red test B3 and B4 showed positive when all other stains are showed negative. In Vogesproskeaur test B3and B4 showed positive when all other stains are showed negative. In citrate utilization B2, B5 and B6 showed negative when all other stains are showed positive. Catalase test showed positive in B1, B3 and when all other stains are showed negative. Bacterial colonies were identified as B1- *E.coli*, B2- *Micrococcus* sp, B3- *B.substilis*, B4- *B.cereus*, B5- *P.fluorescens* and B6- *P.fluorescens.*

Mariyammankoil soil sample and vallam soil sample were collected for isolation of fungi and results were represented. According to the results totally six different morphological fungal strains were observed. In vallam soil samples the bacterial strain counts were high in 10^{-3} dilution when compared to 10^{-4} dilution. F5 and F6 strains were showed nil colonies in both 10^{-4} dilution were observed.

In Mariyammankoil soil sample, fungal strain F4 and F5 were not showed presented their colonies in10^{-3} and 10^{-4} dilution. 10^{-3} dilution of vallam soil samples observed more colonies when compared to mariyammankoil soil sample.

Lactophenol cotton blue Method were preformed to identified fungal isolates and results were identified as F1-*Aspergillus flavus,* F2- *A.terreus,* F3 -*A.niger,* F4-*A.awamori,* F5 - *Fusarium semitectu* andF6- *Trichoderma viride.*

In the present study, the antibiogram susceptibility test the isolated probiotic was tested to standard antibiotic as penicillin, polymixin, gentamycin, rifampicin, sulphamethizole and sterile disc. In all the organisms are *E.coli, S.typhi, Pseudomonas* sp and *B.cereus*were observed in the zone of inhibition of 5, 10, 5 and 10 mm in polymixin and 10, 5, 5 and 10 mm in gentamycin, its compared to others penicillin, rifampicin and sterile disc was detect no zone of inhibition. Whereas sulphamethizole was showed 5 mm zone in *Lactobacillus* sp and others are not shows zone formation. The results revealed in antibiotic susceptibility test with vancomycinand streptomycin were different from the observation madeby Kim *et al*(2006).

Similar results have been reported by Jara *et al*., (2011) and Heikkila and Saris (2003). The spectra of inhibition varied among the species and *L. rhamnosus, L. casei* and *L. plantarum* were the most effective. *S. aureus* was inhibited by 14, 19 and 7 strains of *L. rhamnosus L. casei* and *L. plantarum*, respectively. This confirms the results obtained by Dabiza *et al.* (2006) who isolated strains from dairy products, effective against this target organism. Inhibitory activity was demonstrated by some of all the test strains against *E. coli* 0157:H7 except for *S. thermophilus*. These findings are in agreement with that reported by Ibhanesebhor and Otobo (1996) for inhibitory activities of human colostrums against *S. aureus* and coliform organisms. This may be due mainly to the high immunoglobulins (Igs) content of colostrum.

Phongsopitanun,*et al*., (2014) On the primary screening of antimicrobial activity, the strain D2-1 exhibited activities against *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 27853 while strain D2-2 exhibited activities against both Gram-positive and Gram-negative bacteria but not *Candida albicans* ATCC 10231.

Ashok, *et al*., (2014) studied the only three *Actinomycetes* were tested against ten pathogenic organisms. Five bacteria species *Klebsiella pneumonia, Streptococcus pyogenus, Staphylococcus aureus, Alcaligens* spand *Bacillus subtilis* were tested against three *Actinomycetes.* In the present study the 15 isolates of *Actinomycetes* were found to possess antibacterial potentiality.

Rao, *et al.*, (2013) investigated the antibacterial studies, the isolates KVR 01, KVR 02, KVR 03, KVR 05, KVR 06, KVR 07, KVR 08, KVR 09, KVR 10, KVR 11, KVR 12, KVR 13, KVR 20, exhibited potent antibacterial activity against test pathogens and the maximum inhibition zone 20-18 mm was observed. The remaining isolates KVR 04, KVR 14, KVR 15, KVR 16, KVR 17, KVR 18 and KVR 19 exhibited intermediary activity against the test pathogens with the maximum zone of inhibition was 16-14mm observed for these isolates. Most of the isolates exhibited maximum antibacterial activity against gram positive bacteria used in this study.

Sonashia and Kamat, (2011) reported 30 *Actinomycetes* strains isolated from various locations of soil samples in Goa, out of them 28 isolates exhibited broad spectrum of antimicrobial activity against test pathogens. In the present study 15*Actinomycetes*sp. were isolated from mangrove soil exhibited broad spectrum of antibacterial activities.

The antibacterial activities of *Actinomycetes* (A1, A2, A3, A4, A5, A6, A7 and A8) were determined against four pathogenic bacteria such as *Escherichia coli, Salmonella typhi, Pseudomonas sp.* and *Bacillus cereus,* cultures were represented. According to the antibacterial activity A2 strains showed maximum zone of inhibition measured (22 mm) against *E.coli* and minimum zone of inhibition were observed in A7 strains (12 mm) measured. *S.typhi* showed maximum zone of inhibition A2 (23 mm) and minimum zone of inhibition in A8 (14 mm), whereas *Psueudomonas* sp. showed maximum zone of inhibition measured in A7 (23 mm) and minimum zone of inhibition were observed in A6 (15 mm). *B.cereus* observed maximum zone of inhibition in A1 (23 mm) and minimum zone of inhibition in A8 (12 mm).

