MICROBIAL PRODUCTION OF ANTIBIOTICS FROM MANGROVE ECOSYSTEM

THESIS SUBMITTED IN PARTIAL FULFILMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN MARINE SCIENCE OF THE
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
COCHIN - 682 022

BY
R. RATHNA KALA

POST GRADUATE EDUCATION AND RESEARCH PROGRAMME
IN MARICULTURE
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
POST BOX No. 1603, COCHIN - 682 014, INDIA

DECLARATION

I hereby declare that this thesis entitled **"MICROBIAL PRODUCTION OF ANTIBIOTICS FROM MANGROVE ECOSYSTEM"** is a record of original and bonafide research carried out by me under the supervision and guidance of **Dr. V. Chandrika**, Senior Scientist, Central Marine Fisheries Research Institute, Cochin and that no part there of has been presented before for any other degree in any University.

Rathna Kala

Cochin - 682 014

(R. RATHNA KALA)

C E R T I F I C A T E

This is to certify that the thesis entitled **"MICROBIAL PRODUCTION OF ANTIBIOTICS FROM MANGROVE ECOSYSTEM"** embodies the research of original work conducted by **R. Rathna Kala** (Reg. No.1212) under my supervision and guidance. I further certify that no part of this thesis has previously formed the basis of the award of any degree, diploma, associate-ship, fellowship or other similar titles or recognition.

V Chandrika

Dr. V. CHANDRIKA
Senior Scientist
Central Marine Fisheries Research Institute
Cochin 682 014.

Cochin 682 014

CONTENTS

PREFACE

Antagonistic compounds are widespread in sediments which is a treasure house for many useful micro-organisms and among them streptomycetes are considered the most important group of organisms capable of producing the widely used antibiotic substances. Antibiotics are of considerable interest because they offer a potentially powerful way of selectively inhibiting bacteria, thus permitting one to descriminate between the contributions from various groups of organisms in complex ecological processes. Some antibiotics are active against only Gram-positive or Gram-negative bacteria, others have a broad-spectrum of activity. As different antibiotics have different modes of action, one bacterial process may be inhibited by a given antibiotic but another process may be unaffected (at least over the short term). Moreover, the effectiveness of an antibiotic in inhibiting the bacterial component will depend on a range of environmental conditions (eg. pH, temperature, incubation period etc.) that affect the activity or stability of the chosen compound.

Over a thousand of antibiotic substances have so far been isolated from soil actinomycetes obtained from different type of sediments of the world of which about twenty have come into common use to cure the various diseases of man and animal.

The regular use of low concentrations of antibiotics has become widespread in peneaid shrimp hatcheries. Antibiotics are used in hatcheries to reduce mortalities either by controlling the general level of bacteria

in the culture water or more specifically. Only TETRACYCLINE is used as "cure all" for controlling fish diseases in culture studies so far.

Disease is one of the main factors limiting the survival, growth and production of farmed fishes and shell fishes. The use of antibiotic compounds to control bacterial diseases was studied as early as 1946 by Gutsell. Since then a range of antimicrobial compounds were tested and used to control diseases of Crustacean as well as fish diseases. The control and/or prevention of disease transfer has received an equal importance as that of the diagnosis and determination of the disease in the stocked population. The term "Mariculture Medicine" coined by Khontz (1970) to denote the medical aspect of mariculture, includes (1) recognition that disease does exist, (2) definition of disease, (3) correction of the disease and (4) prevention of recurrence of the disease.

The role of naturally produced antibiotics in ecosystems is debated, even though it is well established that aquatic environments contain substances that inhibit the activity and division of bacteria and that a range of aquatic microbes produce antibiotic compounds. Eventhough several sediments have been examined for the presence of antagonists, a detailed seasonal study and correlation between various physico-chemical factors in mangrove environment are only a few. The studies on varying types of antagonists were mainly oriented towards the isolation of antibiotic substances for use in medicine but not to examine the potentialities of different sediments in harbouring the antagonists. Reports on mangrove antagonistic actinomycetes are few and still fewer are the antagonists against fish-disease pathogens.

The principle interest of the present investigation was to determine seasonal variations of antagonistic actinomycetes in selected mangrove ecosystem. The microbial interrelationship in mangrove sediments was found out by constructing the ratio between bacteria and actinomycetes, bacteria and fungi, fungi and actinomycetes. In addition temperature, pH, salinity, dissolved oxygen and organic carbon were determined seasonally and their possible relationship was statistically analysed and the results are presented. Isolated actinomycetes were subjected to cross streak assay to know their nature of antibiotic activity against test fish pathogens and crude antibiotics were extracted from selected isolates and their inhibitory activity was studied and the results are discussed.

LIST OF TABLES

LIST OF FIGURES

A C K N O W L E D G E M E N T

I am very much indebted to Dr. V. Chandrika, Senior Scientist, Central Marine Fisheries Research Institute, Cochin for her constant help and encouragement, valuable guidance and supervision throughout the research work.

My sincere thanks are due to Dr. P.S.B.R. James, Director, CMFRI, Cochin, for providing me with the facilities to carry out the research work. I wish to record my sincere gratitude to Dr. A. Noble, Head of PGPM.

I wish to take this opportunity to thank Shri M.J. John, Sri C.G. Thomas, PGPM for their administrative help rendered during the tenure of this programme. Special thanks are due to Mr. A. Nandakumar for his prompt help in issuing the required materials and instructions.

I am very much indebted to Dr. K. Rangarajan, CMFRI and Dr. Ebenassar CAS in Botany, Madras University, Madras for their timely help in completing my electron microscopy work. I wish to express my sincere gratitude to Dr. A.D. Diwan, for his earnest help in carrying out the light microscope work.

I acknowledge the Indian Council of Agricultural Research for providing me with Senior Research Fellowship for my doctoral work.

I INTRODUCTION

The mangrove ecosystem comprises a group of floristically diverse trees and shrubs which characterize the intertidal vegetation of many tropical and sub-tropical area. Mangroves are one among the several specialized marine ecosystems in which the productivity at different trophic levels and energy flow assume unusual importance as it has direct influence in enriching the inshore environment (Heald and Odum, 1962).

The mangrove ecosystem is one of the most productive in the world and plays an important part in the ecology of near-shore waters. Mangrove swamps comprising of foilage as a major organic material, support a detrital type of food chain in the tropical marine environment (Odum and Heald, 1975).

Mangrove swamp forests are complex ecosystems that occur along intertidal accretive shores in the tropics dominated by estuarine trees, they draw many of their physical, chemical and biological characterists from the sea, inflowing fresh water, and upland forests. Mangrove swamps serve as ecotones between land and sea, and elements from each are stratified both horizontally and vertically between the forest conopy and subsurface soil (Gerald and Walsh, 1974).

In recent years there is steady increase in awareness of mangrove's ecological significance and benefits to mankind. Many aspects to this ecosystem are still unknown like the distribution of antagonistic actinomycetes. Limited investigations are being made in India on ecology, phyto-geography, microbiology, forestry etc. of mangrove ecosystem.

Mangrove fauna and flora have been extensively reviewed by (Macnae, 1968). Schuster (1952) discussed breakdown and modification of the substratum by bacteria, fungi, actinomycetes and myxomycetes. He mentioned the occurrence of the bacteria Clostridum sp. and Azobacter sp. and the algae Nostoc sp. and Anabena sp. in mangrove swamp and speculated that those organisms are important in nitrogen fixation.

Some observations on the distribution, ecology and the environmental features of the mangroves from 2 major estuarine systems of Goa, have been reported by Untawale et al. (1973). Dwivedi et al. (1973) studied the ecology of mangrove swamps of the Mandovi estuary, Goa. The structure and production in a detrital rich estuarine mangrove swamp in Kollur estuary near Coondapoor (Karnataka) along the Central West Coast of India was studied by Untawale et al. (1977). The distribution of trace elements in the Pichavaram mangroves was done by Ramdhas et al. (1975).

Venkatesan and Ramamurthy (1971) conducted marine microbiological studies of mangrove swamps of killai backwaters and reported the presence of physiologically active groups of bacteria. Natarajan et al. (1979) studied the distribution of V. parahaemolyticus and allied vibrios in backwater and mangrove biotopes at Portonovo.

Antimicrobial properties of alcoholic extracts from Rhizophora mangle was studied by Rojas Hernandez and Coto-Perez (1978).

Matondkar et al. (1981) studied seasonal variation of microflora from mangrove swamps of Goa situated along the Mandovi Zuari estuary. Studies were conducted on heterotrophic bacterial flora by the same authors in

1981. Microorganisms degrading phenolic compounds was studied by Gomes and Mavinkurve (1982) in the mangrove swamps of Goa. Humnadkar and Agate (1985) isolated 21 bacterial species from mud and water collected from mangroves of Sindhu drug and Malvan area in Konkan, Maharashtra. Chandrika et al. (1985) encountered green sulphur bacteria responsible for detritus decomposition from mangrove mud in Karuthedum near Cochin. The distribution of heterotrophic bacteria of mangrove ecosystem in the area Cochin was studied by Surendran (1985). Rhizosphere microflora of Acanthus illicifolius was studied by Mini Raman (1986). Sulfate reducing bacteria from mangrove swamps of Goa, was studied by Saxena et al. (1988). Composition and biological activity of actinomycetes in the mangrove rhizosphere was discussed by Zhen Zhicheng et al. (1989).

Ramamurthy et al. (1990) studied the distribution and ecology of methanogenic bacteria in mangrove sediments of Pitchavaram. Halotolerant Rhizobium strains from mangrove swamps of the Ganges river delta was encountered by Sengupta and Chaudhuri (1990). Lokabharathi et al. (1991) made an attempt to study the ecology and physiology of sulfate - reducing bacteria from mangrove swamps.

Cribb and Cribb (1956) in Australia were the first mycologists to collect marine fungi from mangroves and Swart (1958) did the first comprehensive studies on fungi of soil in east African mangrove vegetation.

Reports on marine actinomycetes are few and still fewer are the studies on actinomycetes in mangroves. Most of the studies have been concentrated on detecting antagonistic actinomycetes producing antibiotics which inhibit root pathogens. Studies on the types of actinomycetes found

in the root region revealed that they are similar to those from the root free soil, usually Streptomyces and Nocardia species predominate. The physiological activities of actinomycetes from rhizosphere and non-rhizosphere soil of several plants have been compared by Abraham and Herr, (1964).

Matondkar et al. (1981) reported that the actinomycetes and yeasts are known to play an important role in mangrove ecosystems where the plant litter decomposition occurs.

Weyland (1986) reported that the mangroves exhibited the highest density of actinomycetes among the areas investigated by him, also within their high salinity regions.

As mangrove ecosystem is an unexplored area for antagonistic compounds from actinomycetes, the present study "Microbial production of antibiotics from mangrove ecosystem" was undertaken not only to examine the occurrence, distribution and seasonal variations of microflora in mangrove sediment but also to compare their quantities with other soil organisms and to correlate their quantity to the various sediment physico-chemical factors. The richness of sediments harbouring the antagonistic actinomycetes also in certain groups of streptomycetes has been well indicated in this study. The study has also facilitated objective screening of the antagonists encountered, so as to select the most potent among them for use in fish disease control which now plague commercial aquaculture. Very encouraging results in this regard were obtained which are reported here.

The study was taken up for a period of one year from January to December 1991. Samplings were done from four fixed mangrove ecosystem

viz. Station I - Mangalavana, Station II - Narakkal, Station III - Puthuvyppu, Station IV - Light house area of Puthuvyppu.

Thesis is presented in 6 Chapters, Chapter I - INTRODUCTION to the topic of study, extensive literature on the subject is summarised and correlated with particular reference to the importance of actinomycetes to bring an awareness of the present status of our knowledge in the subject and the review also clearly states that much work has not been done in the mangrove ecosystem related to antibiotic production from actinomycetes.

Chapter II is on MATERIAL AND METHODS for sample collection, isolation of microflora, maintenance of isolated actinomycete cultures, characterisation of actinomycetes, to study the antagonistic effect of actinomycetes and extraction of crude antibiotics. In addition, regular samples of water and sediments were collected to study some important physico-chemical parameters and to find out their possible relationship if any with the microflora of the mangrove ecosystem.

In Chapter III - RESULTS of the present investigation are presented under seven parts. Results of microbial flora encountered during the period of study are given in Part 1. Under which the distribution of total microbes (bacteria, fungi and actinomycetes) between the microbes and inter-relationship of microflora are given in Part 1.A, 1.B, 1.C, 1.D and 1.E respectively. Part 2 deals with the results of the physico-chemical parameters studied viz., Temperature in Part 2.A, pH in Part 2.B, Salinity in Part 2.C, Dissolved oxygen in Part 2.D and the results of Organic carbon content estimated during the period of study is given in Part 2.E. Results of statistical

analysis of ANOVA are given for each parameter studied at the end of every part of the results to find out the level of significance between stations and seasons. Results of correlation study are also given under each part. Distribution of actinomycetes screened and isolated are in Part 3.A and Part 3.B respectively. Results of sodium chloride tolerance of isolated actinomycetes are given in Part 4. Under Part 5 results of identification of actinomycetes are given in detail. Strain description of 52 identified actinomycetes are given in Part 5.A. Results of Generic composition, Sporophore morphology, Spore morphology, Sporophore and Spore morphology and Pigment production are given in Part 5.B, Part 5.C, Part 5.D, Part 5.E and Part 5.F respectively. Results of antagonistic property of the isolated actinomycetes are given in Part 6. Which are presented in 5 sub parts viz. Part 6.A deals with the results of antibacterial activity, Part 6.B deals with results of antifungal activity, Part 6.C gives the results of antibacterial and antifungal activity and Part 6.D deals with the results of antagonistic nature of the isolated actinomycetes against each test organism and in Part 6.E. Antibiogram of 104 isolated actinomycetes are given in Part 6.F. Part 7 deals with the results of antagonistic activity of the crude antibiotic extracts from selected actinomycete cultures, isolated during the period of study. Part 8 deals with the results of invitro evaluation of pH on solvent in testing the anti-microbial activity of selected isolates.

All data collected and the results of the work done on the above aspects are given either in the form of graphic intensity charts or tables for effective presentation of the results.

Chapter IV - DISCUSSION. All major and minor findings are compared with the previous results obtained by various authors. The properties of these organisms have profound effect on our ideas about the classification, ecology and physiology of this group.

SUMMARY of the results of investigation is presented in the final section of the thesis - in Chapter V which is followed by a detailed list of references (Chapter VI) on the subject.

ACTINOMYCETES

The actinomycetes have recently come to occupy an eminent place because they are important producers of antibiotics, vitamins and enzymes (Waksman, 1957). They are a group of very useful micro-organisms from the points of view of their role in natural cycles of matter. Investigations for their isolation and biological activities, in virgin areas, would reveal their significance further (Ali and Roymon, 1984).

Alexander (1978) stated that the true bacteria are distinctly different from the filamentous fungi and many morphological characters separate the two broad types. There is however a transitional group ie. a connecting link between the simple bacteria and the fungi, a group with boundaries overlapping its more primitive and its more developed neighbours. These are the actinomycetes. Among the procaryotes, a mycelial growth habit is confined to Gram-positive bacteria being characteristic of the organism known as actinomycetes.

Micro-organisms in the order actinomycetales are characterised by being filamentous and branched. This is normally exhibited in some degree

by all the species. None produce endospores of the type found in true bacteria, but many produce mould like spores or conidia. The branched cellular growth (mycelium), together with the specialized methods of sporulation, relates these organisms to the moulds; thus Actinomycetales are referred to as the mould like bacteria. On the bacterial side, they are related to the Gram-positive nonspore formers (Pelczar et al., 1977).

The actinomycetes form an extensive and widely distributed group of micro-organisms. Like the bacteria and the moulds, they occur in nature both as saprophytes and as parasites of plants and animals (Waksman, 1919).

Sieburth (1979) stated that the actinomycetes are highly diverse group of Gram-positive bacteria, which sometimes have acid fast branching filaments, with colonies that range from typical bacterial colonies to colonies having a well-defined coherent mycelium, with specialized structures and spores. The saprophytic actinomycetes have an aerobic metabolism and do not accumulate acids from carbohydrate substrates, whereas the parsite forms are usually micro-aerophilic and convert 50% of their substrate carbon to acid.

OCCURRENCE AND DISTRIBUTION OF ACTINOMYCETES

Actinomycetes are among the most widely distributed groups of micro-organisms in nature. Very few natural substrates are free from them. In some of the substrates as in soils, in lake water and in lake bottoms, in composts, they lead a normal existance. In other substrates, as in sea water and in dust, they are only in transitory state. They are found abundantly in all soils throughout the world especially under dry alkaline conditions,

form a large part of the microbial population of the soil. They also occur on plant residues and in various food stuffs, such as fruits, vegetables, milk and milk products and cocao (Waksman, 1950). And they are almost absent in peatbogs and in the sea (Waksman, 1957).

The soil represents an ideal natural substrate for the development of actinomycetes. They are found so abundantly there, where they are represented by many genera and species. It has been suggested that their major function in the soils is the decomposition of plant and animal residues. In general, a close correlation has been obtained between the abundance of actinomycetes and the amount and extent of decomposition of available organic matter in the soil (Waksman, 1950).

Actinomycetes are present in surface soil and also in the lower horizons to considerable depths. In abundance, they are only second to the bacteria, and the viable counts of the two are sometimes almost equal. In the environments of high pH, a large proportion of the total community consists of actinomycetes (Alexander, 1978).

The review on occurrence and distribution of actinomycetes in nature, in soil, water basins, dust and on exposed surfaces of plants are discussed in brief by Waksman (1919), Lloyd (1969), Watson and Williams (1974), Alexander (1978), Weyland (1986) and reviewed in detail by Waksman (1950, 1963).

The first survey for actinomycete in marine sediments was conducted by Grien and Meyers (1958). The first study to enumerate actinomycetes in fresh sediment samples and to examine off-shore samples was conducted

in the North Sea and in the open Atlantic Ocean by Weyland (1969).

Waksman (1967) stated that actinomycetes belonging to 4 or 5 genera are associated with vegetative matter and sediments in the sea and apparently take an active part in the benthic microflora.

Isolations from marine areas are reported from coastal or shelf regions. Only a few surveys give some knowledge about the occurrence in oceanic sites Zobell (1946), Weyland (1969), Walker and Colwell (1975).

Strains of Nocardia and Streptomycetes were obtained from cordage and fishnets by Freitas and Bhat (1954). Occurrence of actinomycetes in the marine environment is briefly reviewed by Sieburth (1979).

Very little work has been done on marine actinomycetes. Since the environmental conditions of the sea are extremely different from terrestrial conditions, it is felt that marine actinomycetes have different characteristics when compared with their terrestrial counter parts, and might produce different types of antibiotics (Elliah and Reddy, 1987). In addition to antibiotics production, some are useful for the chemical transformation of steroids.

The streptomycetes group of micro-organisms are widely distributed in the water masses. The water mass contains relatively small amounts of streptomycetes; the sediments however, are often rich in them. Mass development of streptomycetes in sediments has a reason for the formation of earthy odours of the water in some areas (Rodina, 1972).

ISOLATION OF ACTINOMYCETES

Of new, biologically active compounds, the ability of these micro-organisms to produce useful antibiotics and to carry out other transformations of commercial interest has focussed attention on factors bearing on their isolation. It would be desirable, therefore to be able to isolate soil inhabiting actinomycetes with a minimum interference from associated bacteria and fungi.

Actinomycetes being filamentous, branching bacteria with a fungal type morphology, are part of the microbial flora of most natural substrates. Numerous methods have been advocated to facilitate the isolation of actinomycetes (El Nakeeb and Lechevalier, 1963).

Methods for the preferential isolation of actinomycetes from soils was suggested by Poter et al. (1960). Lingappa (1961) suggested several different media for isolation of actinomycetes from soil. Most of them contain carbon and nitrogen sources which are utilised by bacteria and moulds as well as by actinomycetes and therefore are not selective for the latter.

Many investigations involving the selective isolation of streptomycetes from soil have been carried out. A brief survey of the literature revealed a total of 21 recommended media. The most frequently used carbon and nitrogen sources were glucose and asparagine (by 13 workers) and glycerol (by 11 workers), potassium nitrate, peptone, casein and starch were employed with moderate frequency. The best media, allowing good development of streptomycetes while supressing bacterial growth, were those containing starch or glycerol as the carbon source with casein, arginine or nitrate

as nitrogen source (Kuster and Williams, 1964).

Shinobu et al. (1958) on testing a large number of strains, found that glycerol and starch (together with glucose) were used as carbon sources by all. Of all the nitrogen sources tested, nitrate turned out as the best inorganic source. Similar results were obtained by Pridham and Gottlieb (1948) who found that all streptomycetes tested were able to utilize starch, glycerol and glucose.

El Nakeeb and Lechevalier (1963) used calcium carbonate, sodium propionate, phenol treatment, centrifugation method and found that calcium carbonate treatment was most effective, as it not only gave highest total counts of actinomycetes, but also the lowest relative numbers of bacteria and fungi.

Hopwood (1960) stated that the pattern of development of the substrate mycelium is markedly influenced by the composition of the medium. Lingappa (1961) reported that chitin-mineral medium was excellent for isolation and estimation of total number of actinomycetes from soil, for growth and maintenance of cultures. Rodina (1972) has suggested 20 media with different combinations for the growth of streptomycetes. Dekleva et al. (1985) developed a defined medium for S. peucetius and methods for reproducible laboratory analysis of its growth and anthracycline production. Two species of streptomycetes isolated from rhizosphere soil were described and cultured on seven different media (Nair and Nair, 1986). A simple synthetic medium for the production of the peptide antibiotic thiostrepton by S. azureius was found by Charry et al. (1989).

TAXONOMY OF ACTINOMYCETES

As everywhere in biology, the most difficult and complicated, but at the same time most important aspect of the study of organisms is the identification of species (Krasilnikov, 1960).

Many authors have attempted to classify members of this group of organisms and the same is also reviewed by many authors. Shirling and Gottlieb (1966) were the one who initiated to standardize the methods for characterization of these organism. Methods for characterization of streptomycetes is reported in detail in (ISP) International Streptomycete Project (1966) by the same authors in the year 1966 and descriptions for each species is given in detail by Shirling and Gottlieb (1968a, 1968b, 1969 and 1972). Morphological, cultural and physiological were the main characters given in ISP for the identification of streptomycetes. Evaluation of criteria used in the ISP co-operative description of type strains of Streptomycetes and Streptoverticillium species was done by Szabo and Marton (1976).

The anatomy of individual colonies of S. coelicolor was studied at various developmental stages in situ by means of surface impressions and thin sections by Wildermuth (1970). Alkalophilic actinomycetes strains were examined by Miyashita et al. (1984) to determine their taxonomic position.

Spore Morphology Studies

Kriss et al. (1945) were probably the first to do electron microscopy of streptomycete spores. Their finding of smooth spores was followed by those of Carvajal (1946) and Bringman (1951), who also examined smooth spores on the cultures they studied. Flaig (1958) described spiny spores on certain species

and also found hairy spores and warty spores on other species. Baldacci and Grien (1955) observed smooth, spiny and hairy spores but failed to mention warty spores. The concept of smooth, warty, spiny and hairy spore surfaces was sufficiently established by Cross and Maclver (1966) and Shirling and Gottlieb (1966) listed these are one of the criteria to be used in characterizing species. Trenser et al. (1966) also stated that spore surface may be characterized according to 4 types, smooth, warty, spiny and hairy. From the studies of Dietz and Mathew (1962, 1968 and 1971) it was shown that, in addition to the 4 recognized spore surface type, a fifth type was designated namely "rugose".

Spore morphology of actinomycetes were studied by many authors, like Hopwood and Glauert (1961), Rancourt and Lechevalier (1963, 1964), Becker et al. (1965), Lechevalier et al. (1966), Willionghby (1966), LIoyd (1969), Wildermuth and Hopwood (1970), Douglas (1970), Attwell and Cross (1972), Mcvittie et al. (1972), Sharples and Williams (1976).

Electron microscopy of cytoplasmic structure in facultative and anaerobic Actinomycete was studied by Overman and Leopine (1963). Williams and Davies (1967) used SEM for the examination of Actinomycetes.

Studies on cell wall - as an aid for identification of actinomycetes

Cell wall composition has been widely accepted as an aid in the identification of genera. Four cell wall types are accepted. Cell-wall compositions of 51 strains of Actinomyces, Nocardia, Streptomyces, Micromonospora, Mycobacterium and Propionibacterium have been investigated by Cummins and Harris (1958). The carbohydrate composition of the cell walls of some lysozyme

resistant streptomycetes was determined by Sohler et al. (1958). Rapid differentiation between Nocardia and Streptomycetes by paper chromatography of whole-cell hydrolysates was done by Becker (1964). Cell-wall preparations were made from more than 140 strains of aerobic actinomycetes all cell-wall preparations contained as major constituents glucosamine, muramic acid, alanine and glutamic acid Becker (1965). A rapid method for characterization of actinomycetes by cell wall composition was done by Boone and Pine (1968). De Weese et al. (1968) found that quantitative data on the amino acid composition of cell walls, can provide definitive identification of some of the species and differentiation of Actinomyces from other members of the Actinomycetales and from morphologically similar genera such as Corynebacterium and Propionibacterium. Staneck and Roberts (1974) used a simplified approach for the identification of aerobic actinomycetes by thin-layer chromatography. The micro-morphology, ultrastructure and cell-wall composition of Streptosporangium corrugation, isolated from beach sand was studied by Williams and Sharples (1976). Meyer (1976) presented the results of a study designed to determine a suitable taxonomic niche for A. dassonvillei. A battery of morphological, physiological and biochemical tests, including paper chromatographic analysis of whole cell hydrolysates was used to study aerobic actinomycetes by Berd (1973). A Nocardioform isolated from soil was studied, on the basis of cell wall composition and physiological characteristics, this organism was placed in the genus Nocardiopsis Shearer (1983). Lechevalier et al. (1986) proposed two new genera Amycolata and Amycolatopsis to accommodate nocardioform actinomycetes having type IV cell-wall composition and lacking mycolic acids. Report on cell-wall chemistry and morphology of the genus Streptalloteichus was stated by Tomita et al. (1987).

Carbon utilisation of actinomycetes

The utilisation of carbohydrates and of other carbon sources has been recommended by many authors as an aid to species differentiation. The ability of different species of actinomycetes to utilize various sources of carbon and nitrogen was considered as an important criterion in the taxonomy.

Carbon utilisation was studied by many authors, Gottlieb (1961), Lacey (1971), Mayer (1976), Iwaski et al. (1981), Diab and Gounaim (1982), Miyashita et al. (1984), Nair and Nair (1986) and Tomita et al. (1987).

New species of actinomycetes

A single mesophilic species of a new genus belonging to the family Streptomycetaceae of the order Actinomycetales was described and named as Waksmania (W. rosea, type sp.) by Lechevalier and Lechevalier (1957). A new genus of Actinomycetales Micropolyspora gen. nov was proposed by Lechevalier and Solotorovsky (1961). Two aerobic mesophilic species of new genus belonging to the family Actinoplanaceae were described under the name Microellobosporia (M. cinerea type species) by Cross et al. (1963). Actinomyces humiferus was proposed by Gledhill and Casida (1969) and the details of occurrence and characterization was studied. A new species S. spinoverrucosus isolated from the air during a study of the distribution of aerobic Actinomycetales strains in the atmosphere of Kuwait was described by Diab and Gounaim (1982). The type strain of a new nocardioform genus Saccharothrix was described by Labeda et al. (1984).

PATHOGENECITY OF ACTINOMYCETES

The actinomycetes commonly isolated from soil and less commonly from fresh water, which are pathogenic to man and animals exist in atleast four families.

Mycobacterium marinus has been isolated from spontaneous tubercular lesions from fish dying in sea water aquaria by Aronson (1926).

GENETICS OF ACTINOMYCETES

The first important contact between genetics and microbes occured in 1941, when Beadle and Tatum succeeded in isolating a series of biochemical mutants from the fungus Neurospora. In 1944, bacterial genetic transfer known as transformation revealed that it is mediated by free deoxyribonucleic acid (DNA). The chemical nature of hereditary material was thus discovered.

Studies on genetics of actinomycete was reviewed by Bradley (1966).

Genetic recombination in S. fradiae by protoplast fusion and cell regeneration was studied by Baltz (1978). DNA were extracted from strains of A. viscosus and A. naeslundii and were compared by DNA-DNA hybridisation Coykendall and Munzenmaier (1979). Polar lipid composition in the classification of Nocardia and related bacteria was studied by Minnikin (1977). Genetic mapping studies with a number of bld mutants and their classification using various criteria was reported by Merrick (1976). The isolation of 3 kinds of rifampicin resistant mutants of S. coelicolor and the identification of probable RNA polymerase mutants among them was studied by Chater (1974). Gordon et al. (1974) reported some of the characteristic of 27 mislabeled strains and demonstrated their close relationship to the type strain of N. autotrophica. The cultural conditions for preparing stable protoplasts of streptomycetes and for reverting them to the filamentous state at a high frequency on the surface of synthetic agar plates was reported by Okanishi (1974).

Fernandez et al. (1989) studied the diversity among 43 isolates of the genus Frankia by determining levels of DNA relatedness and DNA base compositions. The S. rimosus gene has been cloned into E. coli and expressed under control of ph or hpp promoters Reynes (1988). Chung et al. (1985) made an molecular approach to examine the genetic relatedness of 19 Frankia isolates by measuring the extent of DNA-DNA homology and the fidelity of hybrid-duplex molecules. DNA restriction patterns and DNA-DNA solution hybridisation studies of Frankia isolates from Myrica pensylvanica (Bayberry) was made by Bloom (1989). Ibrahim and Abdul-Hajj (1989) reported unique microbial transformation product of 5-hydroxy-flavone, isolation and elucidation of its structure by Spectroscopic techniques. Crameri et al. (1983) reported the restriction fragment analysis of the total chromosomal DNAs of actinomycete strains by one dimensional agarose gel electrophoresis which generate a reproducible and unique finger print for each organism. Biosynthesis of anthracyclines by analysis of mutants of streptomyces sp. Strain C 5 blocked in claunomycin was studied by Bartel et al. (1990).

ACTIVITY AND FUNCTION OF ACTINOMYCETES

Development of actinomycete colonies in selective synthetic media is very slow when compared to most fungi and bacteria, characteristic suggestive of their inability to be effective competitors and of the lack of prominence when the nutrient level is high and the pressure of competition is great. The feeble competitive powers may explain their relative scarcity during the initial stages of plant residue decomposition. When nutrients

become limiting and the pressure of the more effective competitors diminish, the actinomycete become more prominent.

The Order Actinomycetales has received special attention because many strains have the capacity to synthesize toxic metabolites. As many as three fourths of the streptomycete isolates may produce the antimicrobial agents known as antibiotics. The antibiotic substances produced in culture by actinomycetes inhibit the growth or cause the elimination of populations of bacteria, yeast and fungi of many taxonomic categories. Percentage of actinomycetes producing antibiotics varies with the soil and season of year and some test organisms are sensitive to compounds produced by many and some are inhibited by metabolites excreted by only actinomycetes. Despite the great industrial and therapeutic value of these chemical, there is still no clear picture of the significance of compounds in natural process. In addition to production of antimicrobial metabolites, many species of streptomycetes liberate extracellular enzymes which lyse bacteria. The possession of enzymes of this type may be important in the microbiological equilibrium in the environment.

The activities of the actinomycetes in soil transformations still are not clearly defined. Because microscopic examination reveals few actinomycetes in the mycelial stage and since the present evidence indicates that the high plate counts are largely the result of conidial persistence, it seems that the actinomycetes have a lesser biochemical importance than the bacteria and fungi. Nevertheless, there is evidence for the microorganisms participating in the decomposition of resistant components of

plant and animal tissue, formation of humus and transformation of organic matter at high temperature Alexander (1978). Streptomyces are important in the recycling of carbon in polymeric macromolecules Brookes and Mc Grath (1986). Rodina (1972) reported that Streptomycetes also break down proteins, urea, amino acids, and simpler nitrogenous substances. In water masses they effect the decomposition of organic plant and animal remains and the liberation of ammonia from complex proteins.

Chandramohan et al. (1972) stated that marine actinomycetes take an active role in the deterioration of cellulosic substances in the marine environment. Pelczar and Reid (1977) reported that Nocardia, Streptomyces and Micromonospora are responsible for the characteristic musty or earthy odour of a freshly plough field. Evidence for activity against the lignin fraction of straw was produced for a range of actinomycete strains by Ball et al. (1989).

Vitamin and Enzyme production

Vitamin B_{12}, the pernicious anemic factor has been recovered from waste products from the production of some of the antibiotics by Streptomycetes cultures and is found in appreciable amounts in activated sludge Frazier (1958).

Much work has been done on the production of protease by Streptomyces sp. Chahal and Nanda (1976), but only few reports are about pectinase Sata Masayakti et al. (1980) and cellulase (Desai and Betrabet, 1972) production by some members of this group of micro-organism. L. asparaginase production by S. griseus was studied by DeJong (1972). Extra cellular enzyme

activities during lignocellulose degradation by Streptomyces sp. was reported by Ramachandra (1987). Wachinger et al. (1989) surveyed the distribution of cellulase activities and cellulase system associated with mycelia among 160 new streptomycetes isolates.

ANTIBIOTIC PRODUCTION

Antibiotic substances are produced by many microorganisms in various ecological conditions. Producers of biologically active substances can be found among representatives of marine microflora, inhabitants of rivers and lakes, antibiotics are produced by decaying plant and animal remains, by growing plants and live animals, etc. But the major part of micro-organisms that can produce antibiotics inhabits the soil (Egorov (1985).

The observation that one micro-organism could inhibit the growth of another had been made fairly frequently towards the end of the nine-teenth century and it had even been demonstrated that such an interaction might be mediated by the release from one organism of a metabolite which was toxic to the other. Only after the discovery and development of penicillin that a truly wide ranging search for antibiotics was initiated.

The search for chemotherapeutic agents from microbes has resulted in the discovery of an amazing number of antibiotic substances, the majority of which have proved in tests on laboratory animals to be too toxic for them to be of any practical clinical use.

Penicillin was found out in 1928, by Fleming from a stray fungal (Penicillium notatum) contaminant preventing the growth of Staphylococci is a historical fact known to many.

In 1932 Raistrick turned his attention to Fleming's confirming that an interesting antibiotic was liberated into the medium but he was unable to isolate the active substance.

In 1938-39 Florey and Chain included P. notatum in a study of naturally occuring antibacterial substances. Their work at Oxford was encouraging and by 1941 a quantity of material, so active that it was considered to be pure penicillin, had been isolated and used for clinical trials.

Waksman and his team considered that one of the ecological factors involved in the fierce competition of micro-organisms living in the soil might be antibiotic production, started in 1939 to screen soil micro-organisms. They discovered many organisms producing compounds with antibiotic activity, none of these compounds proved to have any medical potential until 5 years and some 10,000 isolates later, streptomycin was isolated from a strain of S. griseus cultured from heavily manured soil(Riviere, 1977).

Grein and Meyers (1958) isolated 166 isolates of actinomycetes obtained from sea water and found that 70% of these were active against both Gram-positive and Gram-negative bacteria. Krassilinilov (1962) tested 326 microbial isolates obtained from oceans throughout the world at a depth of 0 to 3500 m. Isolates exhibited a very large antibacterial spectrum. Bamm et al. (1966) isolated 2 streptomycetes from Bombay waters and the cultures were found to elaborate antibacterial substances. Wood (1967)

recorded a number of actinomycetes from estuarine sources and some have been found to produce antibiotics.

Yagi et al. (1971) reported that the addition of elemental sulfur to the fermentation medium of S. sioyaensis caused marked stimulation of siomycin production. The stimulation appeared to be the result of the utilisation of thiosulfate, which accumulates as an oxidation product ofelemental sulphur.

In a survey of Sagami Bay, 136 strains of actinomycetes were obtained from 37 samples, of which 27% had antimicrobial activity and 17% inhibited a sarcoma cell. The saline tolerance of representative isolates were also tested (Okazaki and Okami, 1972).

S. flavohelwanesis, Strain AS-H-23, isolated from the Egyptian soil of Helwan, showed strong proteolytic activities and an antimicrobial agent AS-H-23A, highly active against Gram-positive bacteria (Abdullah and Fathy, 1976). Vanaja Kumar (1991) screened 386 isolates from various tissues of 5 different molluscs from Portonovo coastal region, out of which 290 Strains (75.1%) exhibited antagonistic properties. It is reported that, of all the animals examined T. telescopium was found to be the best source for antagonistic actinomycetes.

S. coeliocolor was found to produce a third secondary metabolite by Rudd and Hopwood (1980) in addition to the antibiotics methylenomycin A and actinorhodin. It was a red pigmented, highly non polar compound with antibiotic activity against certain Gram-positive bacteria. Production

of new aminoglucoside antibiotics the sannamycin complex from S.sannanensis isolated from soil sample was studied by Iwasaki et al. (1981). A novel antibiotic producing Actinomadura kijaniata sp. nov. was reported by Horan and Brodsky (1982). An antibiotic related to production of the red antibiotic, Undecyl prodigiosin, by S. coeliocolor A 3(2) was studied by DNA cloning and biochemical analysis (Feitelson (1985). β -lactam antibiotic from Streptomyces JA 13 was studied by Daginawala and Wadher (1985).

Effects of metals on S. coeliocolor growth and actinorhodin production was studied by Abbas and Edwards (1990). Vilches et al. (1990) stated the influence of different nutritional compounds on oleandomycin biosynthesis by S. antibioticus, resulting in the design of a chemically defined medium for the production of the antibiotic. Kapurimycins, new antitumour antibiotics produced by Streptomyces was studied by Yoshida (1990). Imai (1990) isolated and studied the structure of a new phenoxazine antibiotic. Exfoliazone produced by S. exfoliatus. A new antitumour substance produced by Streptomycete (isolated from a soil sample collected in Seto, Aichi Prefecture, Japan) was reported by Kojiri (1991). Detection, isolation and structural elucidation of 2 new angucyclinones exhibiting biological activity was studied by Grabley et al. (1991). The producing organism Streptomyces sp. was isolated from soil sample collected near Ajantha (India). Henkel and Zeeck (1991) reported structure and absolute configuration of Napthomevalin, a new dihydro - napthoquinone antibiotic from Streptomyces sp. (isolated from soil sample collected in Strathgordon, Australia). Fermentation, isolation and structural determination of a cyclic

hexadepsipeptide compound from Streptomyces sp. was studied by Hensens et al. (1991), the organism was isolated from rhizosphere soil sample obtained from Japanese Garden. Hydantocidin, a new compound with potent selective herbicidal activity, was found in submerged culture of S. hygroscopicus by Nakajima et al. (1991).

II MATERIAL AND METHODS

1. STUDY AREAS

Four fixed mangrove ecosystem located along 9°55'-10°10' N and 76°10'-76°20' E were selected to study, the occurrence, distribution and seasonal variations of microbial flora in sediment and also production of antimetabolites from antagonistic actinomycetes. Estimation of physico-chemical parameters were also done to know the effect of ecological parameters in the growth and distribution of microbes.

Sampling sites were:- (Fig. A)

Station - I	Mangalavana	-	(Avicennia sp. dominated)
Station - II	Narakkal	-	(Avicennia sp. dominated)
Station - III	Puthuvyppu	-	(Acanthus sp. dominated)
Station - IV	Puthuvyppu-light house	-	(Acanthus sp. dominated)

The sampling sites in these mangrove ecosystem is widely separated and have dissimilar sediment characteristics and is influenced highly by monsoon and tidal cycles.

Regular fortnightly water and sediment samples were collected from these four fixed stations for a period of one year (January 1991 to December 1991) in the early hours of the day. The central portion of the samples were immediately and aseptically transferred to sterile petridishes. The petridishes with samples were kept in a sterile plastic bag and held at 4°C until processing. Sediments of the sampling Station I, was black clayey with deposits of mangrove leaves throughout the sampling period. Sediment samples of Station II was black, sandy silt type; sediment samples of Stations III and IV were brown to black, sandy throughout the sampling period.

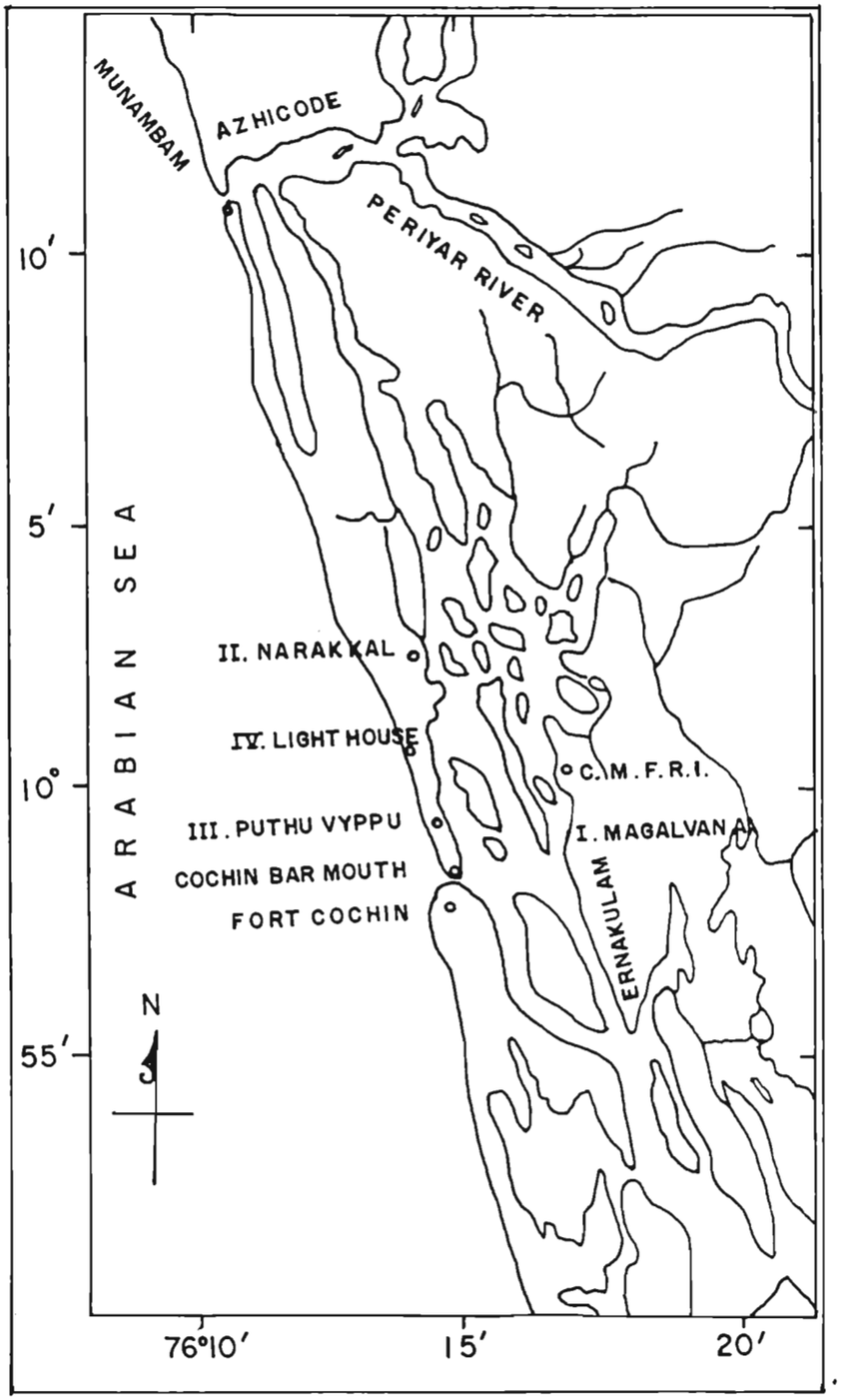


Fig. A. Map showing the sampling stations I – IV.

Soon after the collection, the samples were transported immediately to the bacteriological laboratory. Plating was done for bacteria and fungi using selective medium three hours of collection from the sediment samples. But for the enumeration of actinomycetes - sediment samples were air dried and then used for the study.

2. ISOLATION OF BACTERIA, FUNGI AND ACTINOMYCETES

A. Bacteria

For the quantitative analysis of bacteria sea water agar (SWA) was used.

B. Fungi

For the enumeration of fungi oat meal agar (Himedia) was used.

C. Actinomycetes

Selective media used for the isolation of actinomycetes were oat meal agar - (OMA), Glucose - Asparagine agar - (GAA) Grein and Meyer's agar (GMA) and Kuster's agar (KUST) (Himedia). Apart from this, counts were also taken from sea water agar (SWA).

Pour plate technique

Approximately 1 gm of the sediment sample was aseptically transferred to 99.0 ml sterilised sea water, after thorough mixing and shaking, serial dilutions were made by adopting standard procedures (Rodina, 1972). One ml of the inoculum was transferred to sterile glass petridishes and pour plated with the respective selective media for the isolation of bacteria, fungi and actinomycetes.

The plates were incubated at room temperature ((28±2°C) in a bell jar. The colonies developed in the petridishes were counted after 3-7 days

for the quantitative analysis of bacteria. The actinomycete colonies developed were counted after 7-10 days. The fungal colonies were encountered after 5-6 days incubation and all the colonies counted were expressed on dry weight basis.

3. MAINTENANCE OF CULTURE

Actinomycete colonies growing on the isolation plates were selected based on their morphological characters like colour of aerial mycelium and subcultured in sea water agar. The sub cultures were streaked in fresh sterile plates until pure colonies appeared. Pure cultures were then maintained on slants of sea water agar media at 4°C for further studies. Stock cultures were periodically subcultured and stored at 4°C.

4. CHARACTERISATION OF ACTINOMYCETES

Identification of the selected isolates of actinomycetes were done with the help of the methods recommended by Shirling and Gottlieb (1966), in the International Streptomycetes Project (ISP).

For identification of actinomycetes the main characters suggested in the ISP are as follows:

A. Morphological characterization

B. Melanoid pigment production

C. Carbon Utilisation

Based on these characters the actinomycetes cultures were identified.

A. Morphological Characterization

Sporophore morphology, spore morphology and colour of the isolates were studied according to the methods recommended in ISP.

a. Colour of the isolates

Aerial mycelium colour, reverse side colour, and the soluble pigment were studied in the selective media (oat meal agar, glucose asparagine agar and iron starch salts agar) as recommended in the ISP.

b. Sporophore morphology

Sporophore morphology was studied by the cover slip method (Kawato and Shinobu, 1959).

Cover-slip cultures

A sterilized cover slip was carefully inserted at an angle of about 45° into sea water agar medium in a petridish, until about half of the cover slip was in the medium. Actinomycete was then inoculated along the line where the medium meets the upper surface of the cover slip. After the incubation period (of 10-14 days at RT), the cover slip was carefully removed, its orientation in the medium being noted, and placed upwards on a slide. The organism had grown both on the medium and in a line along the cover slip. The growth adhering to the cover slip when it was removed from the medium was then fixed and stained to study sporophore morphology.

Fixation and Staining procedure

Growth on the cover slip was fixed with few drops of absolute methanol for 15 min and washed with tap water, dried by blotting. Stained

with crystal violet for a minute and again washed and blot dried, the cultures were viewed under light microscope, and photomicrographed.

c. **Spore Morphology**

For identification of actinomycetes, spore morphology was studied at Madras University, Madras and also at CMFRI, Cochin.

Formavar-covered copper grids were gently pressed to the sporulating surface of the organisms on sea water agar. Spore chains adhering to the surface of the grids were then viewed in the transmission electron microscope and the electron micrographs were taken (Tresner et al., 1961).

B. **Melanoid pigment production**

As melanoid pigment production is one of the identification characters, suggested by ISP, the test was done. The cultural condition and method of determination of melanoid pigment production was studied as recommended in ISP.

Media used for the study were, Tyrosine agar (TA), Peptone-yeast extract iron agar (PYIA) and Tryptone yeast extract broth (TYB).

Results were recorded after 10-14 days as follows:

Cultures forming a greenish brown to brown to black diffusible pigment or a distinct brown pigment modified by other colour were recorded as positive (+) and absence of brown to black colours or total absence of diffusible pigment was recorded as negative (-) for melanoid pigment production.

C. **Carbon Utilisation**

The utilisation of various carbon sources is an important character by which species of Actinomycetes are differentiated. To determine the utilisation of various carbon sources, method recommended in ISP was employed.

Basal agar medium was used for the study. After autoclaving the basal agar medium, it was cooled to 60°C and sterile carbon source was added aseptically to give a concentration of approximately 1%. Carbon source and controls used for the study were:-

No Carbon source (negative control)

D - glucose (positive control)

L - arabinose

D - xylose

i - inositol

D - mannitol

D - fructose

Rhamnose

Sucrose and

Raffinose

Agar slants were made with these medium, inoculated and observed after 10-14 days.

Results were recorded as follows:

Strongly positive utilization (++), when growth on tested carbon in basal medium is equal to or greater than growth on basal medium plus glucose.

Positive utilization (+), when growth on tested carbon is significantly better than on basal medium without carbon, but somewhat less than on glucose.

Utilisation doubtful or trace growth (±), when growth on tested carbon is only slightly better than on the basal medium without carbon and significantly less than with glucose.

Utilization negative (-), when growth is similar to or less than growth on basal medium without carbon.

After identifying the (52) cultures upto species level, the descriptions of the strains isolated in the present study were compared with type cultures given in ISP. Since ISP descriptions do not include the antagonistic property of the strains, this was compared with the available literature by Umezawa (1967), Pridham and Tresner (1974).

5. SODIUM CHLORIDE TOLERANCE

Sodium chloride was added to sea water agar at varying concentrations (0%, 0.5%, 3.0%, 4.5%, 7.0%) and autoclaved. One control slant was also maintained without sea water and $NaCl_2$. The inoculated cultures were then incubated at RT (28 ± 2°C) for about 14 days and the results were recorded as follows:-

- \- (No growth)
- \+ (Poor growth)
- ++ (Moderate growth)
- +++ (Good growth)

6. ANTAGONISTIC EFFECT OF ACTINOMYCETES

The isolated actinomycete cultures were screened for antagonistic properties against known (fish) test pathogens provided by CIFT, COCHIN and NCL, POONA.

Vibrio anguillarum, V. cholerae, V. alginoliticus, V. parahaemolyticus Aeromonas, Pseudomonas, Bacillus, Staphylococcus, Salmonella-I, Salmonella-II. E. coli, Rhodotorula rubra, R. marina and Cladosporium sp.

Cross-streak assay suggested by Casida (1968) was followed to study the antagonistic effect of actinomycetes.

Cross streak assay:

The actinomycete isolates were inoculated as a single streak on dried plates of sea water agar and incubated at RT (28 ± 2°C). After seven days, when the actinomycete growth was seen as a ribbon, the test organisms were streaked at right angles to the original streak of the isolates. The plates were then incubated at RT. The zone of inhibition was measured after about 24-48 hours. Control plates of the same medium without antagonistic actinomycete were maintained with test organisms to assess their normal growth.

7. EXTRACTION OF ANTIBIOTICS

After screening the isolated cultures for antimicrobial activity, 6 cultures showing different types of activity were selected and used for the extraction of antibiotics.

1. Active against Gram-positive bacteria, Gram-negative bacteria, filamentous and non-filamentous fungi.
2. Active against Gram-positive bacteria, filamentous and non-filamentous fungi.
3. Active against Gram-negative bacteria, filamentous and non-filamentous fungi.
4. Active against filamentous and non-filamentous fungi.
5. Active against filamentous fungi.

A. **Medium used**

Yeast-extract malt-extract (YME) broth (Himedia) recommended by the ISP was used for growing the cultures. Each culture was inoculated individually into 1.5 litre of medium in Hoffkine flask. To obtain sufficient cellular extracts for antibiotic studies, the actinomycetes were mass-cultured.

B. **Cultural conditions**

The inoculated flasks were incubated at RT for 14 days without agitation. After incubation period, the growth was filtered off and the culture filterate was centrifuged at 5000 rpm to get cell free clear supernatant-solution which was taken for extraction.

C. **Extraction of antibiotics**

1. **Selection of pH**

Culture filterate of each isolate were divided into 3 parts and adjusted to 3 different pH viz. 4.0, 7.0 and 9.0 with IN HCl or IN NaOH, before extracting with various solvents. pH were checked with a pH meter.