However, the extracellular filtrates obtained from these same microorganisms presented low antifungal activity. Numerous Gram-negative bacteria have been used in the control of fungal plant pathogens. These bacteria exert their antifungal activity through theproduction of extracellular lytic enzymes, siderophores, salicylic acid, antibiotics, and volatilemetabolites, such as hydrogen cyanide (Manwar *et al*., 2004; Kishore *et al*., 2005 and Afsharmanesh, *et al*., 2006).

The antagonism going in a negative direction. All the fungal extracts werecapable of inhibiting the growth of the tested bacteria. Both *Morganella* and *Klebsiella* are important bacteriainvolved in the nitrogen cycle. While *Klebsiella* is a nitrogen fixer, *Morganella* is a

denitrifying bacterium (Idriss *et al*., 2002). Growth inhibition of both the bacteria is harmful for the soil fertility and especially for the leguminous soil wherethe crop is largely dependent upon the nitrogen source by the microorganisms.

In the present study, antibacterial activities of Bacterial sp (B1, B2, B3, B4, B5 and B6) were determined against four pathogenic bacteria such as *Escherichia coli, Salmonella typhi, Pseudomonas sp.* and *Bacillus cereus.* The antibacterial activity against *E.coli* showedmaximum zone of inhibition measured in *Klebsiella pneumoniae* (4 mm) and minimum zone of inhibition in *E.coli. Bacillus subtilis* and *Pseudomonas fluorescens* were showed no zone of inhibition. *S.typhi* showed in maximum zone of inhibition in *Pseudomonas fluorescens* (15 mm) and minimum *zone of inhibition* in *Klebsiella pneumoniae* and *Salmonella typhi* (5 mm). In *Psueudomonas* sp. maximum zone of inhibition were observed in *Klebsiella pneumoniae* (17 mm) and minimum zone of inhibition in *Pseudomonas fluorescens* (11 mm). Whereas no zone of inhibition were observed in *Bacillus cereus*.

The antibacterial activities of fungal sp (*A.flavus, A.niger, A.terreus, Aspergillus awamori, Fusarium semitectum* and *Trichoderma viride*) were determined against four pathogenic bacteria such as *Escherichia coli, Salmonella typhi, Pseudomonas sp.* and *Bacillus cereus.* The antibacterial activity against *E.coli* showedmaximum zone of inhibition measured in *Aspergillus awamori* and *Trichoderma viride* (5 mm) and minimum zone of inhibition were observed in *A.terreus* (1 mm) measured. *S.typhi* showed maximum zone of inhibition in *Trichoderma viride* (13 mm) and minimum zone of inhibition in *A.flavus* (4 mm), whereas *Psueudomonas* sp. showedmaximum zone of inhibition *Trichoderma viride* (20 mm) and minimum zone of inhibition in *A.awamori* (2 mm). Whereas no zone of inhibition were observed in *B.cereus*.

The different bacterial*spp.* were isolated from soil samplescollected from Bhopal region of MadhyaPradesh and then the isolated bacterial *spp*.were screened for their potential to generateantimicrobial substance. An agar welldiffusion method was used to assess theproduction of antimicrobial compounds bybacteria isolated from soil samples against 7pathogenic strains of bacteria. Out of 28isolates tested, 12 (42.87%) isolates werefound to exhibit antibacterial activity againstpathogenic strains of bacteria. Most of these substances were partially orcompletely inactivated by proteases and TCA, suggesting that a protein moiety isinvolved in the activity. This may indicatethat the inhibition was due to the

presenceof bacteriocin-like substances. Theantimicrobial substances showed highthermal resistance and low molecularweight, which are characteristics of smallhydrophobic peptides that constitute classII bacteriocins (Riley and Wertz, 2002).

Although the identification of theseisolated bacterial colonies were not yetdone up to the *species* level, but theirmorphological and biochemicalcharacteristics indicate that they belong tothe genus *Bacillus* and *coccus*. Differentantimicrobial compounds are produced bymembers of the genus *Bacillus*, most ofthese identified as peptides, lipopeptidesand phenolic derivatives (Nakano and Zuber.,1990). Different antimicrobialsubstances produced by Bacillus *spp*.isolated from arthropods were recentlydescribed, including aromatic acids, acetylamino acids (amino acid analogs) and peptides (Gebhardt *et al*., 2002). Studies indicated that bacteriocin-likesubstances have been related to Bacillus*spp*. isolated from soil (Oscariz *etal*.,1999; Bizani and Brandelli.,2002) .