2. **Solvents used**

Chloroform, ethyl ether and ethyl acetate were the solvents used for extraction of antibiotics from the supernatant solution. One part of culture filtrate in each pH value were extracted thrice with each solvent at RT (29 ± 2°C).

Solvent - filterate mixture were shaken thoroughly for 15 minutes in a separating funnel and allowed to settle for another 15 minutes. After extraction with solvents, the aquous phase was tested for their antibiotic activity against the test organisms and all the aquous phase did not show any activity. Solvent layer were separated and evaporated at 50 ± 2°C using a Rota Vapour. The residue were then dissolved in 80% methanol.

8. **ANTIMICROBIAL ACTIVITY OF THE EXTRACTS**

Antimicrobial activity of these methanol extracts were tested against the test pathogens selected for the study by agar diffusion method (Casida, 1968).

The zone of inhibition (diameter) was measured in mm (excluding diameter of cup - 3 mm) after the incubation period (24 hours). Sea water agar was the medium used for the test, as it was found best for isolation, maintenance and also to test the antimicrobial activity in the present study.

9. **PHYSICO-CHEMICAL PARAMETERS**

An attempt was made to study some important physico-chemical parameters and find out their possible relationship, if any, with the micro-flora of the mangrove ecosystem.

The following parameters have been studied.

A - Temperature of water and sediment

B - pH of water and sediment

C - Salinity of water

D - Dissolved oxygen and

E - Organic Carbon

A. **Temperature**

Temperature was determined immediately after collection of water and sediment samples at the collection site itself with an accuracy of ± 0.1°C using precision mercury thermometer.

B. **pH (Hydrogen - Ion - Concentration)**

pH was estimated using an Toshinwal pH meter (Cat. No. CL 47) immediately after the transportation of the samples to the laboratory.

C. **Salinity**

Salinity was estimated by Mohr titration (Strickland and Parsons, 1968). The outline of Mohr-Knudson method is as follows:

Ten millilitres of water samples were titrated against the silver nitrate solution with potassium chromate as an indicator. Care was taken to arrive at the exact end point colouration in all the samples and every set of titration, silver nitrate was standardised using standard sea water supplied by the Oceanography Institute, Copenhagen. Each sample was titrated and the mean values were taken. Salinity of the sample was calculated using the following formula.

$$\text{Salinity (\%)} = \frac{V_1 S}{V_2}$$

Where

V_1 = Volume of silver nitrate for 10 ml standard sea water

V_2 = Volume of silver nitrate for 10 ml sample

S = Salinity of standard sea water

D. **Dissolved Oxygen**

Dissolved oxygen samples were collected using 125 ml 'reagent' bottle with BOD stopper. Traditional Winkler method with azide modification was used for the determination of dissolved oxygen content. The outline of this method is as follows (Claude and Pillai, 1984).

To a sample in 125 ml bottle, 2.0 ml of manganese sulphate solution and 2.0 ml of alkali iodine - azide solution were added. The bottle was stoppered with care to prevent air bubbles. The solution was mixed by shaking and inverting the bottle several times. The precipitate was allowed to settle and dissolved in the laboratory using 2.0 ml of concentrated sulphuric acid.

From this, 100 ml of sample was taken for filteration and was poured into a 250 ml flask, titrated with standard sodium thiosulphate (6.3 g/lit) solution to a pale straw colour. About 5 drops of starch indicator solution was added to this and titrated until blue colour disappears. The following equation was used to calculate the dissolved oxygen concentration.

$$\text{Dissolved oxygen (mg/L)} = \frac{(T) \cdot (N) \cdot (8{,}000)}{S}$$

Where,

T = Volume in millilitre of sodium thiosulphate

N = Normality of sodium thiosulphate

S = Volume in millilitres of sample

E. **Organic Carbon**

Organic carbon of the sediment sample was estimated by standard titration procedure (Khanna and Yadav, 1979).

Organic carbon present in organic matter is oxidised to CO_2 in the presence of potassium dichromate and sulphuric acid. Potassium dichromate produces nascent oxygen when combined with organic carbon to produce CO_2. The excess of potassium dichromate not reduced by the organic matter of sediment was then determined by titration with standard ferrous ammonium sulphate.

10. STATISTICAL ANALYSIS

The data collected for each parameter in different stations in different months were pooled seasonwise and tabulated. The same tables were used for ANOVA analysis with the help of computer to find out the level of significance among the stations, seasons and stations in a season. Correlation study was also done among the parameters and the correlation matrix with the level of significance for correlation co-efficients was obtained from the computer. The better correlated parameters are presented.

For statistical correlationship among the parameters B/F, B/A, F/A were transformed to

$$\log_e \frac{1 + B}{1 + F} \qquad \log_e \frac{1 + B}{1 + A} \qquad \log_e \frac{1 + F}{1 + A}$$

respectively.

This transformation was done, as in few cases the observations for bacteria, fungi and actinomycetes was 0, to find out the relation between these parameters. Hence a Unit (1) was added to both parameters to get better statistical relations (to avoid infinitive or not determined values which could not be used for statistical analysis).

III RESULTS

1. MICROBIALFLORA ENCOUNTERED DURING THE PERIOD OF STUDY

A. Bacteria

Total number of bacteria in the sediment of 4 fixed stations in general ranged from $1.0x10^4$/gm to $260x10^4$/gm of the sediment. The highest count ($240x10^4$/g) was recorded in the month of July 1991. In the pre-monsoon and post-monsoon seasons the bacterial population ranged from 1.0-$156x10^4$/gm and 1.0-$160x10^4$/gm respectively.

Station I

In this typical mangrove habitat, the total bacterial population in Mangalavana ranged from 1.0-$140x10^4$/gm. The highest count was recorded in the month of November 1991 (post-monsoon) and lowest in the month of April 1991 (pre-monsoon). Counts ranged from $1x10^4$/gm to $50x10^4$/gm during monsoon.

Station II

The bacterial flora recorded at Station II ranged from 1.0-$260x10^4$/gm (in monsoon), 1.0-$160x10^4$/gm in the post-monsoon and in the pre-monsoon season ($2x10^4$/gm - $82x10^4$/gm).

Station III

Highest number of bacteria was recorded in the month of May ($180x10^4$/gm) followed by $120x10^4$/gm in the month of September.

Station IV

The bacterial population ranged from 1.0-$220x10^4$/gm. The highest count was recorded in the post-monsoon ($220x10^4$/gm) followed by $80x10^4$/gm

in the monsoon and the lowest count was recorded in the pre-monsoon at the light house area of Puthuvyppu ($58x10^4$/gm).

Analysis of variance did not show any significant difference in the distribution of bacteria between the seasons or between stations sampled during the study period (Table 2).

Distribution of bacteria showed positive correlations at 5% level with B/F and B/A in Station I and correlation coefficients were found to be 0.743 and 0.552 respectively. Similar relationships were also found in all the stations. For B/F and B/A ratio the correlation coefficients found were 0.743 and 0.503; 0.828 and 0.820; and 0.706 and 0.456 for Station II, III and IV respectively (Table 5. 1-4).

B. **Fungi**

The overall range of fungal population recorded in all the stations during the study period was $1.0\text{-}28x10^4$/gm. The highest count was encountered in the month of August. The lowest count was recorded in the post-monsoon ($1.0x10^4$/gm) and $1.0\text{-}22x10^4$/gm of fungi was recorded in the pre-monsoon.

Station I

Maximum number of fungi was recorded in the month of March $22x10^4$/gm. The lowest count was recorded in post-monsoon ($1.0\text{-}4x10^4$/gm).

Station II

The fungi recorded in this station was generally low when compared to other stations throughout the period of study. The maximum population recorded in the month of January 1991 was $4x10^4$/gm.

Table 2. Analysis of variance for microbiological parameters among different seasons, stations and between seasons and stations.

	Source	df	SS	MS	F	P	Remarks
I	A	2	654.438	327.219	0.1361		NS
	B	3	2958.198	986.066	0.4100		NS
	AB	6	17087.896	2847.983	1.1842	0.3226	NS
	E	84	202016.375	2404.957			
	T	95	222716.906				
II	A	2	17.063	8.531	0.5614		NS
	B	3	17.042	5.681	0.3738		NS
	AB	6	76.021	12.670	0.8338		NS
	E	84	1276.500	15.196			
	T	95	1386.625				
III	A	2	11565.438	5782.719	4.2230	0.0179	*
	B	3	4208.083	1402.694	1.0244	0.3861	NS
	AB	6	5255.979	875.997	0.63977		NS
	E	84	115025.000	1369.345			
	T	95	136054.500				

I - Total bacterial count,

II - Total fungal count

III - Total actinomycete count

A - Season, B - Station, E - Error, T - Total

NS - Not Significant,

* - Significant at 5% level

Station III

The maximum number of fungi recorded in Station III (Puthuvyppu) was $8x10^4$/gm in the month of July 1991. In the pre-monsoon and post-monsoon almost similar range was recorded, $1-4x10^4$/gm.

Station IV

Maximum number of fungi ($28x10^4$/gm) was recorded in this station during the period of study. The highest count was noted in the monsoon ($28x10^4$/gm).

Analysis of variance of number of fungi did not show any significant difference between the stations or seasons (Table 2).

In Station I the distribution of fungal flora (Table 5.1) had a positive relationship with F/A (r=0.405), direct relationship was also noted in Narakkal (Table 5.2) with correlation coefficient of 0.650 and in Station III (Table 5.3) being 0.639. But in the light house area of Puthuvyppu the fungal distribution had a negative correlation with B/F and B/A with correlation coefficients of -0.595 and -0.416 and a positive correlation with F/A (r=0.716) (Table 5.4).

C. **Actinomycetes**

The actinomycete population ranged from $1.0x10^4$/gm to $243x10^4$/gm of sediment sample. The maximum population ($243x10^4$/gm) was recorded in the month of September 1991 (monsoon).

Station I

Maximum number of actinomycete was recorded in this typical mangrove ecosystem was $187x10^4$/gm in the month of August 1991 (monsoon). During pre-monsoon, $2-12x10^4$/gm of actinomycetes was recorded and in post-monsoon $2.0-62.0x10^4$/gm of actinomycetes was recorded (Fig.1).

Station II

Maximum count was encountered in the monsoon season ($243x10^4$/gm). During post-monsoon $1.0-114x10^4$/gm of actinomycete was recorded and the minimum count was encountered in the pre-monsoon ($2.0-8x10^4$/gm) season (Fig.2).

Station III

Similar seasonal cycle in the distribution of number of actinomycete was also recorded in Stations I, II and III. Maximum number was recorded in monsoon $94x10^4$/gm in the month of August 1991. The range in the post-monsoon was low ($8.0-38x10^4$/gm) and lowest count was recorded in the pre-monsoon ($1.0-13x10^4$/gm). The distribution pattern of actinomycetes, was found to be similar in 3 Stations (I, II and III) (Fig.3).

Station IV

Unlike the other 3 stations maximum counts was recorded in the month of November ($63x10^4$/gm). In monsoon $1.0-32x10^4$/gm and in the pre-monsoon period $1.0-3x10^4$/gm of actinomycetes were recorded at Station IV (Fig.4).

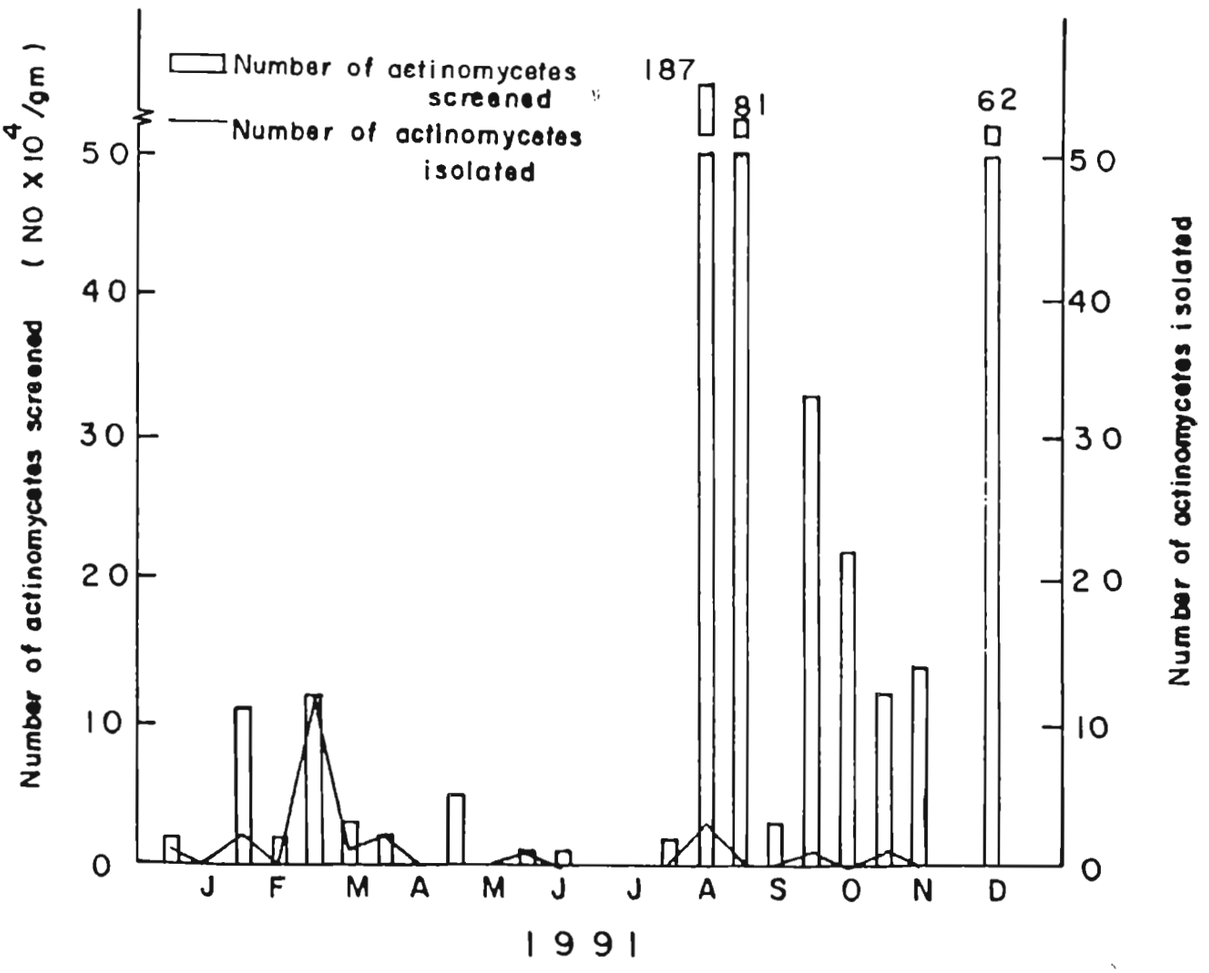


Fig. 1 Total number of actinomycetes screened and isolated at Station - I.

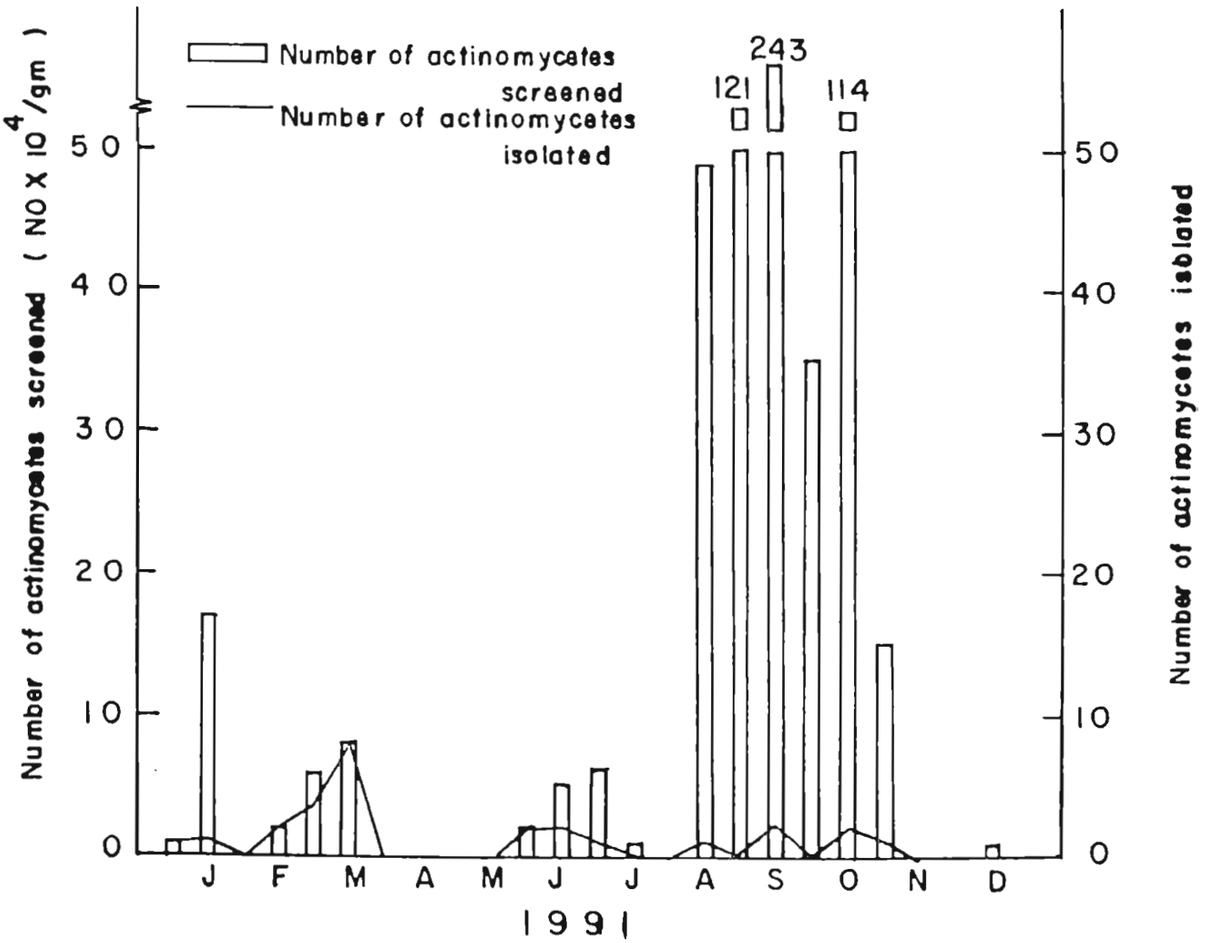


Fig. 2. Total number of actinomycetes screened and isolated at Station - II.

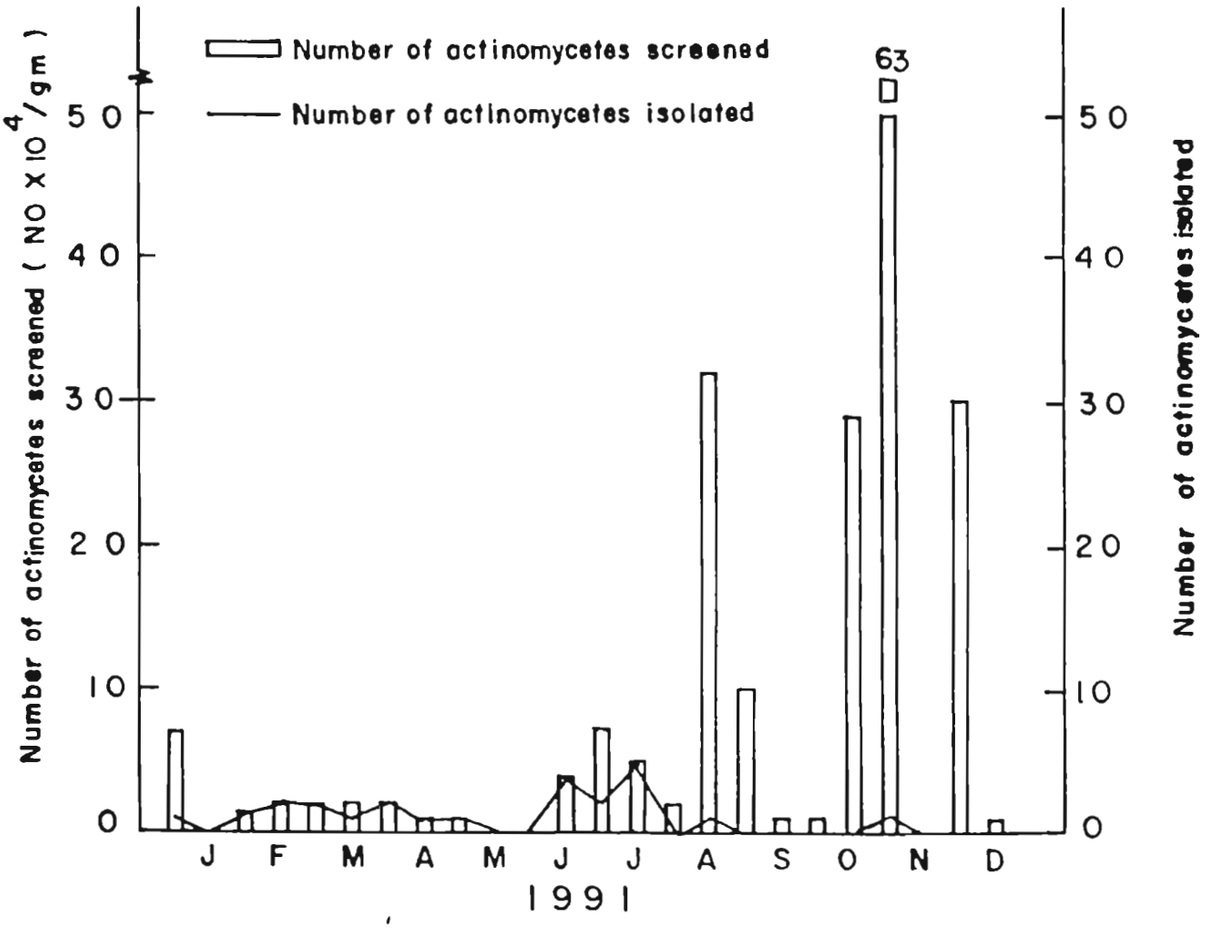


Fig. 3 Total number of actinomycetes screened and isolated at Station - III.

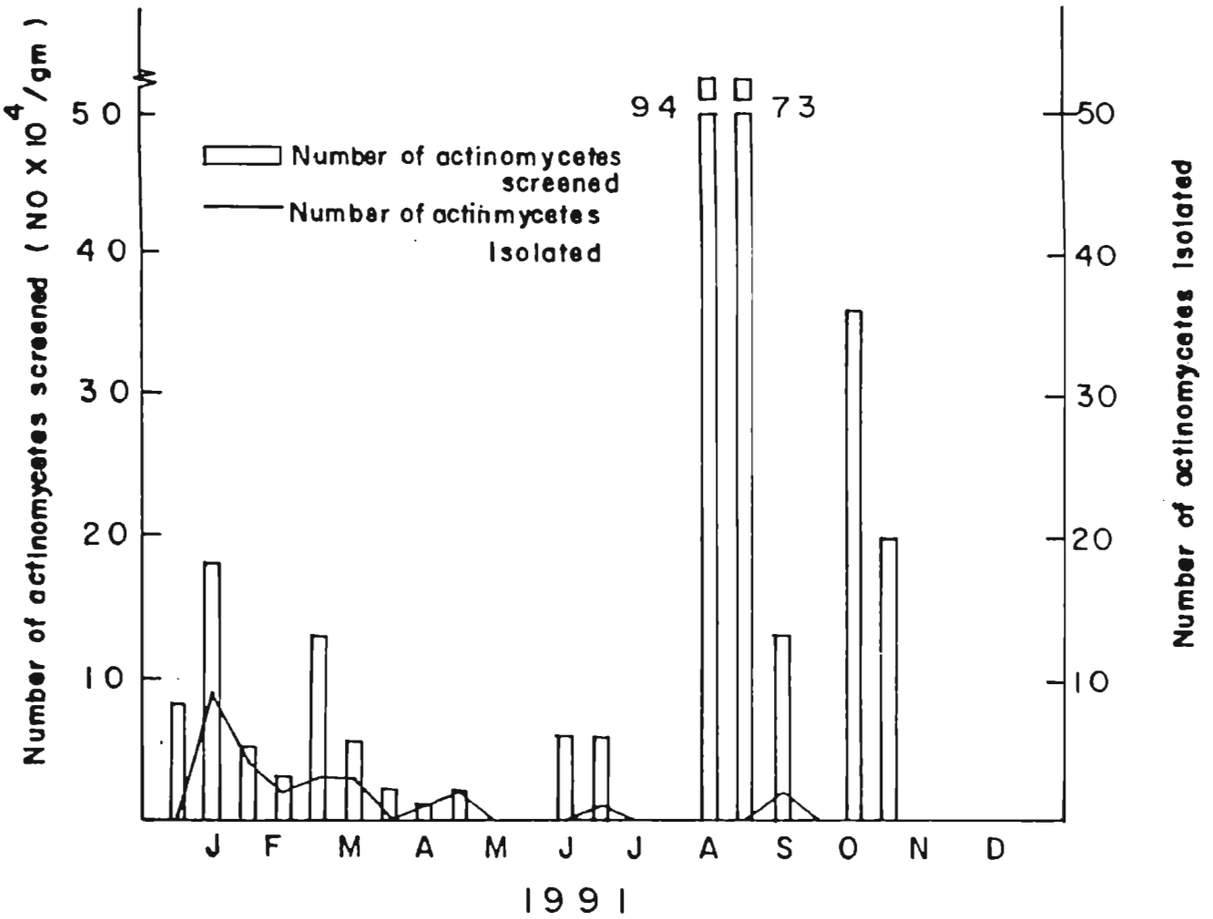


Fig. 4. Total number of actinomycetes screened and isolated at Station - IV.

Analysis of variance of actinomycetes did not show any significant difference neither between the stations nor between the seasons in the distribution of total number of actinomycetes (Table 2).

Negative correlation was found between the distribution of actinomycetes and B/A and F/A at Mangalvana during the period of study. The correlation coefficient were found to be -0.608 and -0.786 for B/A and F/A (Table 5.1).

Distribution of actinomycetes at Station II Table 5.2 (Fig.5) showed a positive correlation with pH of water and sediment, organic carbon and negative correlation with B/A. The correlation co-efficients being 0.430, 0.411, 0.657 and -0.619.

In the Station III (Puthuvyppu), distribution of actinomycete did not show any relation with any of the parameters studied (Table 5.3).

In the light house area of Puthuvyppu (Table 5.4) total actinomycetes showed a positive relationship at 5% level with the total bacteria with correlation coefficient of 0.706 (Fig.6).

D. **Relationship between microbial parameters**

a. **Correlation between actinomycetes and bacteria**

Positive correlation at 5% level was found between bacteria and actinomycete at Puthuvyppu in post-monsoon ($r=0.552$) also at the light house area of Puthuvyppu (Station IV) in the same season ($r=0.715$). The pooled data for all the season showed positive relationship ($r=0.618$) with 5% significant difference at Station IV for total counts of bacteria and actinomycetes (Table 4).

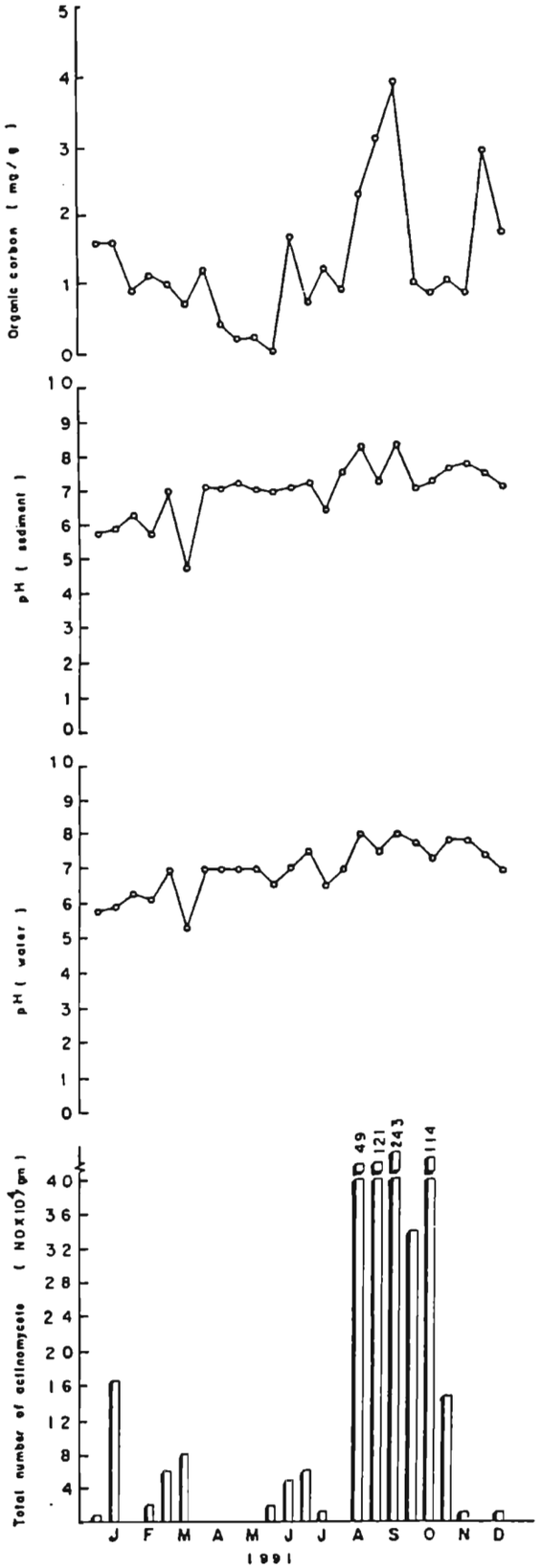


Fig.5 Total number of actinomycetes and physicochemical parameters at Station - II.

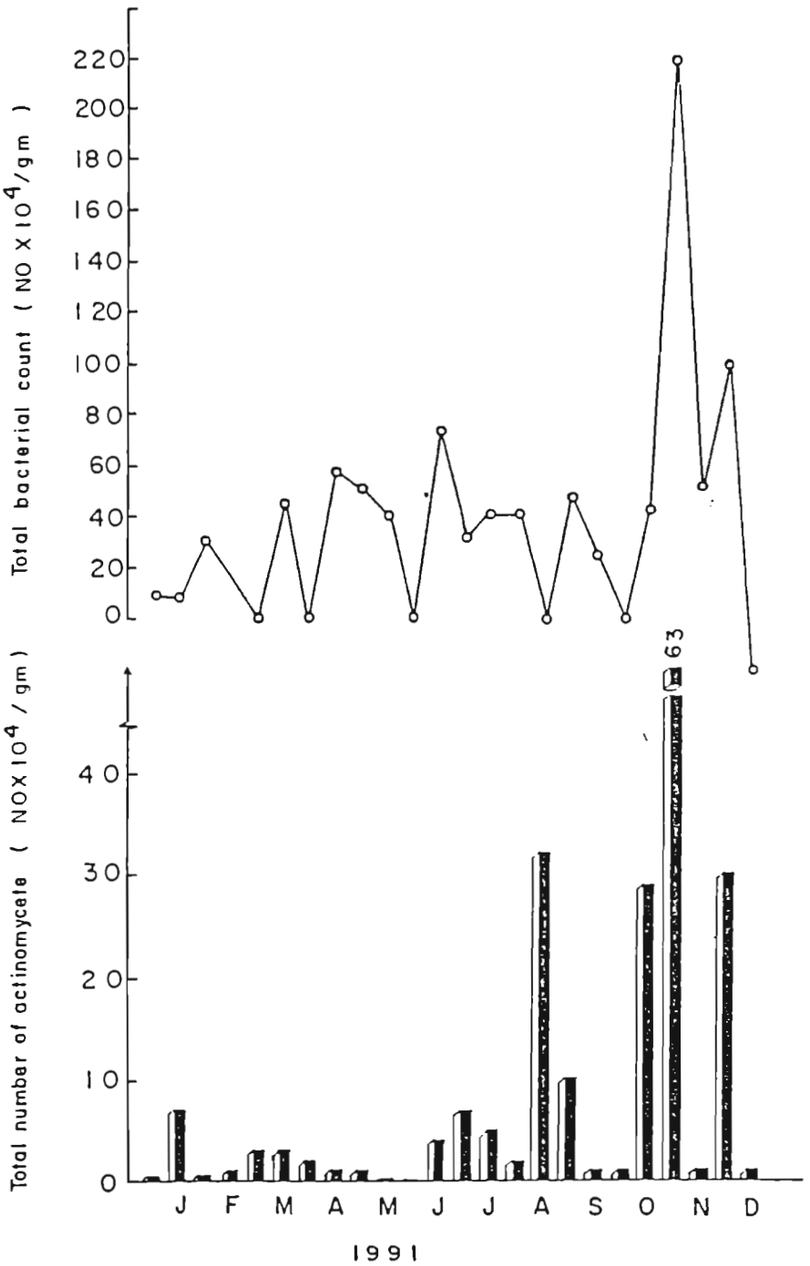


Fig. 6 Total number of actinomycetes and bacteria at Station IV.

b. **Correlation between actinomycetes and fungi**

Positive correlation existed between the total counts of fungi and actinomycetes at 5% level in the pre-monsoon season at Mangalavana and Narakkal and correlation coefficients were found to be 0.634 and 0.756 respectively. During monsoon similar correlation was observed between fungi and actinomycetes at Narakkal, Puthuvyppu and light house area, with r values 0.606, 0.649 and 0.603 respectively. Significant relationship was noted in Station I and Station II during post-monsoon with correlation coefficients of 0.761 and 0.505 respectively (Table 4).

E. Interrelationship of microflora

ANOVA analysis of B/F ratio did not show any significant difference between seasons or stations, whereas B/A ratio showed a significant difference at 5% level between seasons, and not within the stations (Table 3). F/A ratio showed only less significant difference at 10% level between the stations but not for seasons.

B/F ratio showed a positive relationship with total count of bacteria (r=0.743) at Mangalavana. B/A ratio showed positive relationship with bacterial count (r=0.552) and with B/F (r=0.629) and a negative correlation with total actinomycetes r=-0.608. And F/A ratio showed a positive relationship with total fungal count (r=0.405) and a negative relationship with total actinomycetes (r=-0.786) (Table 5.1).

At Narakkal B/F ratio showed positive relationship with bacterial count (r=0.743) and B/A ratio (r=0.621). B/A ratio showed positive relationship with bacteria (r=0.508). F/A ratio showed positive relationship with fungi (r=0.650) (Table 5.2).

Table 3. Analysis of variance for interrelationship of microbiological parameters among different seasons, stations, and between seasons and stations.

	Source	df	SS	MS	F	P	Remarks
I	A	2	3.657	1.829	0.5601		NS
	B	3	5.445	1.815	0.5559		NS
	AB	6	17.196	2.866	0.8778		NS
	E	84	274.268	3.265			
	T	95	300.566				
II	A	2	21.509	10.755	3.1868	0.0463	*
	B	3	4.845	1.615	0.4785		NS
	AB	6	18.153	3.025	0.8965		NS
	E	84	283.474	3.375			
	T	95	327.981				
III	A	2	0.747	0.374	0.4129		NS
	B	3	6.237	2.079	2.2983	0.0834	
	AB	6	7.898	1.316	1.4551	0.2037	NS
	E	84	75.990	0.905			
	T	95	90.872				

I - B/F Ratio, II - B/A Ratio, III - F/A Ratio

A - Season, B - Station, E - Error, T - Total

NS - Not Significant, * - Significant at 5% level

Table 4. Correlation between microbiological parameters

	Season	Station I	II	III	IV
I	Pre-monsoon	0.18	0.157	0.113	0.136
	Monsoon	0.41	0.061	0.057	0.344
	Post-monsoon	0.143	0.134	0.552*	0.715*
	Pooled	0.121	0.068	0.084	0.618*
II	Pre-monsoon	0.634*	0.756*	0.216	0.354
	Monsoon	0.335	0.606	0.649*	0.603*
	Post-monsoon	0.761*	0.505*	0.087	-
	Pooled	0.070	0.114	0.071	0.549*

I Correlation between actinomycete and bacterial counts.

II Correlation between actinomycete and fungal counts.

* Significant at 5% level.

In order to assess the correlation and extent of influence between the parameters studied the following characters were selected and correlation coefficient 'r' was calculated.

1. Atmospheric temperature
2. Water temperature
3. Water pH
4. Salinity
5. Dissolved oxygen
6. Sediment temperature
7. Sediment pH
8. Organic carbon
9. Total bacterial count
10. Total fungal count
11. Total actinomycete count
12. B/F Ratio
13. B/A Ratio
14. F/A Ratio

The result of these parameters during January to December 1991 in the four selected mangrove ecosystems are presented as correlation matrix in the Tables 5.1 - 4.

Table 5.1 Correlation matrix of physico-chemical and microbiological parameters (Station I)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.	1.000													
2.	- 0.167	1.000												
3.	- 0.122	0.178	1.000											
4.	0.316	- 0.210	- 0.386	1.000										
5.	0.230	- 0.159	- 0.177	- 0.051	1.000									
6.	0.586*	- 0.279	0.199	- 0.089	0.210	1.000								
7.	- 0.123	0.145	0.910*	- 0.339	- 0.092	0.195	1.000							
8.	- 0.022	- 0.019	0.483*	- 0.282	- 0.245	- 0.061	0.414*	1.000						
9.	0.413*	- 0.050	- 0.050	0.069	0.190	0.246	- 0.034	- 0.111	1.000					
10.	0.176	- 0.069	- 0.298	0.218	- 0.059	- 0.034	- 0.216	0.068	- 0.049	1.000				
11.	- 0.368	- 0.111	0.038	- 0.281	0.009	- 0.149	0.173	0.182	- 0.140	- 0.055	1.000			
12.	0.224	0.107	0.119	0.202	0.225	0.136	0.096	- 0.348	0.743*	- 0.387	- 0.182	1.000		
13.	0.156	0.146	- 0.155	0.371	0.128	0.015	- 0.223	- 0.396	0.552*	- 0.084	- 0.608*	0.629*	1.000	
14.	0.373	0.027	0.082	0.170	- 0.223	0.050	- 0.063	0.175	0.142	0.405*	- 0.786*	- 0.067	0.353	1.000

Table 5.2 Correlation matrix of physico-chemical and microbiological parameters (Station II)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.	1.000													
2.	0.915*	1.000												
3.	0.092	0.157	1.000											
4.	0.150	0.148	- 0.485*	1.000										
5.	0.338	0.559*	0.138	0.195	1.000									
6.	0.844*	0.933*	0.020	0.156	0.491*	1.000								
7.	- 0.007	0.085	0.931*	- 0.379	0.232	- 0.003	1.000							
8.	- 0.069	- 0.103	0.330	- 0.302	0.139	- 0.115	0.328	1.000						
9.	- 0.078	0.026	0.331	- 0.371	- 0.045	- 0.102	0.177	- 0.100	1.000					
10.	- 0.272	- 0.279	- 0.143	0.159	- 0.249	- 0.341	- 0.160	- 0.010	- 0.010	1.000				
11.	0.258	0.307	0.430*	- 0.374	0.162	0.294	0.411*	0.657*	0.059	- 0.165	1.000			
12.	0.329	0.216	0.231	- 0.269	0.005	0.156	0.106	- 0.104	0.743*	- 0.241	0.067	1.000		
13.	- 0.089	- 0.192	- 0.112	0.140	- 0.023	- 0.245	- 0.113	- 0.411*	0.508*	0.161	- 0.619*	0.621*	1.000	
14.	- 0.179	- 0.188	0.098	0.228	0.061	- 0.286	0.242	0.079	- 0.178	0.650*	- 0.007	- 0.247	0.065	1.000

Table 5.3 Correlation matrix of physico-chemical and micro-biological parameters (Station III)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.	1.000													
2.	0.831*	1.000												
3.	- 0.009	0.003	1.000											
4.	0.272	0.125	- 0.519*	1.000										
5.	0.464*	0.515*	0.179	0.088	1.000									
6.	0.693*	0.904*	0.004	0.246	0.517*	1.000								
7.	- 0.105	- 0.043	0.952*	- 0.608*	0.090	- 0.040	1.000							
8.	- 0.634*	- 0.590*	- 0.193	- 0.081	- 0.447*	- 0.427*	- 0.005	1.000						
9.	0.122	0.013	0.151	0.079	0.378	- 0.025	- 0.009	- 0.366	1.000					
10.	- 0.131	- 0.149	- 0.079	- 0.295	0.049	- 0.192	0.056	0.187	0.005	1.000				
11.	- 0.129	0.074	0.193	- 0.217	- 0.133	0.139	0.262	0.316	0.013	- 0.157	1.000			
12.	0.107	- 0.016	- 0.009	- 0.034	0.262	- 0.080	- 0.182	- 0.466*	0.828*	- 0.080	- 0.085	1.000		
13.	0.048	- 0.047	0.033	- 0.136	0.305	- 0.079	- 0.077	- 0.397	0.820	0.191	- 0.095	0.924*	1.000	
14.	- 0.184	- 0.069	0.294	- 0.496*	- 0.047	- 0.154	0.402	0.049	0.219	0.639*	- 0.018	0.113	0.310	1.000

Table 5.4 Correlation matrix for physico-chemical and microbiological parameters (Station IV)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.	1.000													
2.	- 0.230	1.000												
3.	- 0.028	- 0.130	1.000											
4.	- 0.229	0.271	- 0.518*	1.000										
5.	0.218	0.312	- 0.300	0.282	1.000									
6.	- 0.199	0.825*	- 0.222	0.460*	0.570*	1.000								
7.	0.030	- 0.103	0.954*	- 0.462*	- 0.106	- 0.134	1.000							
8.	- 0.146	- 0.337	0.235	- 0.111	- 0.394	- 0.409*	0.156	1.000						
9.	0.094	- 0.324	0.054	- 0.381	- 0.164	- 0.435*	- 0.037	0.041	1.000					
10.	- 0.085	0.129	0.047	0.136	0.373	0.054	0.188	- 0.119	- 0.225	1.000				
11.	- 0.067	- 0.145	0.171	- 0.338	- 0.113	- 0.338	0.224	0.147	0.706*	0.291*	1.000			
12.	0.187	- 0.370	- 0.101	- 0.262	- 0.171	- 0.280	- 0.199	0.018	0.706*	- 0.595*	0.177	1.000		
13.	0.229	- 0.279	- 0.185	- 0.117	0.052	- 0.135	- 0.243	- 0.222	0.456*	- 0.416*	- 0.146	0.844*	1.000	
14.	- 0.433*	0.211	0.017	0.240	0.089	0.083	0.078	0.051	- 0.160	0.716*	0.246	- 0.509*	- 0.393	1.000

B/F ratio showed positive relationship with bacterial count (r=.829) and B/A (r=0.924) in Puthuvyppu. B/A ratio also showed positive relationship with bacteria (r=0.820). F/A ratio showed positive correlation with total actinomycetes and the correlation co-efficient being 0.639 (Table 5.3).

In the light house area of Puthuvyppu, B/F ratio showed positive relationship with bacterial count and B/A ratio, the 'r value' being 0.706 and 0.844 respectively. An indirect relationship was also obtained with fungal count (r=-0.595) and F/A ratio (r=-0.509) (Table 5.4).

B/A ratio showed positive relationship with total bacteria (r=0.456) and a negative correlation with fungi (r=-0.416). Whereas F/A ratio showed a positive relation with fungal count (r=0.716) and a negative correlation with B/F ratio (r=-0.509) (Table 5.4) at Station IV.

2. PHYSICO-CHEMICAL PARAMETERS

A. Temperature

a. Atmospheric temperature

During the period of study atmospheric temperature ranged from 23.0°C to 38.5°C. The peak value was recorded during the month of April 1991 and the lowest temperature was recorded in August 1991.

In the pre-monsoon season the lowest temperature was recorded in the month of May 1991. In monsoon, atmospheric temperature ranged between 20°C to 35.0°C. And in the post-monsoon it ranged from 24.0°C to 37.0°C.

Analysis of variance did not show any significant difference either between the seasons or stations (Table 1.1).

Table 1. Analysis of variance for physico-chemical and microbiological parameters among different seasons, stations and between seasons and stations.

Table 1.1 Atmospheric temperature

Source	df	SS	MS	F	P	Remarks
A	2	373.078	186.539	0.5026		NS
B	3	1081.570	360.523	0.9714	0.3647	NS
AB	6	2467.234	411.206	1.1080		NS
E	84	31174.531	371.125			
T	95	35096.414				

A - Season

B - Station

E - Error

T - Total

NS - Not Significant

At Mangalavana, atmospheric temperature showed a positive relationship with sediment temperature (r=0.586) and also with the total bacterial count (r=0.413) during the period of study (Table 5.1).

Atmospheric temperature showed a positive correlation with sediment temperature (r=0.844) at Narakkal (Table 5.2). At Puthuvyppu (Table 5.3) atmospheric temperature showed a positive relation with water temperature, dissolved oxygen, sediment temperature and their correlation coefficients were found to be 0.831, 0.464 and 0.693 respectively, whereas it showed a negative relationship with organic carbon (r=-0.634). In the light house area of Puthuvyppu, atmospheric temperature showed a negative relationship with F/A ratio (r=-0.433) (Table 5.4).

b. **Water temperature**

Surface water temperature of the mangrove stations ranged between 23°C to 36°C. Highest water temperature was recorded in the month of October 1991 (36°C) and the lowest in December 1991 (23°C).

Station I

At Mangalavana water temperature ranged from 24°C to 32°C. The highest temperature was observed in monsoon (32°C) in July 1991 and lowest during pre-monsoon 24°C in the month of December 1991.

Station II and III

Surface water temperature did not vary much in these 2 stations. Temperature was high (36°C) in the month of October in both the stations and low in the month of December 1991 (24°C in Narakkal and 23°C in Puthuvyppu).

Table 1.2 Water Temperature

Source	df	SS	MS	F	P	Remarks
A	2	559.943	279.91	0.7714		NS
B	3	957.479	319.160	0.8794		NS
AB	6	2416.536	402.756	1.1097	0.3637	NS
E	84	30486.875	362.939			
T	95	34420.833				

A - Season, B - Station, E - Error, T - Total,
NS - Not Significant

Table 1.3 Sediment Temperature

Source	df	SS	MS	F	P	Remarks
A	2	82.333	41.167	4.5927	0.0128	*
B	3	73.281	24.427	2.7252	0.0493	*
AB	6	47.188	7.865	0.8774		NS
E	84	752.938	8.964			
T	95	955.740				

A - Season, B - Station, E - Error, T - Total
* Significant at 5% level, NS - Not Significant

Station IV

In light house area of Puthuvyppu, water temperature ranged from 24°C to 35°C. In the pre-monsoon season the temperature ranged between 28.5°C to 35.0°C and with the onset of monsoon the temperature declined and reached minimum in July. During post-monsoon the temperature again increased and reached maximum in October 1991 (34°C).

Analysis of variance did not show any significant difference in the water temperature between the seasons or between stations sampled during the period of study (Table 1.2).

Correlation matrix of water temperature did not show relationship with microbiological and physico-chemical parameters studied at Mangalavana (Table 5.1).

Water temperature at Narakkal showed a positive correlation with dissolved oxygen and sediment temperature and their correlation co-efficients being 0.559 and 0.933 respectively (Table 5.2).

A positive correlation was found to exist between water temperature and dissolved oxygen, sediment temperature their correlation coefficients being 0.515 and 0.904 respectively. A negative relationship was found between water temperature and organic carbon (r=-0.590) at Puthuvyppu (Table 5.3).

In Station IV water temperature showed a positive relationship only with sediment temperature, the correlation coefficient being 0.825 (Table 5.4).

c. Sediment temperature

Sediment temperature was recorded always high than that of the water temperature and ranged between 24°C to 38°C. The maximum tem-

perature was recorded during the post-monsoon season (38°C) in the month of October and minimum in the month of December 1991 (24°C).

Station I

At Mangalavana the temperature ranged from 27-32°C during pre-monsoon season. The highest temperature of 32°C was recorded in the month of April. With the onset of monsoon, the temperature declined and reached a minimum (25.5°C) in August 1991 and again increased and reached a peak of 31°C in post-monsoon.

Station II

Highest temperature was recorded in October at Narakkal, and lowest temperature was recorded in December 1991. In the pre-monsoon season temperature ranged from 31°C to 35°C and decreased to a minimum of 25.5°C in the month of August 1991 due to monsoon effect.

Station III and IV

Sediment temperature did not vary much among these 2 stations. The maximum temperature 35°C was recorded in Station III during October and in Station IV during February. In both the stations temperature declined during monsoon and again increased in the post-monsoon season.

ANOVA analysis showed significant difference at 5% level between the seasons and among the stations but no difference was observed between seasons and stations (Table 1.3).

It was noted that the sediment temperature showed positive relationship with atmospheric temperature at Mangalavana (Table 5.1).

At Narakkal sediment temperature showed a positive relationship with dissolved oxygen and the correlation co-efficient was found to be 0.491 (Table 5.2).

Sediment temperature showed a positive relationship with dissolved oxygen (r=0.517) and a negative relationship (r=-0.427) with organic carbon at Puthuvyppu (Table 5.3).

In the light house area of Puthuvyppu sediment temperature showed a positive relationship with salinity and dissolved oxygen and their correlation co-efficients being 0.460 and 0.570 respectively. Sediment temperature showed a negative correlation with organic carbon content (r=-0.409) and total bacterial count (r=-0.435) during the period of study (Table 5.4).

B. **pH (Hydrogen-ion-concentration)**

a. **pH (Water)**

The average pH value recorded in the 4 stations during the period of study ranged between 5.1 to 8.4. Maximum pH was noted during post-monsoon (8.4) in the month of January and lowest in pre-monsoon (5.1) in March.

Station I

Highest water pH (8.4) was recorded at Mangalavana during post-monsoon. Range of pH in the pre-monsoon season was between 5.8 to 7.5 and increased with the onset of monsoon to 6.5 to 8.1.

Station II, III and IV

Similar fluctuations in pH like Mangalavana was noticed in the other stations also, where the highest pH recorded was 8.0 in all the three stations

Table 1.4 Water pH

Source	df	SS	MS	F	P	Remarks
A	2	7.854	3.927	5.9876	0.0037	**
B	3	2.117	0.706	1.0759	0.3638	NS
AB	6	2.628	0.438	0.6678		NS
E	84	55.091	0.656			
T	95	67.690				

A - Season, B - Station, E - Error, T - Total

** Significant at 1% level, NS - Not Significant

Table 1.5 Sediment pH

Source	df	SS	MS	F	P	Remarks
A	2	11.539	5.770	7.4144	0.0011	**
B	3	2.966	0.989	1.2706	0.2898	NS
AB	6	2.577	0.430	0.5520		NS
E	84	65.366	0.778			
T	95	82.449				

A - Season, B - Station, E - Error, T - Total

** Significant at 1% level, NS - Not Significant

during the period of monsoon. The lowest pH values recorded were 5.3 (in Station II); 5.1 (in Station III) and 5.2 (in Station IV) in the pre-monsoon season.

Analysis of variance of water pH showed significant difference (at 5% level) between seasons but no significant difference was observed among the stations or between season and stations (Table 1.4).

At Mangalavana pH of water showed a positive correlation with pH of sediment and organic carbon, their correlation co-efficients being 0.910 and 0.483 respectively (Table 5.1).

pH of water showed a negative relationship with salinity (r=-0.485), but a positive correlation also existed with pH of sediment (r=0.931) and total actinomycetes (r=0.430) at Narakkal (Table 5.2).

At Station III (Table 5.3) and IV (Table 5.4) a similar type of relationship existed where pH of water showed a negative relationship with salinity (r=-0.519) at Station III, (r=-0.518 at Station IV) but showed a positive correlation with sediment temperature (r=0.952 at Station III, r=0.954 at Station IV).

b. **pH (Sediment)**

Sediment pH ranged from 4.3 to 9.7. The maximum pH was noted during post-monsoon and lowest was recorded in pre-monsoon.