Fungi belonging to different groups, have been studied for production of some secondary metabolites in laboratory and natural conditions (Ekesi *et al*., 2005) that led to introduction of genus and species which are superior in studied characteristics (Lotfi *et al*., 2010). Metabolites can enhance abundance and biological components of soils or some of them can be effective in reducing population of soil living organisms. Also, metabolites can have some values as well as commercial and industrial importance (Petit *et al*., 2009). Most of the bacterial isolates used in this study were gram negative and plant pathogenic. *Penicillium* isolates were obtained from

5. SUMMARY AND CONCLUSION

In our study Soil samples were collected from Vallam (VM) and Mariyammankovil (MK), Thanjavur (Dt). These samples were subjected to analysis of physico-chemical parameters such as pH, electrical conductivity (dsm^{-1}), organic carbon (%), organic matter (%), Avaiable nitrogen, Available phosphorus (mg/kg), Available potassium (mg/kg), Available zinc (ppm), Available copper (ppm), Available iron (ppm), Available manganese (ppm) and cation exchange capacity (C.mole $proton^{+}$/kg), calcium, magnesium, sodium and potassium. Isolation of actinomycetes, Isolated actinomycetes sp were subjected to biochemical analysis such as gram's staining, motility, indole, MR- Methyl red, VP- Vogesproskeaur, TSI- Triple Sugar Iron, S- Sucrose, L- Lactose, M-Mannitol, St- Starch (+) - Positive, (-) - negative, (+/-)– invalid. Isolation of bacteria. Isolated bacterial sp were subjected to biochemical analysis such as gram's staining, shape, motility, indole, MR, VP, citrate and catalase results were

tabulated. Isolation of fungi. Antibacterial activities of Actinomycetes, Bacteria and Fungi were analysed and results were tabulated.

According to the physico- chemical results, Mariyammankovil (MK), soil sample showed maximum level in electrical conductivity (dsm^{-1}), organic carbon (%), organic matter (%),Available potassium (mg/kg), Available zinc (ppm), Available copper (ppm), Available manganese (ppm) and cation exchange capacity (C.mole $proton^{+}$/kg), whereas Vallam soil samples showed maximum in pH, Avaiable nitrogen, Available iron (ppm), calcium, magnesium and sodium.

According to the results totally eight different morphological actinomycetes strains were observed. In vallam soil samples the actinomycetes strain counts were high in 10^{-2} dilution when compared to 10^{-3} dilution. In A5, A6, A7andA8. actinomycetes strain counts were not presented in 10^{-2} but in 10^{-3}4cfu/ml were observed. In Mariyammankoil soil sample actinomycetes strain counts were high in 10^{-2} dilution when compared to 10^{-3} dilution. In A6, A_7 andA_8. actinomycetes strain counts were not presented in 10^{-3}. Mariyammankoil soil samples observed cfu/ml in all strains when compared to vallam soil sample.

According to the tabulated results totally six different morphological bacterial strains were observed. In vallam soil samples the bacterial strain counts were high in 10^{-4} dilution when compared to 10^{-5} dilution. In Mariyammankoil soil sample bacterial strain counts were high in 10^{-4} dilution when compared to 10^{-5} dilution.

According to the results totally six different morphological fungal strains were observed. vallam soil samples observed more colonies when compared to mariyammankoil soil sample.

Lactophenol cotton blue Method were preformed to identified fungal isolates and results were identified as F1-*Aspergillus flavus*, F2- *A.terreus*, F3 -*A.niger*, F4-*A.awamori*, F5 - *Fusarium semitectu* and F6- *Trichoderma viride.*

Effect of antimicrobial activities of probiotic bacteria were analysed against some antibiotic sensitivity test also determined against penicillium, polymixin, gentamycin, rifampicin and sulphamethizole antibiotics were used. Among the antibiotic susceptibility test was polymixin and gentamycin also maximum zone of inhibition recorded.

The antibacterial activities of *Actinomycetes* (A1, A2, A3, A4, A5, A6, A7 and A8) were determined against four pathogenic bacteria such as *Escherichia coli, Salmonella typhi, Pseudomonas sp.* and *Bacillus cereus,* results were represented. *S.typhi, Pseudomonas* sp and *B.cereus* showed maximum zone of inhibition23mm when *E.coli and B. cereus* showed minimum zone of inhibition.

The antibacterial activities of Bacterial sp (B1, B2, B3, B4, B5 and B6) were determined against four pathogenic bacteria such as *Escherichia coli, Salmonella typhi, Pseudomonas sp.* and *Bacillus cereus. Pseudomonas sp* showed maximum zone of inhibition in all *bacterial sp* but *B.cereus* were showed no zone of inhibition.

The antibacterial activities of fungal sp (*A.flavus, A.niger, A.terreus, A.awamori, Fusarium semitectum* and *Trichoderma viride*) were determined against four pathogenic bacteria such as *Escherichia coli, Salmonella typhi, Pseudomonas sp.* and *B. cereus. Pseudomonas* spshowed maximum zone of inhibition in allbacterial spwhen compared to *Escherichia coli* and *Salmonella typhi,* but *B.cereus* wereshowed no zone of inhibition.

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