Station I

At Mangalavana pH range was from 5.2 to 9.7. The maximum pH was recorded in the month of December 1991. pH range in the pre-monsoon

season was found to be 5.9 to 7.8 and slight increase to alkaline range in pH was recorded with the onset of monsoon (6.5 to 8.3).

Station II

At Narakkal, fluctuation in pH was noted between 4.7 to 8.3. The maximum pH was recorded in monsoon. In pre-monsoon pH ranged from 4.7 to 7.2 and in post-monsoon 5.8 to 7.7.

Station III and IV

Sediment pH in these 2 stations did not vary much. Highest value was recorded in pre-monsoon 8.2 and 8.1 at Station III and IV respectively. With the onset of monsoon, pH declined in both the stations. 5.5 to 7.5 and 5.2 to 7.9 were the range of pH recorded in Station III and IV respectively during post-monsoon.

ANOVA analysis of sediment pH during the study period showed significant difference at 1% level for seasons but no difference was observed among the stations or between seasons and Stations (Table 1.5).

pH of sediment recorded during the study period at Mangalavana showed a positive relationship with organic carbon (r=0.414) (Table 5.1).

At Narakkal (Table 5.2) pH of sediment showed a positive relationship with total actinomycetes and correlation coefficients was found to be 0.411.

Sediment pH showed a negative relationship with salinity of water at both Stations III and IV (r=-0.608 for Station II and r=-0.462 for Station IV) (Table 5.3 and 4).

C. **Salinity**

Salinity showed an obvious seasonal variation in all the 4 mangrove stations studied during January to December 1991. Overall salinity ranged from 0.8‰ to 34.0‰. Salinity was maximum during pre-monsoon and peak value was recorded as 34‰ in the month of March at Puthuvyppu followed by 33.6‰ at light house area of Puthuvyppu 28.1‰ at Narakkal and 20.4‰ at Mangalavana. During monsoon salinity was found to be decreasing and in all the 4 stations minimum salinity was recorded in monsoon. Range of salinity recorded in the 4 stations during the monsoon months were 0.9 to 7.7‰, 1.0 to 15.9‰, 0.8-20.0‰ and 2.2 to 18.6‰ at Station I, II, III and IV respectively. A sudden decrease in salinity was observed during July 1991 in all the 4 stations. Again increase in salinity was recorded as 1.6 to 16.8‰, 1.4 to 18.1‰, 1.4 to 30‰ and 1.4 to 30.0‰ in Station I, II, III and IV respectively during post-monsoon.

Analysis of variance studied for the salinity showed a very high significant difference for seasons and highly significant difference among the stations but no difference between seasons and the stations (Table 1.6).

At Mangalavana (Table 5.1) salinity did not show any relationship with any of the environmental parameters studied.

D. **Dissolved oxygen**

The highest dissolved oxygen was recorded in the month of August 1991 at Narakkal. Range of dissolved oxygen during pre-monsoon and post-monsoon were 1.5 ml/l to 13.4 ml/l and 0.4 ml/l to 5.4 ml/l respectively. In all the other three stations a similar pattern of increasing trend was

Table 1.6 Salinity

Source	df	SS	MS	F	P	Remarks
A	2	4869.006	2434.503	60.5560	0.0000	***
B	3	727.498	242.499	6.0319	0.0009	**
AB	6	351.296	58.549	1.4564	0.2032	NS
E	84	3377.012	40.203			
T	95	9324.813				

A - Season, B - Station, E - Error, T - Total

*** Very High Significant, ** Significant at 1% level

NS Not Significant

Table 1.7 Dissolved Oxygen

Source	df	SS	MS	F	P	Remarks
A	2	322.466	161.233	12.3579	0.0000	***
B	3	83.176	27.725	2.1250	0.1031	NS
AB	6	30.609	5.102	0.3910		NS
E	84	1095.946	13.047			
T	95	1532. 97				

A - Season, B - Station, E - Error, T - Total

*** Very High Significant, NS - Not Significant

noticed with the peak values during pre-monsoon (17.3 ml/l at Mangalavana, 11.5 ml/l at Puthuvyppu and 9.4 ml/l at light house area) and the oxygen content declined with the onset of monsoon ranging from 1.5 ml/l to 15.9 ml/l (Mangalavana); 0.5 ml/l to 9.6 ml/l (Puthuvyppu) and from 0.2 ml/l to 5.2 ml/l at the light house area of Puthuvyppu. Lowest oxygen content was recorded during the post-monsoon season invariably in all the three stations as 1.5 ml/l, 0.5 ml/l and 0.2 ml/l at Mangalavana, Puthuvyppu and light house area of Puthuvyppu respectively.

ANOVA analysis for dissolved oxygen showed very high significant difference between seasons but no significant difference was observed among the stations or in a season between the Stations (Table 1.7).

At Mangalavana dissolved oxygen did not show any correlation with any of the environmental parameters studied (Table 5.1).

E. **Organic carbon**

Organic carbon content 13.5% was recorded as highest value during post-monsoon season at Mangalavana followed by 9.6% at light house area of Puthuvyppu in the same season and it was also observed that in both these stations organic carbon content showed a similar increasing trend with lowest values recorded in the pre-monsoon months as 1.4% to 3.1% (Mangalavana) and 1.9% to 2.8% at light house and with a slight increase in the organic carbon was noted with the onset of monsoon at Mangalavana but at the same time a slight decline was noticed at light house (0.3% to 2.7%).

Table 1.8 Organic carbon

Source	df	SS	MS	F	P	Remarks
A	2	39.151	19.576	5.4395	0.0060	**
B	3	105.891	35.297	9.8080	0.0000	***
AB	6	28.734	4.789	1.3307	0.2527	NS
E	84	302.298	3.599			
T	95	476.073				

A - Season, B - Station, E - Error, T - Total

** Significant at 1% level, *** Very High Significant

NS - Not Significant

In Station II and III, minimum value of organic carbon was recorded during the pre-monsoon months as 0.2% to 1.2% and 0.1% to 2.0% respectively. Maximum organic carbon value was obtained in the monsoon period with peak values of 3.8% and 4.0% at Station II and III respectively. Organic carbon content declined during the post-monsoon season in both the stations to 0.8% at Narakkal, to 0.7% at Puthuvyppu.

3. DISTRIBUTION OF ACTINOMYCETES

A. Distribution of total actinomycetes encountered during the period of study

In total, 1591 actinomycetes were encountered from the sediment samples collected during the period of study at four fixed mangrove stations namely Mangalavana (Station I) Narakkal (II), Puthuvyppu (III) and light house area of Puthuvyppu (IV). Among these four stations studied highest number was recorded at Narakkal 626 (39.35%), followed by 454 (28.5%) at Mangalavana, 308 (19.36%) at Puthuvyppu. The lowest number of actinomycetes was recorded at the light house area of Puthuvyppu, 203 (12.76%) (Table 6).

a. Seasonal distribution of actinomycetes

Maximum number of actinomycetes was recorded in the monsoon the value being 955 (60.03%) followed by 541 (34.00%) in post-monsoon. And the lowest number of actinomycetes was recorded during the pre-monsoon season the value being 95 (5.97%) (Table 6).

Monsoon

During the monsoon, out of 955 actinomycetes encountered, maximum number was recorded at Narakkal 427 (44.71%), followed by 274 (28.69%)

Table 6. Seasonal distribution of total actinomycetes in the study area

Station / Season	I	II	III	IV	Total
Pre-monsoon	35 (36.84%)	16 (16.84%)	33 (34.74%)	11 (11.58%)	95 (5.97%)
Monsoon	274 (28.69%)	427 (44.71%)	193 (20.21%)	61 (6.39%)	955 (60.03%)
Post-monsoon	145 (26.80%)	183 (33.83%)	82 (15.16%)	131 (24.21%)	541 (34.00%)
Total	454 (28.54%)	626 (39.35%)	308 (19.36%)	203 (12.76%)	1591 (100%)

at Mangalavana and 193 (20.21%) at Puthuvyppu. Minimum was encountered at the light house area of Puthuvyppu 61 (6.39%) (Table 6).

Post-monsoon

The maximum number recorded during the post-monsoon season was at Narakkal 183 (33.83%) followed by 145 (26.80%) at Mangalavana 131 (24.21%) at the light house area of Puthuvyppu, 82 (15.16%) at Puthuvyppu out of 541 actinomycetes encountered in this season (Table 6).

Pre-monsoon

Unlike the other two seasons pre-monsoon recorded maximum number of actinomycetes at Mangalavana 35 (36.84%) followed by Puthuvyppu 33 (34.74%) 16 (16.84%) at Narakkal and the lowest number was recorded at the light house area of Puthuvyppu 11 (11.58%) among 95 total actinomycetes encountered in pre-monsoon (Table 6).

b. **Distribution of actinomycetes according to colour series**

Among the total number of actinomycetes recorded from the sediment samples of fixed mangrove stations in the year 1991 five colour series of actinomycetes were observed namely white, grey, red, orange and green. It was found that grey coloured actinomycetes dominated [840 (52.8%)] than the other 4 colours observed, followed by white 676 (42.5%), 70 (4.4%) of red colour series, only 4 (0.25%) number of orange coloured actinomycetes was noted and green coloured actinomycete was very rare and it was possible to encounter only one (0.06%) green coloured actinomycete (Table 7).

Table 7. Seasonal distribution of total actinomycetes based on the colour series.

Colour / Season	White	Grey	Red	Orange	Green	Total
Pre-monsoon	65 (68.42%)	19 (20.00%)	8 (8.42%)	2 (2.11%)	1 (1.05%)	95 (5.97%)
Monsoon	293 (30.68%)	598 (62.62%)	62 (6.49%)	2 (0.21%)	0 (0%)	955 (60.03%)
Post-monsoon	318 (58.78%)	223 (41.12%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	541 (34.00%)
Total	676 (42.50%)	840 (52.80%)	70 (4.40%)	4 (0.25%)	1 (0.06%)	1591 (100%)

Grey colour series

Totally, 428 (50.95%) grey coloured actinomycete was encountered out of total 840 actinomycetes encountered at Narakkal followed by 195 (23.21%) at Puthuvyppu, 169 (20.12%) at Mangalavana and the least at the light house area of Puthuvyppu 48 (5.71%) (Table 8).

White colour series

It was found that among 676 actinomycetes recorded 278 turned into (41.12%) white coloured actinomycete at Mangalavana followed by 154 (22.78%) at the light house area of Puthuvyppu, 133 (19.68%) at Narakkal and the minimum number was encountered 111 (16.42%) at Puthuvyppu (Table 8).

Red colour series

Among 70 red colour series of actinomycetes encountered [62 (88.57%)] maximum number of red coloured actinomycete were recorded at Narakkal, followed by 6 numbers (8.57%) at Mangalavana and the lowest number at Puthuvyppu 2 (0.29%). In the light house area of Puthuvyppu red coloured actinomycete was found to be completely absent (Table 8).

Orange colour series

Among 4 orange coloured actinomycetes observed during the period of study 3 (75%) were from Narakkal and 1 (25%) from Mangalavana and none were recorded in other two stations (Table 8).

Green colour series

Only one actinomycete of green colour was recorded at the light house area of Puthuvyppu during the period of study (Table 8).

Table 8. Spatial distribution of total actinomycetes in the study area based on the colour series

Station / Colour	I	II	III	IV	Total
White	278 (41.12%)	133 (19.68%)	111 (16.42%)	154 (22.78%)	676 (42.50%)
Grey	169 (20.12%)	428 (50.95%)	195 (23.21%)	48 (5.71%)	840 (52.80%)
Red	6 (8.57%)	62 (88.57%)	2 (0.29%)	0 (0.00%)	70 (4.40%)
Orange	1 (25.00%)	3 (75.00%)	0 (0.00%)	0 (0.00%)	4 (0.25%)
Green	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (100.00%)	1 (0.06%)
Total	454 (28.50%)	626 (39.35%)	308 (19.36%)	203 (12.76%)	1591 (100%)

c. Seasonal distribution of actinomycetes according to colour series

Monsoon

During the period of study in monsoon, grey coloured actinomycetes dominated 598 (62.62%) followed by white colour series 293 (30.68%), red coloured actinomycetes 62 (6.49%) and only 2 (0.21%) number of orange coloured actinomycetes were recorded. Green coloured actinomycetes was absent in monsoon during 1991 (Table 7).

In monsoon out of 598 grey colour actinomycetes recorded 290 (48.50%) was from Narakkal followed by 153 (25.59%) at Puthuvyppu, 132 (22.07%) at Mangalavana and only 23 numbers (3.85%) of actinomycetes of grey coloured was recorded at the light house area of Puthuvyppu. Whereas out of 293 actinomycetes encountered during monsoon white colour series dominated 140 (47.78%) at Mangalavana followed by 75 (25.60%) at Narakkal, 40 (13.65%) at Puthuvyppu and 38 (12.97%) in the light house area of Puthuvyppu. Among the 62 red coloured actinomycetes recorded in monsoon, it was found that 60 (96.77%) were observed at Narakkal and 2 (3.23%) from Mangalavana and in all other stations it was absent. Two orange coloured actinomycetes was recorded only at Narakkal. Green pigmented actinomycete was absent in all the four stations studied (Table 9).

Post Monsoon

In the post monsoon season white coloured actinomycetes dominated 318 (58.78%) followed by grey colour actinomycete 223 (41.12%). And the other 3 coloured actinomycetes (red, orange and green) were not recorded throughout the period of study in the post-monsoon season (Table 10).

Table 9. Distribution of total actinomycetes in the study area based on colour series during monsoon.

Colour / Station	White	Grey	Red	Orange	Green
I	140 (47.78%)	132 (22.07%)	2 (3.23%)	0 (0.00%)	0 (0.00%)
II	75 (25.60%)	290 (48.50%)	60 (96.77%)	2 (100.00%)	0 (0.00%)
III	40 (13.65%)	153 (25.59%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
IV	38 (12.97%)	23 (3.85%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Total	293	598	62	2	0

White coloured actinomycete dominated in the post-monsoon season, while studying the spatial distribution of actinomycete, grey coloured actinomycete was found to be predominant at Station II 137 (61.44%) followed by 112 nos. (35.22%) of white coloured actinomycetes at Mangalavana, 108 (33.96%) at Station IV, 52 (16.35%) at Station III, 46 (14.47%) at Puthuvyppu. Minimum numbers of grey coloured actinomycetes were recorded in the other three stations as 33 (14.80%) at Station I, 30 (13.45%) at Station III and 23 (10.3%) at Station IV. The other three colour series were not present in all the 4 studied stations in the post-monsoon season (Table 10).

Pre-monsoon

White coloured actinomycetes dominated 65 (68.42%) in the pre-monsoon season followed by grey coloured actinomycete 19 (20.00%) red coloured 8 (8.42%), orange coloured 2 numbers (2.11%) and only one (1.05%) green coloured actinomycete was recorded in pre-monsoon (Table 11).

Among 65 white coloured actinomycetes recorded in pre-monsoon, 26 (40.0%) were encountered from the sediment samples at Mangalavana and 12 (18.46%) from Narakkal, 19 (29.23%) from Puthuvyppu and 8 (12.31%) at the light house area of Puthuvyppu (Table 11).

Out of 19 grey coloured actinomycetes encountered maximum number [12 (63.16%)] were from Puthuvyppu followed by 4 (21.03%) at Mangalavana and 2 (10.33%) at the light house area of Puthuvyppu. And only one (5.26%) grey coloured actinomycete was recorded from Narakkal during pre-monsoon (Table 11).

Table 10. Distribution of total actinomycetes in the study area based on colour series during post-monsoon.

Colour / Station	White	Grey	Red	Orange	Green
I	112 (35.22%)	33 (14.80%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
II	46 (14.47%)	137 (61.44%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
III	52 (16.35%)	30 (13.45%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
IV	108 (33.96%)	23 (10.31%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Total	318	223	0	0	0

Table 11. Distribution of total actinomycetes in the study area based on colour series during pre-monsoon

Colour / Station	White	Grey	Red	Orange	Green
I	26 (40.00%)	4 (21.05%)	4 (50.00%)	1 (50.00%)	0 (0.00%)
II	12 (18.46%)	1 (5.26%)	1 (25.00%)	1 (50.00%)	0 (0.00%)
III	19 (29.23%)	12 (63.16%)	2 (25.00%)	0 (0.00%)	0 (0.00%)
IV	8 (12.31%)	2 (10.53%)	0 (0.00%)	0 (0.00%)	1 (100%)
Total	65	19	8	2	1

During pre-monsoon 8 red coloured actinomycetes were encountered, out of which 4 (50%) were from Mangalavana and 2 were (25%) from Narakkal and 2 (25%) from Puthuvyppu, samples were devoid of red colour series at Station IV (Table 11).

Two orange coloured actinomycetes one at Mangalavana and another at Narakkal were recorded in the pre-monsoon. And only one green pigmented actinomycete was recorded throughout the study period which was encountered from the sediment samples of the light house area of Puthuvyppu in the premonsoon season (Table 11).

B. **Distribution of the isolated actinomycetes**

Among the 1591 actinomycetes encountered from the sediment samples of 4 fixed stations during the period of study, only 104 were isolated based on their morphological appearance and aerial mass colour. 26 (25%) cultures were isolated from Mangalavana 26 (25%) from Narakkal, 27 (25.96%) from Puthuvyppu and 25 (24.04%) from the light house area of Puthuvyppu (Table 12).

a. **Seasonal distribution of the isolated cultures of actinomycetes**

Out of 104 cultures maximum number of actinomycetes were isolated during pre-monsoon 55 (52.89%) followed by monsoon 27 (25.96%) and 22 (21.15%) in the post-monsoon season (Table 12).

Pre-monsoon

Out of 55 cultures isolated in the pre-monsoon season 17 cultures (30.90%) were from Mangalavana, 13 (23.64%) from Narakkal, 15 (27.27%)

Table 12. Seasonal distribution of isolated actinomycetes (104 Nos.) in the study area.

Station / Season	I	II	III	IV	Total
Pre-monsoon	17 (30.90%)	13 (23.64%)	15 (27.27%)	10 (18.18%)	55 (52.89%)
Monsoon	4 (14.82%)	8 (29.63%)	3 (11.11%)	12 (44.44%)	27 (25.96%)
Post-monsoon	5 (22.73%)	5 (22.73%)	9 (40.90%)	3 (13.64%)	22 (21.15%)
Total	26 (25.00%)	26 (25.00%)	27 (25.96%)	25 (24.04%)	104 (100%)

from Puthuvyppu and only 10 (18.18%) were isolated from the light house area of Puthuvyppu (Table 12).

Monsoon

In the monsoon period among 27 actinomycetes encountered, maximum number 12 (14.44%) actinomycetes were isolated from the sediment samples of the light house area of Puthuvyppu, followed by 8 (29.63%) cultures from Narakkal and 4 (14.82%), 3 (11.11%) actinomycetes were isolated from Mangalavana and Puthuvyppu respectively (Table 12).

Post-monsoon

Out of 22 cultures maintained maximum number [9 (40.9%)] of actinomycetes were isolated from Puthuvyppu area, followed by Mangalavana and Narakkal where 5 (22.73%) were isolated from each of these stations whereas only 3 (13.64%) were isolated from the light house area of Puthuvyppu during the period of study (Table 12).

b. **Distribution of the isolated actinomycetes according to their colour series**

Out of 1591 actinomycetes encountered during the period of study, 104 were isolated and maintained for further studies. Among these 104 cultures 5 colour series were observed namely white, grey, red, orange and green. White colour series dominated (where 60 (57.69%) numbers were isolated) followed by grey coloured actinomycetes 29 (27.89%), red colour 10 (9.62%), orange 4 (3.85%) and only one (0.96%) green coloured actinomycete was isolated and maintained (Table 13).

Total 13. Spatial distribution of isolated actinomycetes (104 Nos.) in the study area based on the colour series.

Station / Colour	I	II	III	IV	Total
White	15 (25.00%)	16 (26.67%)	17 (28.33%)	12 (2.00%)	60 (57.69%)
Grey	7 (24.14%)	5 (17.24%)	5 (17.24%)	12 (41.38%)	29 (27.89%)
Red	4 (40.00%)	3 (30.00%)	2 (20.00%)	1 (10.00%)	10 (9.62%)
Orange	0 (0.00%)	2 (50.00%)	2 (50.00%)	0 (0.00%)	4 (3.85%)
Green	0 (0.00%)	0 (0.00%)	1 (100%)	0 (0.00%)	1 (0.96%)
Total	26 (25.00%)	26 (25.00%)	27 (25.96%)	25 (24.04%)	104 (100%)

Among 60 white coloured actinomycetes isolated during the period of study, maximum 17 (28.33%) number of isolates were recorded from the sediment samples of Puthuvyppu, followed by Narakkal 16 (26.67%), Mangalavana 15 (25%) and 12 (2.0%) from the light house area of Puthuvyppu (Table 13).

Twelve (41.38%) grey coloured actinomycetes were isolated from the light house area of Puthuvyppu, 7 (24.14%) from Mangalavana and 5 (17.24%) from Station II and III, out of 29 cultures isolated during the period of study (Table 13).

Out of 10 red coloured actinomycetes isolated 4 (40%) were from Mangalavana, 3 (30%) from Narakkal, 2 (20%) from Puthuvyppu and one (10%) from the light house area of Puthuvyppu (Table 13).

Only four orange coloured actinomycetes were isolated, 2 (50%) from Station II and another 2 (50%) from Station III. And only one green coloured actinomycete was isolated from the light house area of Puthuvyppu (Table 13).

Seasonal distribution of actinomycetes according to colour series

Pre-monsoon

In the pre-monsoon season out of 55 isolated actinomycetes, the maximum number 35 (63.64%) were of white colour series followed by grey colour 11 (20.0%) red colour 8 (14.55%) and only one (1.8%) orange coloured actinomycete was isolated and no green coloured actinomycete was isolated in this season (Table 14).

Table 14. Seasonal distribution of isolated actinomycetes (104 Nos.) based on colour series.

Colour / Season	White	Grey	Red	Orange	Green	Total
Pre-monsoon	35 (63.64%)	11 (20.00%)	8 (14.55%)	1 (1.80%)	0 (0.00%)	55
Monsoon	9 (33.33%)	16 (59.26%)	1 (3.70%)	1 (3.70%)	0 (0.00%)	27
Post-monsoon	16 (72.73%)	2 (9.10%)	1 (4.55%)	2 (9.10%)	1 (4.55%)	22

Among 35 actinomycetes of white colour series isolated in the pre-monsoon season 10 (28.57%) were from Station I and 10 from III, 9 (25.71%) from Station II and 6 (17.14%) from Station IV. Out of 11 grey coloured actinomycetes, 4 (36.36%) were isolated from Station I, 3 (27.27%) from Station III and IV each and only one (9.09%) from Station II. Three (37.5%) red pigmented actinomycete was isolated from Mangalavana, 2 (25%) from Narakkal and Puthuvyppu and only one (12.5%) from the Station IV. Only one orange colour actinomycete was isolated from Station II and none in other stations (Table 15).

Monsoon

During monsoon 27 actinomycetes were isolated out of which 16 (59.26%) were of grey colour, 9 (33.33%) of white colour one red and one (3.7%) orange colour (Table 14).

Out of 9 white coloured actinomycetes isolated during the monsoon season, one (11.11%) was from Mangalavana, 3 (33.33%) was from Station II and 2 (22.22%) from Station III and 3 (33.33%) from Station IV. Among 16 grey coloured actinomycetes maximum were isolated from Station IV [9 (56.25%)], followed by 4 (24.06%) from Station II, 2 (12.5%) from Station I and only one (6.25%) from Station III. Only one red and one orange coloured actinomycetes were isolated from Station I and II respectively (Table 16).

Post-monsoon

In the post-monsoon season 22 actinomycetes were isolated out of which 16 (72.73%) were of white colour 2 (9.1%) of grey, 2 (9.10%) of red and one (4.55%) red and one of green colour (Table 14).

Table 15. Distribution of isolated actinomycetes (104 Nos.) in the study area based on colour series during pre-monsoon.

Colour / Station	White	Grey	Red	Orange	Green
I	10 (28.57%)	4 (36.36%)	3 (37.50%)	0 (0.00%)	0 (0.00%)
II	9 (25.71%)	1 (9.09%)	2 (25.00%)	1 (100.00%)	0 (0.00%)
III	10 (28.57%)	3 (27.27%)	2 (25.00%)	0 (0.00%)	0 (0.00%)
IV	6 (17.14%)	3 (27.27%)	1 (12.50%)	0 (0.00%)	0 (0.00%)
Total	35	11	8	1	0

Table 16. Distribution of isolated actinomycetes (104 Nos.) in the study area based on colour series during monsoon.

Station / Colour	White	Grey	Red	Orange	Green
I	1 (11.11%)	2 (12.50%)	1 (100%)	0 (0.00%)	0 (0.00%)
II	3 (33.33%)	4 (25.00%)	0 (0.00%)	1 (100%)	0 (0.00%)
III	2 (22.22%)	1 (6.25%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
IV	3 (33.33%)	9 (56.25%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Total	9	16	1	1	0

Table 17. Distribution of isolated actinomycetes (104 Nos.) in the study area based on colour series during post-monsoon.

Colour / Station	White	Grey	Red	Orange	Green
I	4 (25.00%)	1 (50.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
II	4 (25.00%)	0 (0.00%)	1 (100.00%)	0 (0.00%)	0 (0.00%)
III	5 (31.25%)	1 (50.00%)	0 (0.00%)	2 (100.00%)	1 (100%)
IV	3 (18.75%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Total	16	2	1	2	1

Among 16 white coloured actinomycetes isolated 4 (25%) were from Station I, 4 (25%) from Station II, 5 (31.25%) from Station III and 3 (18.75%) from Station IV. Out of 2 grey coloured, one was isolated from Station I and another from Station III. One red coloured actinomycete was isolated from Station II. The 2 orange colour actinomycetes isolated were from Station III and the one isolate of green colour was from Station III (Table 17).

4. SODIUM CHLORIDE TOLERANCE OF ISOLATED ACTINOMYCETES

To study the sodium chloride tolerance of the isolated actinomycetes from the mangrove sediments, 5 concentrations of sodium chloride was incorporated into the medium (seawater agar). Along with this one control was also maintained for each of the actinomycete culture tested for sodium chloride tolerance in which only distilled water was added to prepare the media and neither seawater nor sodium chloride was added to this control media. Such a control was maintained to know whether actinomycetes grow even without the source of sodium chloride or seawater to identify their origin.

Out of 104 isolates tested for the sodium chloride tolerance, 72 (69.23%) cultures exhibited good growth at 0%, 29 (27.85%) showed moderate growth at 0%, only 3 isolates showed poor growth at 0% and none of the isolates tested showed negative result (no growth) at 0% (Table 19).

When 0.5% of sodium chloride was added to SWA and tested for sodium chloride tolerance, out of 104 cultures tested 53 isolates (50.96%) exhibited good growth, 46 (44.23%) moderately grew at 0.5%, and only 5 of the tested cultures showed poor growth at 0.5% and none of the isolates showed negative result.

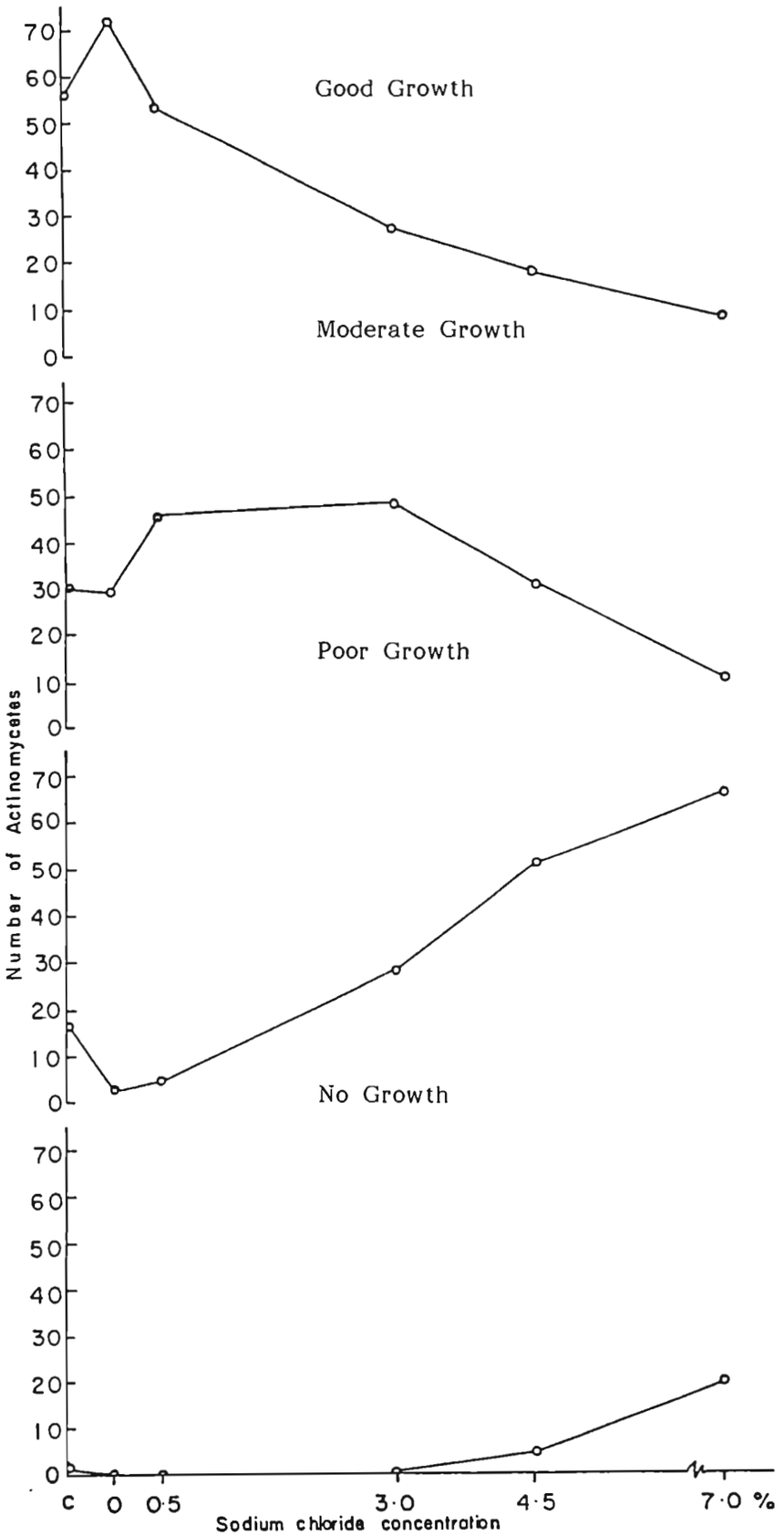


Fig. 7 Sodium chloride tolerance test for 104 isolated actinomycetes

Table 18. Growth pattern of sodium chloride tolerance of 104 isolated actinomycetes.

Strain No.	Control	0%	0.5%	3.0%	4.5%	7%
7	+	+	++	++	++	+
8	+++	+++	+++	+	+	+
9	+++	+++	+++	++	+	+
12	+++	++	++	+	+	-
22	-	++	++	+	+	+
23(1)	+++	+++	+++	++	++	+
23(2)	+++	+++	+++	++	++	+
23(A)	+++	+++	++	++	+	+
23(B)	+++	+++	+++	++	++	+
24	++	++	++	++	++	+
25	++	++	++	++	++	+
26A	+++	+++	++	++	++	+
26B	+++	+++	++	++	++	+
28	+++	++	+	+	+	+
28A	++	++	++	+	+	+
30	+++	+++	++	+	+	+
34	+++	+++	++	++	+	+
38	++	+++	++	++	+	+
45	++	+++	++	+	+	-
46	++	+++	++	++	+	+
47	++	++	++	+	+	+

Strain No.	Control	0%	0.5%	3.0%	4.5%	7%
48	++	++	++	++	+	+
52	+++	++	++	+	+	+
64	+	++	++	++	++	++
64A	+++	+++	++	++	+	-
68	+++	+++	+++	+++	++	++
70	++	+++	+++	++	++	+
73	+++	+++	+++	++	+	+
74A	++	+++	+	+	+	-
74B	+	++	++	+	+	+
75	+	+++	+++	++	+	+
76	+++	++	++	++	++	++
77	+++	++	++	++	-	-
78	+++	++	++	+	+	+
79	+++	+++	+++	+++	+++	++
81	++	+++	+++	+++	+++	++
82	++	+++	+++	+++	+++	+++
83	++	+++	+++	++	++	+
84	+++	++	++	++	++	+
87	+	++	++	+	+	+
88	+++	+++	+++	+++	+	-
89	++	+++	+++	++	++	-
91	+++	+++	++	+	+	+
92A	++	+++	+++	+++	+++	+++

Strain No.	Control	0%	0.5%	3.0%	4.5%	7%
92B	+	+++	+++	+	+	+
93A	+++	+++	+++	+++	+++	++
93B	+++	+++	+++	+++	++	++
94	++	+++	++	++	++	+
95	+++	++	+++	++	++	++
98	++	++	++	++	+	+
101A	++	++	++	++	+	+
101B	+++	+++	+++	++	++	+
103	+++	+++	+++	+++	++	+
103A	+++	+++	+++	+++	+++	+
103B	++	+++	+++	+++	+++	+
104	+++	+++	+++	+	+	+
104B	++	+++	++	+	+	+
105	+++	+++	+++	-	-	-
106A	+++	+++	+++	+++	+++	++
106B	++	+++	+++	+++	++	+
107	+++	+++	+++	++	+	+
108	+++	+++	+++	+++	+++	+
108A	+++	+++	+++	++	++	+
110	+++	+++	+++	++	-	-
111	++	+++	++	++	++	+
129	+++	++	++	++	++	+
130	++	++	++	++	+	+
104A	+++	++	+++	++	++	++

Strain No.	Control	0%	0.5%	3.0%	4.5%	7.0%
134	+	++	++	++	++	-
135	+++	+++	+++	++	+	+
140	+	+++	+++	++	++	+
142	+	+++	+++	+	+	+
148	+	+++	+++	+++	++	+
151	+	+	+	+	+	-
158	+	+++	+++	+++	+	-
158A	+	+++	+++	++	+	+
159	+++	+++	++	+	+	-
159A	+++	++	++	++	+	-
160	+++	++	+	+	+	+
161	+++	++	+++	+++	+++	+
162	+++	+++	++	++	+	-
163	++	++	++	++	++	+
164	++	+++	+++	+	+	-
165	+	++	++	+	+	-
168	+	++	++	+	+	+
171	+++	+++	++	+++	-	-
172	+	+++	+++	+++	+++	+++
174	-	+	+	+	-	-
175	++	+++	++	+	+	-
176	+++	+++	+++	++	+	+
186	+++	+++	+++	+++	+++	-
187	+++	+++	+++	+++	+++	+++

Strain No.	Control	0%	0.5%	3.0%	4.5%	7.0%
188	++	++	++	++	+	+
189	++	+++	+++	+	+	+
190	+++	+++	+++	+++	+++	++
191	+++	+++	++	++	++	+
192	+++	+++	+++	+++	+++	+++
193	+++	+++	+++	+++	+	+
194	+++	+++	+++	+++	+++	+++
195	++	+++	+++	++	++	+
199	+++	+++	++	++	+	+
200	+++	+++	+++	++	+	+
202	+++	+++	+++	+++	+++	+++
203	++	+++	+++	+++	++	+

(-) No Growth

(+) Poor Growth

(++) Moderate Growth

(+++) Good Growth

Table 19. Percentage of sodium chloride tolerance test of (104) isolated actinomycetes

Concentration of NaCl / Growth pattern	Control	0.0%	0.5%	3.0%	4.5%	7.0%
(-)	2 (1.92%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	5 (4.81%)	21 (20.19%)
(+)	16 (15.39%)	3 (2.85%)	5 (4.81%)	28 (26.92%)	51 (49.03%)	66 (63.35%)
(++)	30 (28.85%)	29 (27.85%)	46 (44.23%)	48 (46.15%)	30 (28.85%)	10 (9.62%)
(+++)	56 (53.85%)	72 (69.23%)	53 (50.96%)	27 (25.96%)	17 (16.35%)	7 (6.73%)

(-) No Growth
(+) Poor Growth
(++) Moderate Growth
(+++) Good Growth

When the sodium chloride concentration increased from 0% to 3.0% only 27 (25.96%) of 104 isolates tested showed good growth whereas 48 (46.15%) isolates exhibited moderate growth and 28 (26.92%) isolates showed poor growth and it was noted that none of the isolates gave negative result.

It was found that only 17 (16.35%) of the isolates could tolerate 4.5% concentration of sodium chloride and exhibited good growth, whereas 30 (28.85%) showed only moderate growth and 51 (49.03%) showed poor growth and 5 (4.81%) were not able to tolerate (4.51%) concentration of sodium chloride in the media.

At the higher NaCl concentration (7.0%) tested, only 7 (6.73%) isolates were able to exhibit good growth and 10 (9.62%) showed moderate growth, whereas more than 50% of the isolates showed poor growth 56 isolates (63.35%) and 21 (20.19%) were not able to tolerate the higher concentration of sodium chloride.

And in the control group where no sodium chloride and seawater was added to the medium, 56 (53.85%) of isolates were able to grow well and showed good growth, 30 (28.85%) showed moderate growth, 16 (15.39%) cultures showed poor growth and only 2 (1.92%) cultures were not able to grow in the absence of sodium chloride and seawater (Table 19) (Fig.7).

5. IDENTIFICATION OF ACTINOMYCETES

Among 104 cultures isolated during the period of study from the sediment samples of 4 fixed mangrove stations, 52 (50%) isolates were identified using ISP-methodology, on the basis of morphological and physiological

characteristics, upto species level. Out of 52 cultures identified, totally 34 different species were recorded, of which 18 were of genus Actinomyces and 34 of Streptomyces.

34 different species identified are as follows:

S. No.	Species composition	No. of isolates identified
1.	Actinomyces aureomonopodiales	- 2 -
2.	A. aureofasiculus	- 3 -
3.	A. aureocirculatus	- 2 -
4.	A. albovinaceus	- 1 -
5.	A. candidus	- 2 -
6.	A. flavescens	- 3 -
7.	A. griseomycini	- 1 -
8.	A. mutabilis	- 2 -
9.	A. umbrinus	- 1 -
10.	A. vilochromogenes	- 1 -
11.	Streptomyces alboniger	- 2 -
12.	S. albidoflavus	- 1 -
13.	S. cacaoi	- 2 -
14.	S. cinereoruber	- 1 -
15.	S. carnosus	- 1 -
16.	S. craterifer	- 3 -
17.	S. echinatus	- 1 -
18.	S. flavochromogenes	- 1 -
19.	S. galtieri	- 4 -
20.	S. gougeroti	- 3 -
21.	S. griseolavendus	- 1 -

22.	S. indigoferus	- 1 -
23.	S. mirabilis	- 1 -
24.	S. nitrosporeus	- 1 -
25.	S. noblis	- 1 -
26.	S. orientalis	- 2 -
27.	S. pyridomyceticus	- 1 -
28.	S. roseus	- 1 -
29.	S. rishiriensis	- 1 -
30.	S. sindensis	- 1 -
31.	S. sclerotialus	- 1 -
32.	S. spadicis	- 1 -
33.	S. thermovulgaris	- 1 -
34.	S. vastus	- 1 -

A. **Strain description**

Strain 28 was identified as Actinomyces aureomonopodiales. Spore chain morphology was found to be Rectiflexibile (RF) type. Mature spore chains were short with 3 to 10 spores per chain formed open loops and imperfect spirals of 1 to 3 turns (Plate 1.a). Sporulating aerial mycelium was absent on iron starch salts agar (ISSA). Spore surface was of smooth type (Plate 1.b). Aerial mass colour was in the white colour series on oatmeal agar (OMA) and glucose asparagine agar (GAA). Brownish black colour was noticed on the reverse side of the colony on OMA and GAA, and no distinct pigment was recorded on ISSA. Melanoid pigments was formed in tryptone-yeast broth (TYB) and tyrosine agar (TA). No pigment was found in the medium in OMA, GAA or ISSA. D-glucose, L-arabinose,

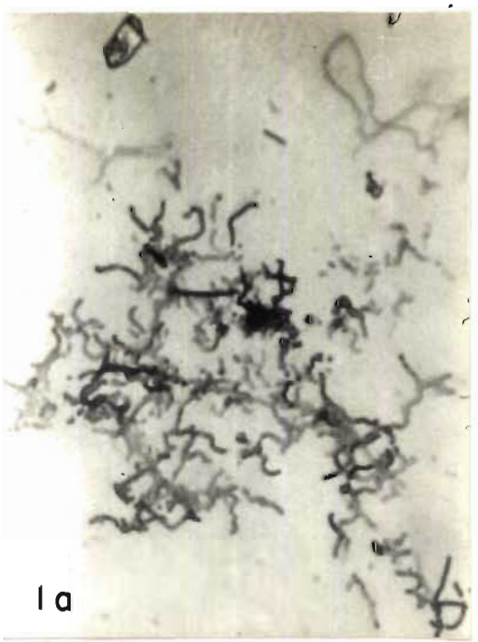
MORPHOLOGY OF ACTINOMYCETES

Sporophore morphology by light microscope (LM)

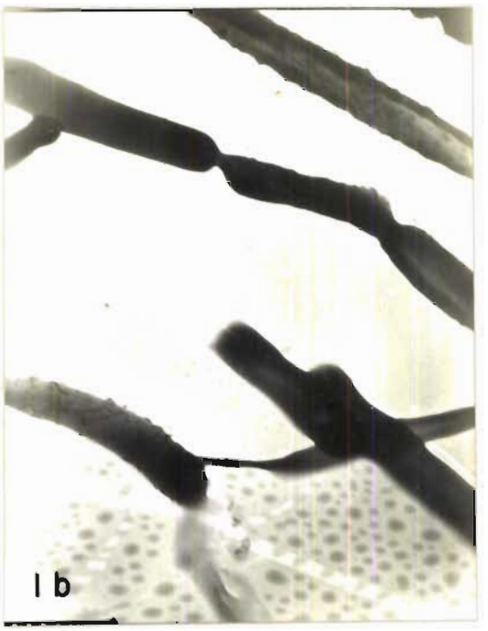
Spore morphology by electron microscope (EM)

Plate 1	Strain No.28				
1.a	RF spore chains	x	400	(LM)
1.b	Smooth spores	x	15,000	(EM)

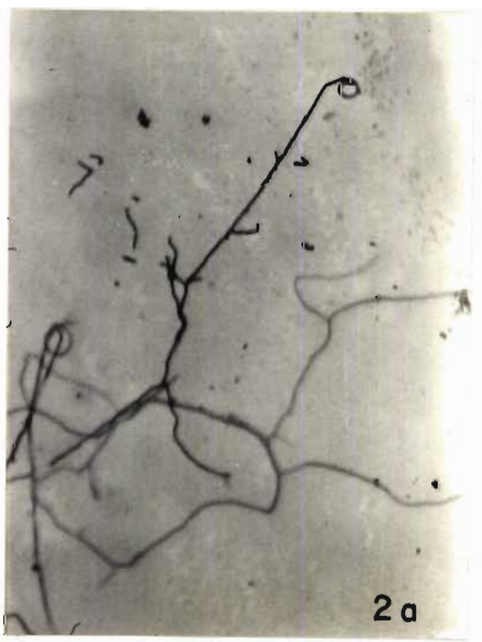
Plate 2	Strain No.79			
2.a	RF spore chains	x	200	(LM)
2.b	Smooth spores	x	5,000	(EM)



1a



1b



2a

2b

i-inositol, D-mannitol, D-fructose and raffinose were the sugars utilised for growth, only a trace of growth was seen with xylose and no growth with rhamnose or sucrose. The strain was antagonistic towards filamentous and non-filamentous fungi tested.

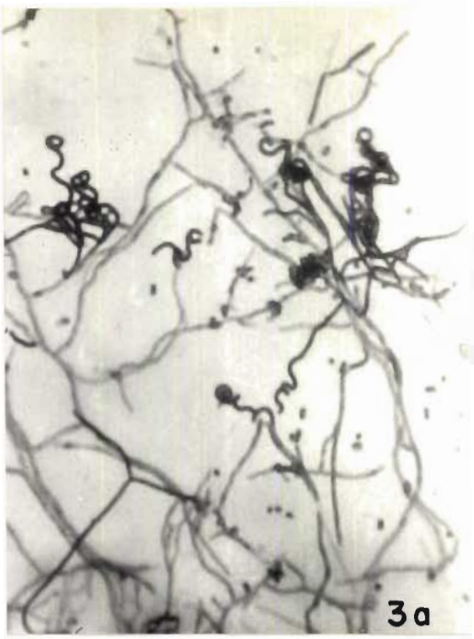
Strain 79 was also identified as A. aureomonopodiales. Yellowish brown pigment was noticed on the reverse side of colony. Trace growth was seen with sucrose and rhamnose, all other sugars tested were utilised for growth. Other characters studied were similar to that of Strain 28.

Strain 38 was identified as A. aureofasiculus. Spore chain morphology was found to be Rectiflexible type. Mature spore chains when present were generally short, with extended spiral of small diameter and open loops (Plate 3.a). Spore surface was of smooth type (Plate 3.b). Aerial mass colour was found to be in the white colour series, which was observed in OMA. Aerial mass colour was absent in GAA. In ISSA dull white coloured aerial mass growth was noticed. Reddish orange colour was noticed on the reverse side colony on ISSA, but not on OMA or GAA. The reddish orange pigment observed on ISSA was not a pH indicator. Melanoid pigments was formed in (PYIA) peptone-yeast iron agar and TYB, but not in TA. No pigment was found in OMA or GAA. D-glucose, L-arabinose, D-xylose, i-inositol, D-mannitol, D-fructose, rhamnose and raffinose were the carbon sources utilised for growth. But only a trace of growth was found with sucrose. The culture was active against filamentous fungi tested.

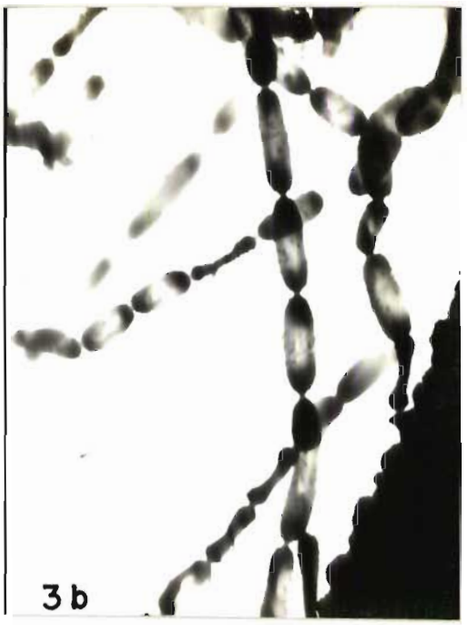
Strain 76 was also identified as A. aureofasiculus. All the characters studied were similar to that of Strain 38, except the spore chain morphology

Plate 3		Strain No.38			
	3.a	RF spore chains	x	400	(LM)
	3.b	Smooth spores	x	10,000	(EM)

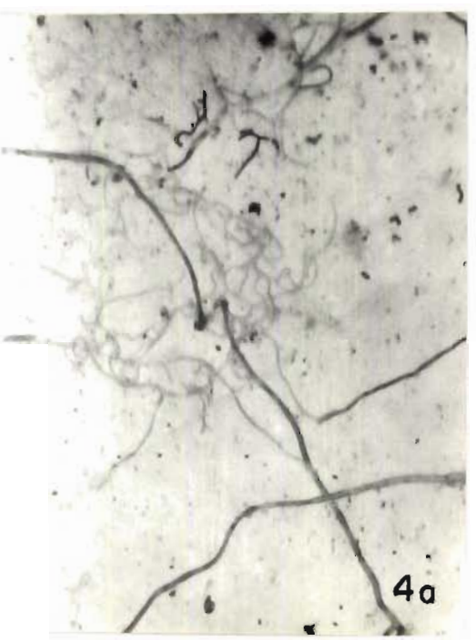
Plate 4		Strain No.76			
	4.a	RF spore chains	x	400	(LM)
	4.b	Smooth spores	x	10,000	(EM)



3a



3b



4a

4b

(Plate 4.a), (straight to flexuous) and it was antagonistic towards both filamentous and non-filamentous fungi tested.

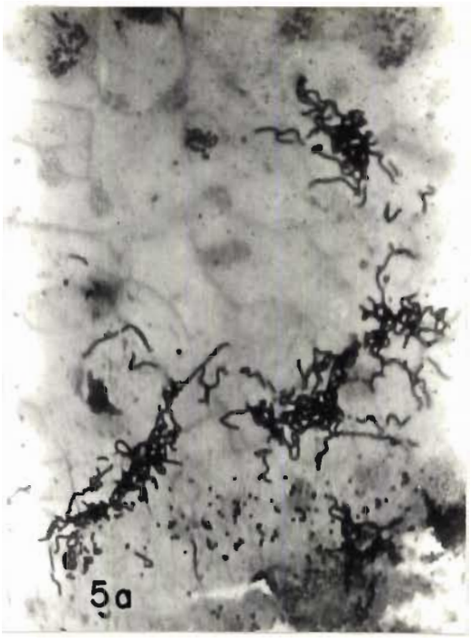
Strain 140 was also identified as A. aureofasiculus. This culture also exhibited straight to flexuous spore chain morphology (Plate 5.a). Pale yellowish brown to deep brown pigment was noticed on the reverse side of colony unlike strain 76 and 140. The strain was active against gram-negative bacteria (only against V. parahaemolyticus), filamentous and non-filamentous fungi tested.

Strain 78 was identified as A. aureocirculatus. Spore chain morphology was Rectiflexible type. Straight to flexuous spore chains with open loops were seen. Mature spores were long with more than 10 spores per chain (Plate 6.a). Spore surface was of smooth walled type (Plate 6.b). Aerial mass colour was in the white colour series. But scanty aerial mycelium development was found on OMA. And sporulating aerial mycelium was inadequate for determination of aerial mass colour on ISSA. No distinctive pigments were found on the reverse side of colony on OMA, GAA or ISSA. Melanoid pigments was not formed in PYIA, TA or TYB. D-glucose, D-xylose, D-mannitol, and D-fructose were utilised for growth. Trace growth was found in L-arabinose, i-inositol, rhamnose, sucrose and raffinose. The strain was active against filamentous and non-filamentous fungi tested in the present observation.

Strain 92A was also identified as A. aureocirculatus. All the characters were similar to that of Strain 78 except for carbon utilisation and its antagonistic property. D-glucose, L-arabinose, D-xylose, i-inositol, D-

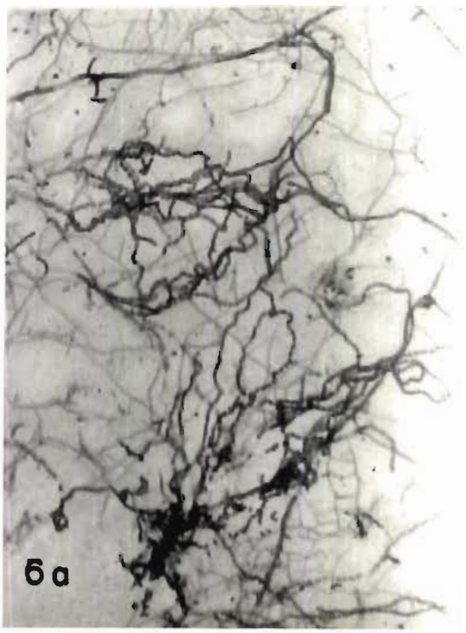
Plate 5		Strain No.140			
	5.a	RF spore chains	x	200	(LM)
	5.b	Smooth Spores	x	15,000	(EM)

Plate 6		Strain No.78			
	6.a	RF spore chains	x	200	(LM)
	6.b	Smooth spores	x	20,000	(EM)



5a

5b



6a

6b

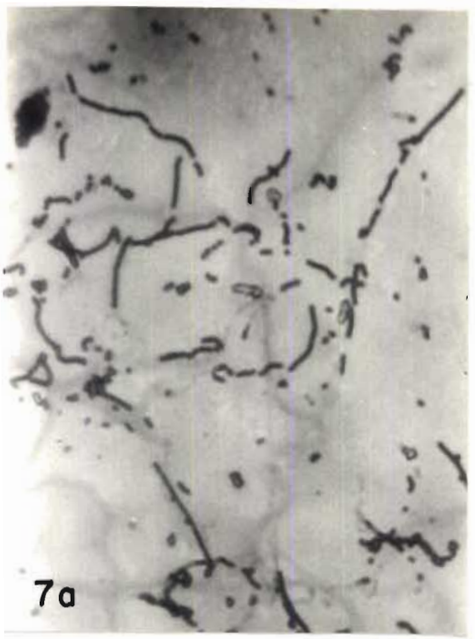
mannitol, D-fructose and rhamnose were utilised for growth and only trace growth was seen with sucrose and raffinose. The strain was active against bacteria (both gram positive and gram negative bacteria) also fungi (both filamentous and non-filamentous fungi).

Strain 201 was identified as A. albovinaceus. Spore chain morphology was of Rectiflexible type. Spore chains were straight to moderately flexous, with open loops. Mature spore chains were short with 3 or more spores per chain (Plate 8.a). Spore surface was of smooth type (Plate 8.b). Aerial mass colour was in the white colour series on OMA, GAA, and ISSA. Faint brown coloured pigment was noticed on the reverse side colony of OMA, GAA and ISSA. The pigment was not pH indicator. Melanoid pigments was not formed in TYB, TA or PYIA. No pigment was found in the medium on OMA, GAA or ISSA. D-glucose, L-arabinose, D-xylose and D-mannose were the carbon sources utilised for growth. Only trace of growth was seen with D-fructose, rhamnose and raffinose and no growth with i-inositol or sucrose. The strain was antagonistic towards filamentous and non-filamentous fungi tested.

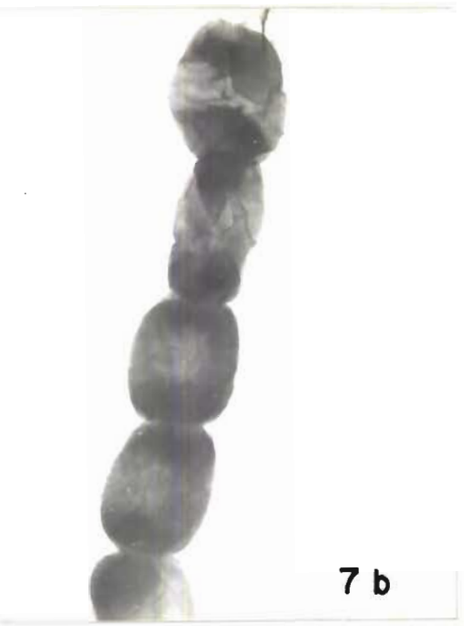
Strain 8 was identified as A. candidus. Spore chain morphology was of Rectiflexible type. Spore chains were long and moderately flexuous, mature spore chain with about 10 to 50 spores per chain (Plate 9.a). Aerial mycelium was poorly developed in ISSA. Spore surface was of smooth walled type (Plate 9.b). Aerial mass colour was in the white colour series on OMA and GAA. No distinctive pigments (light yellow on OMA and GAA)

Plate 7	Strain No.92A				
7.a	RF spore chains	x	400	(LM)
7.b	Smooth spores	x	19,000	(EM)

Plate 8	Strain No.201			
8.a	RF spore chains	x	200	(LM)
8.b	Smooth spores	x	19,000	(EM)

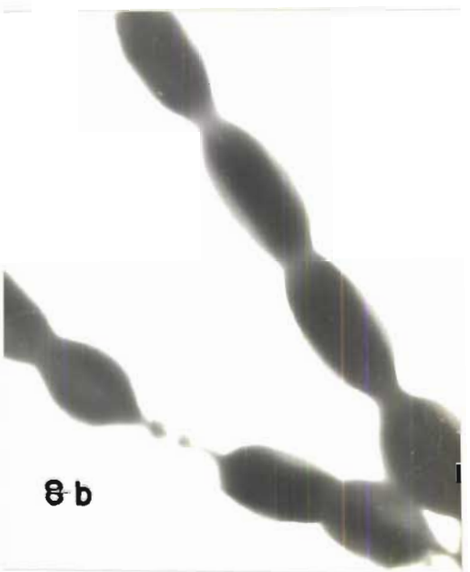


7a



7 b

8a



8 b

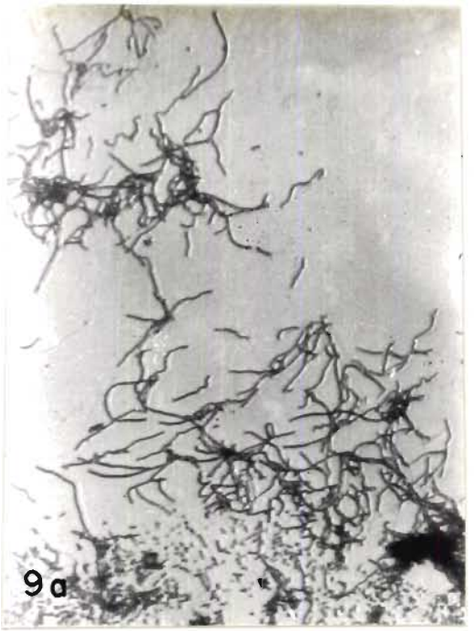
on OMA, GAA or ISSA. Melanoid pigments was not formed on PYIA, TA or TYB. No pigments were found in the medium on OMA, GAA or ISSA. D-glucose, L-arabinose, D-xylose, D-mannitol were utilised for growth. Only a trace of growth was found on i-inositol, rhamnose, sucrose and raffinose, and no growth was noticed with D-fructose. The culture was active against gram-negative bacteria, filamentous and non-filamentous fungi tested.

Strain 70 was also identified as A. candidus. Mature spore chains of this culture was moderately short with 3 to 10 spores per chain. Spore chains were slightly flexuous with short hooks, open loops and compact imperfect spirals with 2 turns per chain (Plate 10.a). Apart from D-glucose, L-arabinose, D-xylose, D-mannitol, rhamnose was also utilised for growth. Trace of growth was seen with i-inositol, D-fructose and sucrose and no growth was seen with raffinose. The culture was antagonistic only towards fungi and not towards bacteria. All the other morphological and physiological characters were similar to that of strain 8.

Strain 12 was identified as A. flavescens. Spore chain morphology was of RAS type. Open spirals with 3 or more turns, strongly flexuous spore chains and chains terminating with hooks (Plate 11.a). Mature spore chains was observed with about 50 spores per chain. Spore surface was of smooth walled type (Plate 11.b). Aerial mass colour in the white colour series on OMA, GAA and ISSA. On reverse side of colony, light yellowish brown pigment was noticed on OMA, GAA but not on ISSA. Melanoid pigment was not formed in PYIA, TA or TYB. Yellowish brown pigment was found in the medium on OMA and GAA. This was not pH sensitive when

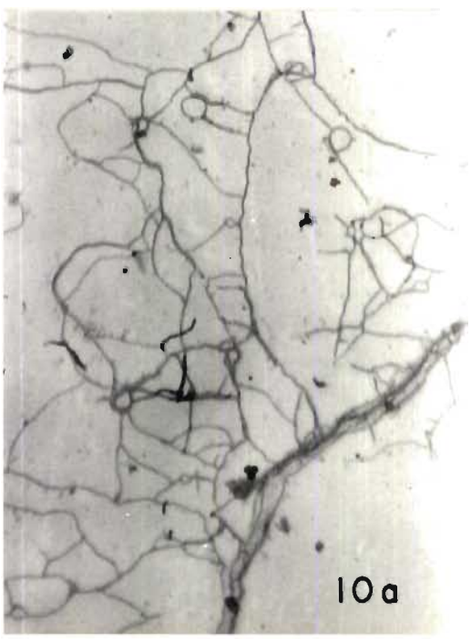
Plate 9		Strain No.8			
	9.a	RF spore chains	x	200	(LM)
	9.b	Smooth spores	x	8,000	(EM)

Plate 10		Strain No.70			
	10.a	RF spore chains	x	200	(LM)
	10.b	Smooth spores	x	19,000	(EM)



9a

9b



10a

10b

tested with 0.05 N NaOH or HCl. D-glucose, L-arabinose, were utilised for growth. Only a trace of growth was seen in i-inositol, D-mannitol, D-fructose, sucrose and raffinose. No growth was seen with rhamnose or D-xylose. Active against filamentous and non-filamentous fungi tested.

Strain 92B was also identified as A. flavescens. It differed from that of Strain 12 in carbon utilisation. Trace of growth was seen with D-xylose, D-fructose, sucrose and raffinose. Whereas all other sugars were utilised for its growth. Antagonistic towards both gram-positive and gram-negative bacteria, filamentous and non-filamentous fungi tested.

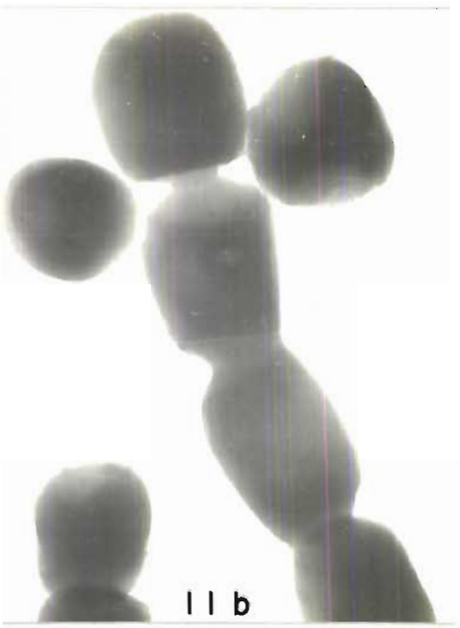
Strain 190 was also identified as A. flavescens. D-glucose and L-arabinose were utilised for growth, only trace of growth was noted with other sugars tested. The strain was active against filamentous fungi. All the other characters were similar to that of strain 12.

Strain 26A was identified as A. griseomycini. Spore chain morphology was of Retinaculiaperti, Spirales (RAS) type, hooks and loops with small diameter (Plate 14.a). Mature spore chains were short with 3 to 10 spores per chain. Spore surface were of smooth type (Plate 14.b). Aerial mass colour was in the green colour series on OMA, but white greyish green on GAA. Aerial mycelium was absent on ISSA. Faint grey to yellow colour pigment was seen on the reverse side colony on OMA and GAA. Substrate pigment was not a pH indicator. Melanoid pigments was formed in TA, TYB and PYIA. No pigment was found in the medium on OMA, GAA or ISSA. D-glucose, L-arabinose, i-inositol, D-fructose, rhamnose and raffinose were utilised for growth. Only trace of growth was seen with D-xylose

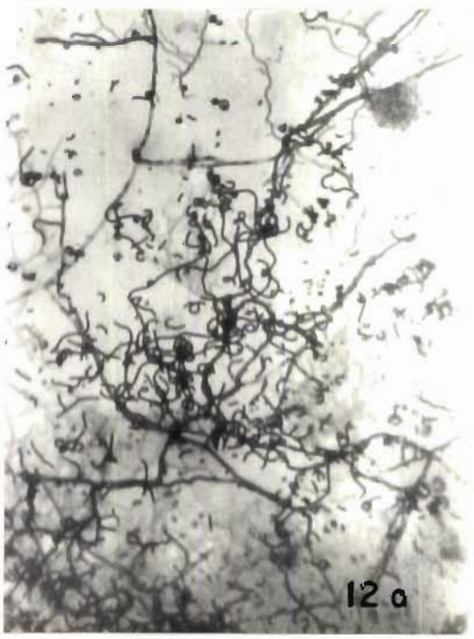
Plate	**11**	Strain No.12			
	11.a	RAS spore chains	x	200	(LM)
	11.b	Smooth spores	x	25,000	(EM)

Plate	**12**	Strain No.92B			
	12.a	RAS spore chains	x	200	(LM)
	12.b	Smooth spores	x	5,000	(EM)

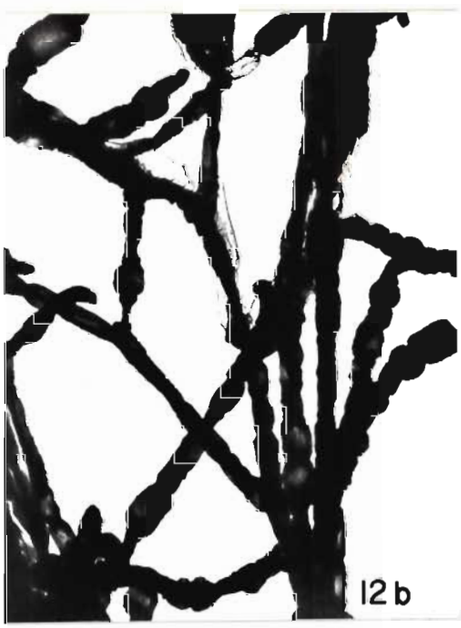
11a



11b



12a



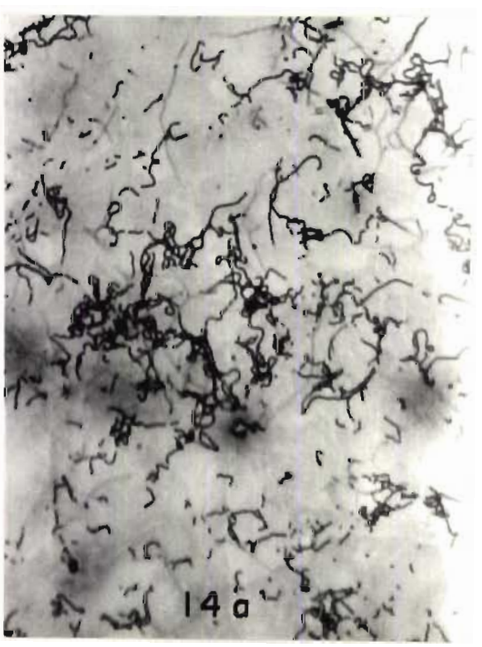
12b

Plate	**13**	Strain No.190			
	13.a	RAS spore chains	x	200	(LM)
	13.b	Smooth spores	x	25,000	(EM)

Plate	**14**	Strain No.26A			
	14.a	RAS spore chains	x	200	(LM)
	14.b	Smooth spores	x	19,000	(EM)

13a

13 b



14 a

14 b

and no growth with sucrose. The culture was active against gram-negative bacteria, filamentous and non-filamentous fungi tested.

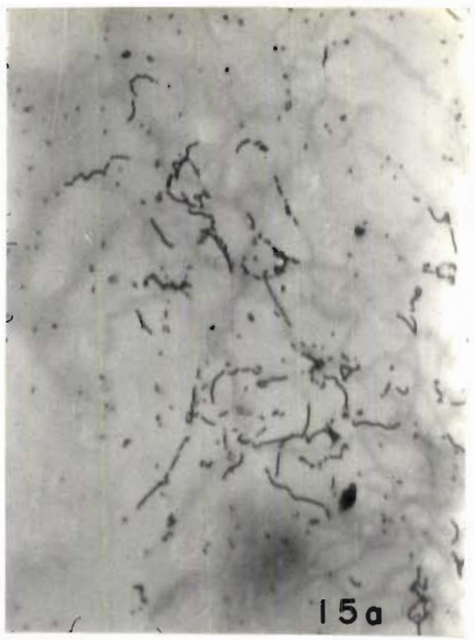
Strain 200 was identified as A. mutabilis. Spore chain morphology was found to be of Retinaculiaperti, Spirales (RAS) type. Spore chains were straight to flexuous compact spirales with small diameter, of about 2 turns per chain, with hooks and open loops (Plate 15.a). Mature spore chains were short with 3 to 10 spores per chain. Spore surface was of smooth type (Plate 15.b). Aerial mass colour was in the white colour series on OMA and GAA. Aerial mycelium was absent on ISSA. No distinctive pigments were found on the reverse side of colony on OMA, GAA or ISSA. Melanoid pigments was not formed on TA, PYIA or on TYB. No pigments were found in the medium on OMA, GAA or ISSA. D-glucose, L-arabinose, D-xylose, i-inositol and D-fructose were utilised for growth. Only a trace of growth were seen with D-mannitol, rhamnose, sucrose and raffinose. Active against filamentous and non-filamentous fungi tested.

Strain 202 was also identified as A. mutabilis. Carbon utilisation and antagonistic activity differed from that of Strain 200. And all the other characters were similar to Strain 200. D-glucose, L-arabinose, D-xylose, i-inositol, D-mannitol, D-fructose and rhamnose were utilised for growth. Only a trace of growth was seen with sucrose and no growth with raffinose. Its antagonistic property was similar to that of Strain 200.

Strain 23A was identified as A. umbrinus. Spore chain morphology was found to be Rectiflexible type (Plate 17.a). Flexuous, mature spore chains were moderately long with about 10 spores per chain. Spore surface

Plate 15		Strain No.200			
	15.a	RAS spore chains	x	200	(LM)
	15.b	Smooth spores	x	5,000	(EM)

Plate 16		Strain No.202			
	16.a	RAS spore chains	x	200	(LM)
	16.b	Smooth spores	x	19,000	(EM)



15a

15b

16b

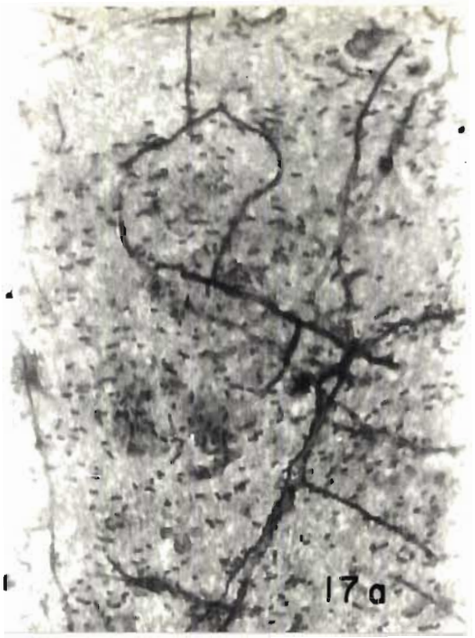
16b

was of smooth type (Plate 17.b). Aerial mass colour was in the red colour series on OMA, GAA and ISSA. Deep reddish brown pigment was noted on the reverse side colony on OMA, GAA and ISSA. The pigment was not a pH indicator. Melanoid pigment was formed in TYB, TA and PYIA. Reddish brown pigment was found in the medium on OMA, GAA and ISSA. The pigment was not pH sensitive when tested with 0.5 N NaOH or HCl. D-glucose, L-arabinose, D-xylose, i-inositol, D-mannitol, D-fructose, rhamnose, sucrose and raffinose were utilised for growth. The culture was active against gram-negative bacteria, filamentous and non-filamentous fungi tested.

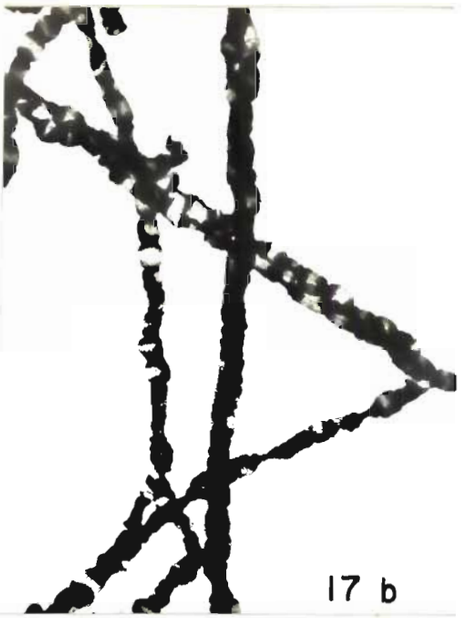
Strain 23.2 was identified as A. vilochromogenes. Spore chain morphology was of spirale type. Extended spirals with 1 or 2 turns, hooks were also seen (Plate 18.a). Mature spore chains were short with 3 to 10 or more spores per chain. Spore surface was of smooth type (Plate 18.b). Aerial mass colour was in the red colour series (Greyish pink on OMA, GAA and greyish red on ISSA). Reddish to deep brown colour pigment was found on the reverse side colony on OMA, GAA and ISSA. Reverse side pigment changed from reddish brown to pale yellowish brown with addition of 0.05 N HCl. Melanoid pigments was formed in TA, PYIA and TYB. Deep yellowish brown pigment was found in the medium on OMA, GAA and ISSA. The pigment was slightly pH sensitive, changing from deep yellowish brown to yellow with addition of 0.05 N HCl. D-glucose, L-arabinose, D-xylose, i-inositol, D-mannitol, D-fructose, rhamnose, raffinose and sucrose were all utilised for growth. The culture was active against filamentous and non-filamentous fungi tested.

Plate	**17**	Strain No.23A			
	17.a	RF spore chains	x	200	(LM)
	17.b	Smooth spores	x	5,000	(EM)

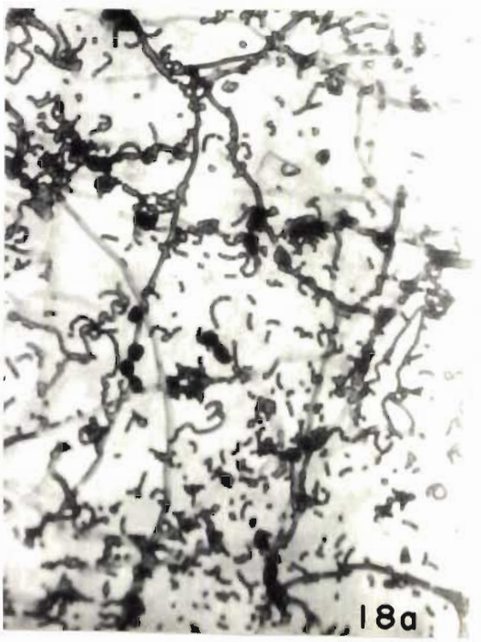
Plat	**18**	Strain No.23.2			
	18.a	Spirale spore chains	x	400	(LM)
	18.b	Smooth spores	x	19,000	(EM)



17 a



17 b



18a

18b

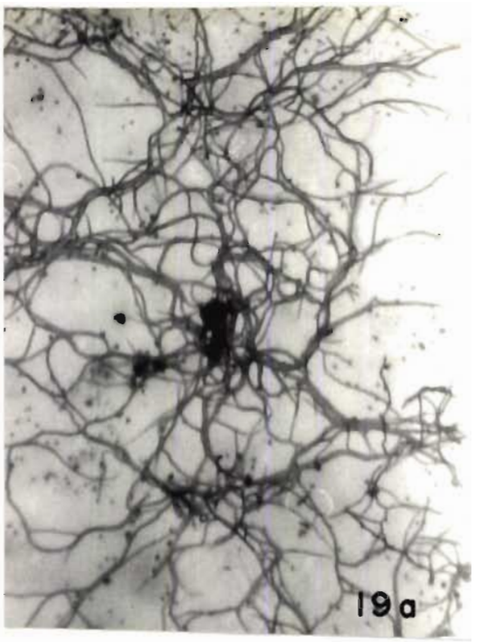
Strain 75 was identified as S. alboniger. Spore chain morphology was found to be Rectiflexible type. Mature spore chains were long with 10 to 50 spores per chain, and were strongly flexuous (Plate 19.a). Spore surface was of smooth type (Plate 19.b). Aerial mass colour was in the white colour series on OMA and GAA. and aerial mycelium was poorly developed on ISSA. No distinctive pigments on the reverse side colony of OMA, GAA or on ISSA were observed. Melanoid pigments was not formed (but light yellowish brown pigment on OMA) in TA, PYIA or TYB. D-glucose, L-arabinose, i-inositol, D-mannitol and raffinose were utilised for growth. Only trace of growth was seen with D-xylose, rhamnose and sucrose, and no growth with D-fructose. The culture was active against gram-negative bacteria (Aeromonas) and filamentous fungi tested.

Strain 77 was also identified as S. alboniger. Spore chain morphology, spore surface, colour of colony, reverse side of colony, colour in medium were similar to that of strain 75. The brown pigment found in the medium on OMA and GAA changed from brown to pale brown to yellow on addition with 0.05 N HCl and NaOH. Carbon utilisation, slightly differed from strain 75. D-glucose, L-arabinose, i-inositol, D-mannitol were utilised for growth. Only trace of growth was seen with D-xylose and raffinose and no growth with D-fructose, rhamnose or sucrose. And the strain was active against gram-negative bacteria, filamentous and non-filamentous fungi tested.

Strain 82 was identified as S. albidoflavus. Spore chain morphology was of Rectiflexible type (Plate 21.a). Straight to flexuous, mature spore chains, when formed were short with 3 to 10 spores per chain. Spore surface was of smooth type (Plate 21.b). Aerial mass colour was in the white

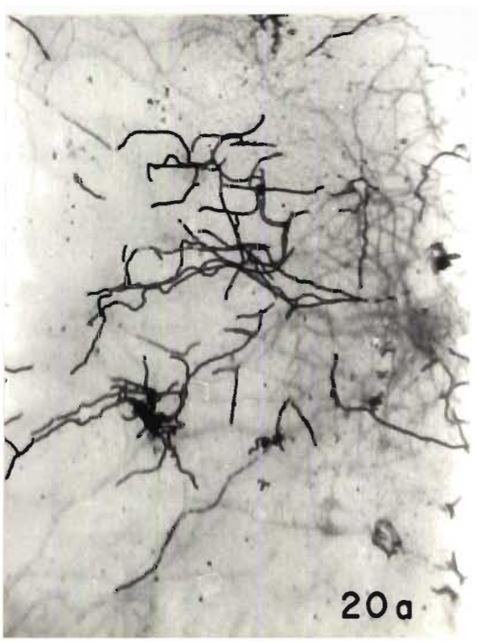
Plate 19		Strain No.75			
	19.a	RF spore chains	x	200	(LM)
	19.b	Smooth spores	x	19,000	(EM)

Plate 20		Strain No.77			
	20.a	RF spore chains	x	200	(LM)
	20.b	Smooth spores	x	19,000	(EM)

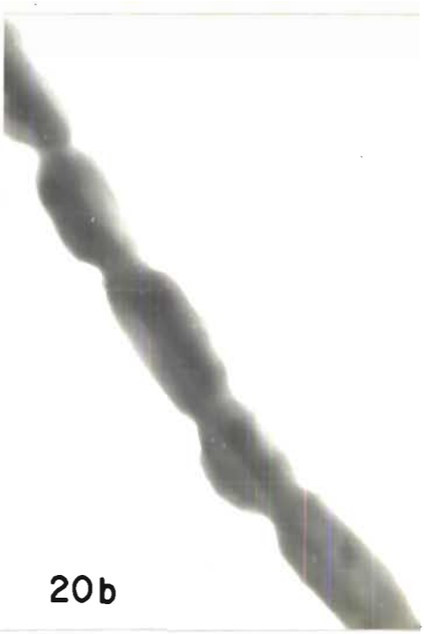


19a

19 b



20a



20b

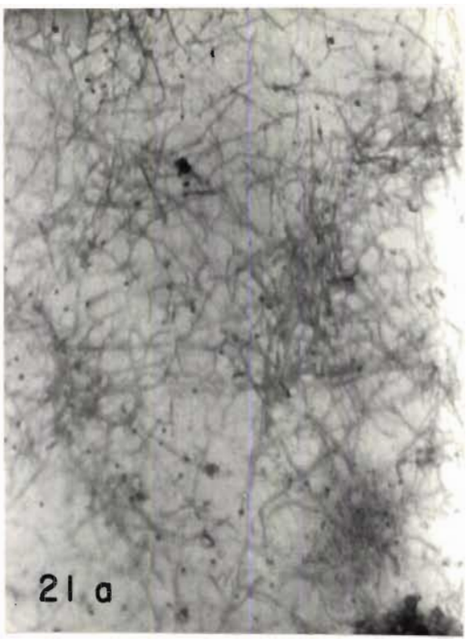
colour series on OMA. No distinctive pigments on the reverse side of colony on any of the medium was noted. Melanoid pigments was not formed in PYIA, TA or TYB. No soluble pigment was found in any of the medium. D-glucose, L-arabinose, D-xylose, D-mannitol and D-fructose were utilised for growth. Only slight growth in rhamnose and no growth with i-inositol, sucrose and raffinose. The culture was active against filamentous and non-filamentous fungi tested.

Strain 30 was identified as S. cacaoi. Spore chain morphology was of spirale type (Plate 22.a). Spirals were compact and long with 2 to 4 turns per chains with open loops. Mature spore chains are long with 10 to 50 spores per chain. Spore surface was smooth type (Plate 22.b). Aerial mass colour was in the white colour series on OMA. Aerial mycelium was poorly developed on GAA and was absent on ISSA. No distinctive pigments was seen on the reverse side of colony on OMA, GAA or ISSA. Melanoid pigment was not formed in PYIA, TA or TYB. No pigment was found in the medium on OMA, GAA or ISSA. D-glucose, L-arabinose, D-xylose, D-mannitol, D-fructose and raffinose were utilised for growth. Only a trace of growth was seen with sucrose and no growth with i-inositol and rhamnose. The culture was active against gram-negative bacteria, filamentous and non-filamentous fungi tested.

Strain 187 was also identified as S. cacaoi. All the characters were similar to that of strain 30, except for carbon utilisation. D-glucose, L-arabinose, D-xylose, D-mannitol and D-fructose were utilised for growth. Only a trace of growth was seen with i-inositol and raffinose and no growth with rhamnose and sucrose.

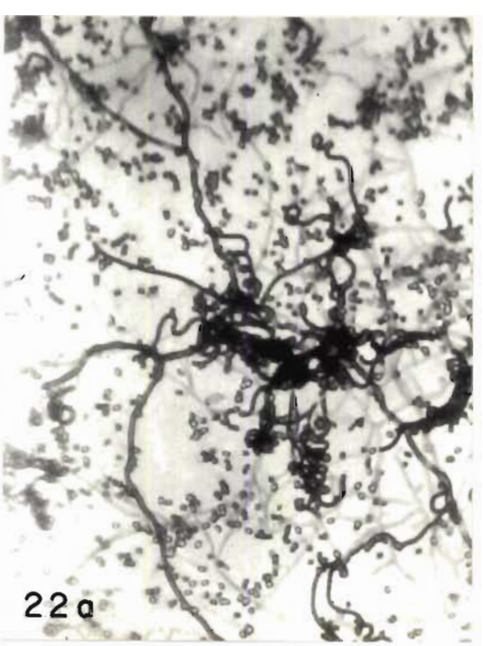
Plate 21	Strain No.82				
21.a	RF spore chains	x	200	(LM)
21.b	Smooth spores	x	5,000	(EM)

Plate 22	Strain No.30			
22.a	Spirale spore chains	x	400	(LM)
22.b	Smooth spores	x	6,000	(EM)



21 a

21 b



22a

22b

Strain 108 was identified as S. cinereoruber. Spore chain morphology was of Rectiflexible type (Plate 24.a). Spore chains were straight to flexuous with open loops and hooks. Mature spore chains were long with about 50 spores per chain. Spore surface was of smooth type (Plate 24.b). Aerial mass colour was in the grey colour-series on OMA and ISSA. Aerial mycelium was absent on GAA. Greyish yellow coloured pigment was noticed on the reverse side colony on ISSA but not on OMA or GAA. Melanoid pigments was formed on TA but not in PYIA or TYB. No pigments were found in the medium on OMA, GAA or ISSA. D-glucose, L-arabinose and D-xylose were utilised for growth. Only a trace of growth was seen with D-mannitol and raffinose, and no growth with i-inositol, D-fructose, rhamnose or sucrose. The culture was active against gram-negative bacteria, filamentous and non-filamentous fungi tested.

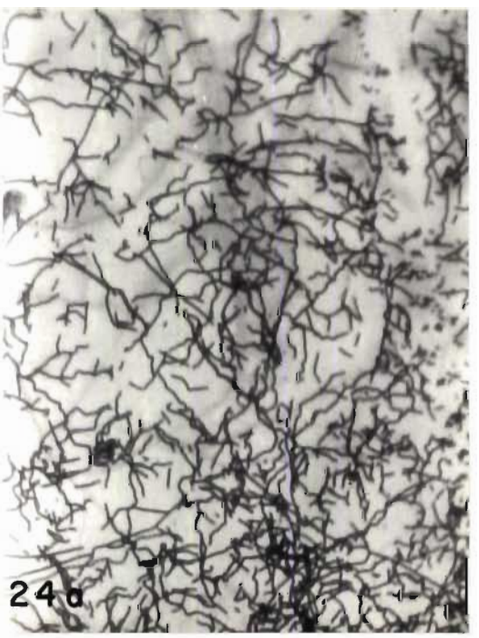
Strain 171 was identified as S. carnosus. Spore chain morphology was of Retinaculiaperti, spirale type (Plate 25.a). Extended spirals, with hooks. Spore chains were moderately flexuous. Mature spore have about 10 or more spores per chain. Aerial mycelium was absent on GAA. Spore surface was of smooth type (Plate 25.b). Aerial mass colour was in the grey colour-series on OMA and ISSA. No distinctive reverse side colony pigment (colourless to pale yellowish brown colour was noted on OMA and GAA). Melanoid pigments are not formed in PYIA, TA or TYB. Pale yellow pigment was found in the medium on OMA, and this pigment was not pH sensitive when tested with 0.05 N HCl or NaOH. D-glucose and rhamnose were utilised for growth. Trace of growth with L-arabinose and D-xylose, and no growth with i-inositol, D-mannitol, D-fructose, sucrose, or raffinose.

Plate 23	Strain No.187			
23.a	Spirale spore chains	x	200	(LM)
23.b	Smooth spores	x	25,000	(EM)

Plate 24	Strain No.108			
24.a	RF spore chains	x	200	(LM)
24.b	Smooth spores	x	10,000	(EM)

23 a

23 b



24 a

24 b

The culture was active against gram-negative bacteria filamentous and non-filamentous fungi tested.

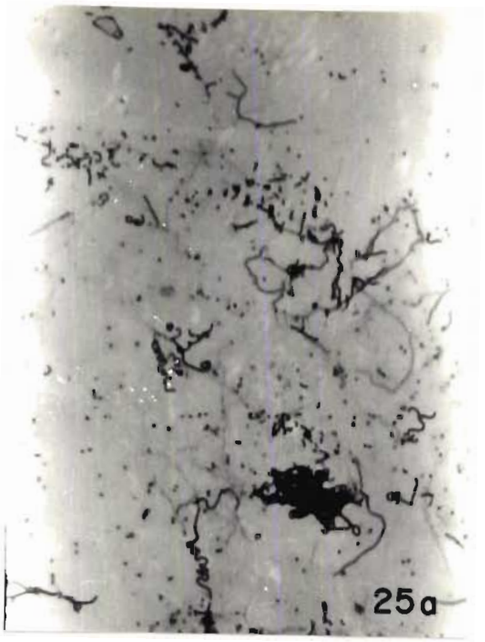
Strain 192 was identified as S. craterifer. Spore chain morphology was of Retinaculiaperti, spirale type (Plate 26.a). Flexuous spore chains with extended spirals and open loops were noted. Spore chains were short with 3 or more spores per chain. Spore surface was of spiny type (Plate 26.b). Aerial mass colour was in the white colour-series on OMA, GAA and ISSA. No distinctive pigments (pale yellowish brown) were noticed on the reverse side of colony on OMA or GAA. Melanoid pigments was not formed on TA, PYIA or on TYB. No pigment was found in the medium on OMA, GAA or ISSA. D-glucose, D-xylose, and rhamnose were utilised for growth. Only a trace of growth was seen with L-arabinose, D-mannitol, and D-fructose and no growth was seen in i-inositol, sucrose and raffinose. The culture was active against bacteria (both gram-positive and gram-negative) filamentous and non-filamentous fungi tested.

Strain 193 was also identified as S. craterifer. All the characters were similar to that of strain 192 except for carbon utilisation. D-glucose, D-xylose and rhamnose were utilised for growth. Only a trace of growth was seen with L-arabinose and no growth with i-inositol, D-mannitol, D-fructose sucrose or raffinose.

Strain 88 was also identified as S. craterifer. Spore chain morphology was of flexible type (Plate 28.a) which were moderately flexuous, short with about 3 spores per chain, sometimes with extended spirals was also seen. Spore surface was of spiny type (Plate 28.b). D-glucose, L-arabinose

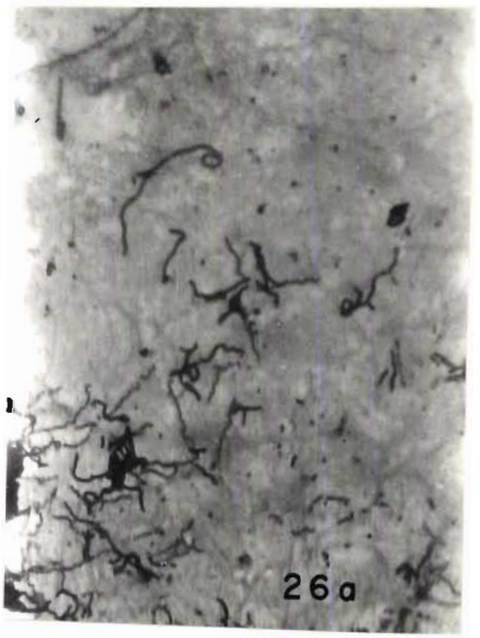
Plate 25	Strain No.171			
25.a	RAS spore chains	x	200	(LM)
25.b	Smooth spores	x	19,000	(EM)

Plate 26	Strain No.192			
26.a	RAS spore chains	x	200	(LM)
26.b	Spiny spores	x	10,000	(EM)

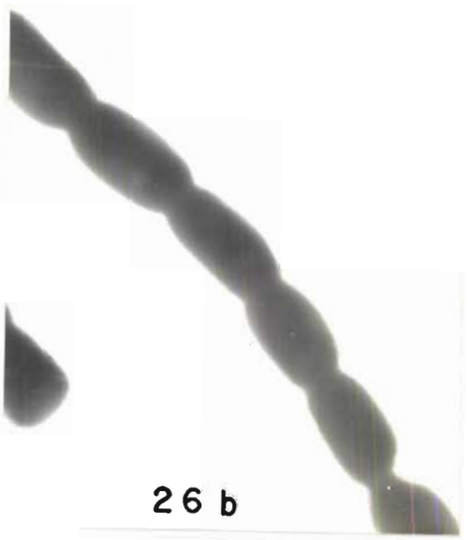


25a

25 b



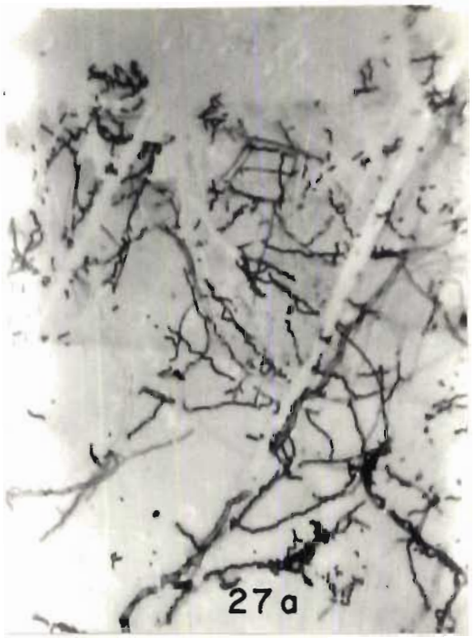
26a



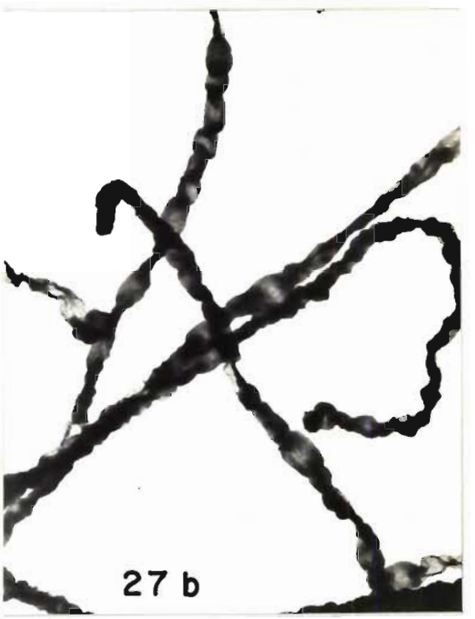
26 b

Plate 27	Strain No.193				
27.a	RAS spore chains	x	200	(LM)
27.b	Spiny spores	x	5,000	(EM)

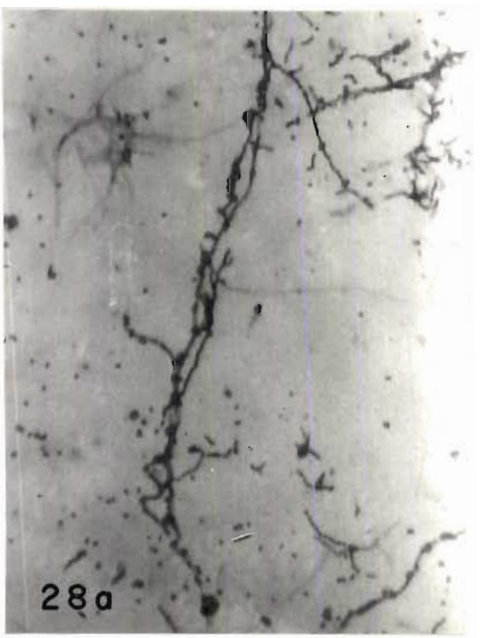
Plate 28	Strain No.88			
28.a	RAS spore chains	x	200	(LM)
28.b	Spiny spores	x	25,000	(EM)



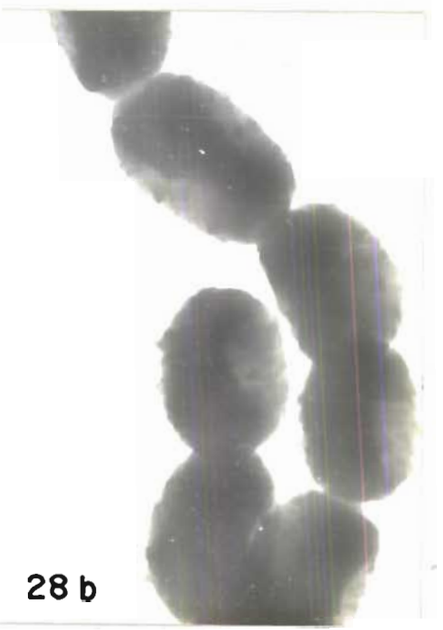
27a



27 b



28a



28 b

were utilised for growth. Only a trace of growth was seen with D-xylose, i-inositol, D-mannitol, D-fructose, rhamnose and raffinose. All other characters were similar to that of strain 192.

Strain 106B was identified as S. echinatus. Spore chain morphology was of Recinaculiaperti, spirale type (Plate 29.a). Extended spirals with open loops, with flexuous spore chains were observed and mature spore chains were moderately long with about 10 spores per chain. Spore surface was of smooth type (Plate 29.b). Aerial mass colour was in the grey colour series on OMA, GMA and ISSA. Greyish yellow pigment was observed on the reverse side of colony on OMA and GAA but not on ISSA. Substrate pigment was not a pH indicator. Melanoid pigment was formed in TYB but not in TA or PYIA. No soluble pigment was found in the medium in OMA, GAA or ISSA. D-glucose, L-arabinose, D-xylose, i-inositol, D-mannitol, D-fructose, and raffinose were utilised for growth. Only trace of growth was seen with rhamnose and no growth with sucrose. The culture was active against (V. alginoliticus) gram-negative bacteria, filamentous and non-filamentous fungi tested.

Strain 135 was identified as S. flavochromogenes. Spore chain morphology was of Rectiflexible type (Plate 30.a). Straight to flexuous spore chains with closed loops were noted. Mature spore chains were short with 3 to 10 spores per chain. Spore surface was of smooth type (Plate 30.b). Aerial mass colour was in the white colour-series on OMA and ISSA. Aerial mycelium was poorly developed on GAA. No distinctive pigments were noticed on the reverse side colony on OMA, GAA or ISSA. Melanoid pigments was formed on TA and PYIA but not in TYB. Yellowish brown

Plate 29	Strain No.106B				
29.a	RAS spore chains	x	200	(LM)
29.b	Smooth spores	x	5,000	(EM)

Plate 30	Strain No.135				
30.a	RF spore chains	x	200	(LM)
30.b	Smooth spores	x	5,000	(EM)

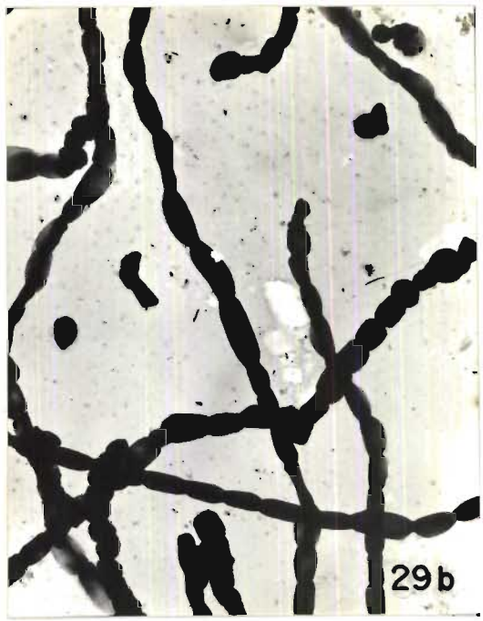
pigment was found in the medium on OMA, GAA and ISSA. The pigment was not pH sensitive when tested with 0.05 N HCl or NaOH. D-glucose, L-arabinose, D-xylose and rhamnose were utilised for growth. Only a trace of growth was seen on raffinose but no growth with i-inositol, D-mannitol, D-fructose or sucrose. The culture was active against filamentous and non-filamentous fungi tested.

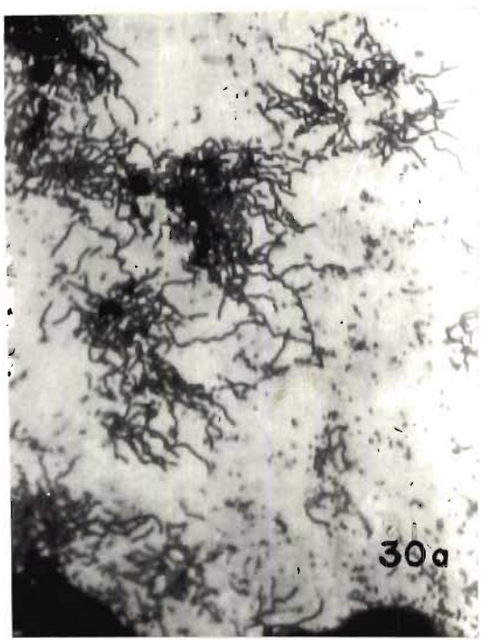
Strain 34 was identified as S. galtieri. Spore chain morphology was of Rectiflexible type (Plate 31.a). Spore chains were flexuous, short with 3 to 10 spores per chain. Spore surface was of smooth type (Plate 31.b). Aerial mass colour was in the white colour series on OMA. No distinctive pigments (on reverse side of colony) was noted on OMA, GAA or ISSA. Melanoid pigments are not formed in TA, PYIA or TYB. D-glucose was utilised for growth. Only a trace of growth was seen with D-fructose and raffinose and no growth with i-inositol, L-arabinose, D-xylose, D-mannitol, rhamnose and sucrose. The culture was active against gram-negative bacteria, filamentous and non-filamentous fungi tested.

Strain 105, 186 and 191 were also identified as S. galtieri. All these cultures slightly differed from strain 34. The dissimilar characters alone are stated below for these three strains.

Strains 105: Light yellow to yellowish brown pigment was observed on the reverse side of colony on OMA. Glucose was utilised for growth, but no growth was seen with L-arabinose, D-xylose, i-inositol, D-mannitol, rhamnose, sucrose or raffinose. The culture was antagonistic only towards fungi tested.


29 a


29b


30a

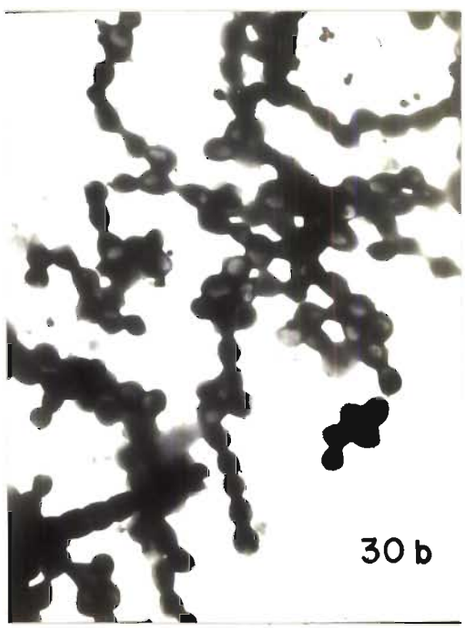
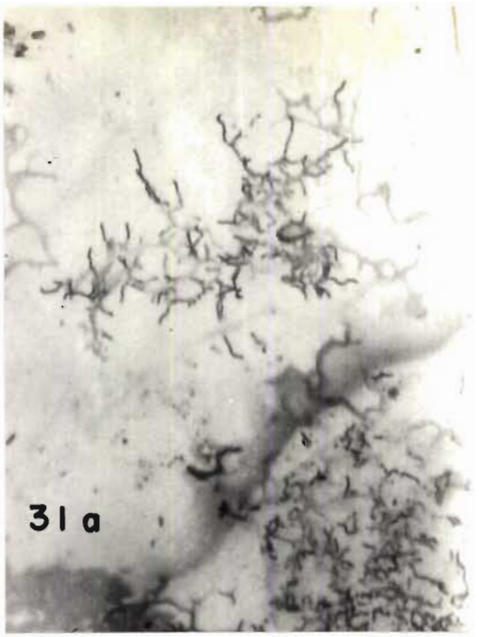

30 b

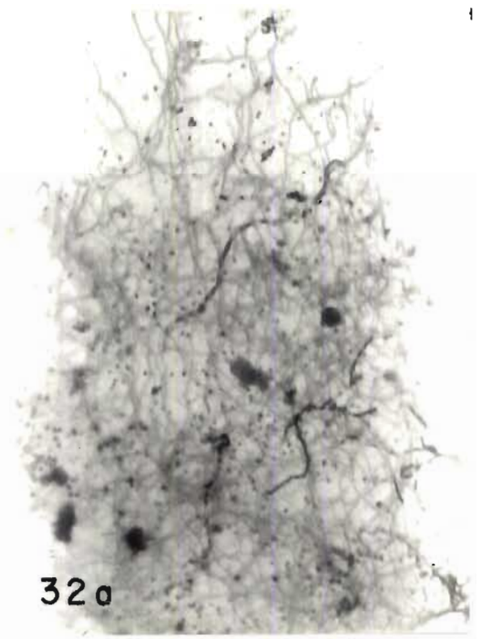
Plate 31	Strain No.34				
31.a	RF spore chains	x	200	(LM)
31.b	Smooth spores	x	5,000	(EM)

Plate 32	Strain No.105			
32.a	RF spore chains	x	200	(LM)
32.b	Smooth spores	x	15,000	(EM)



31 a

31 b



32 a

32 b

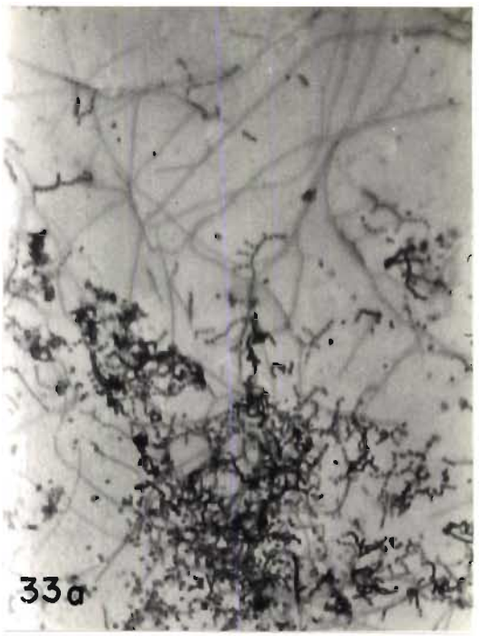
Strain 186: Spore chain morphology - Mature spore chains were molerately long with 10 or more spores per chain and were flexuous with extended spirals (Plate 33.a). Grey colour pigment was noticed in TA on aged culture. D-glucose, D-fructose were utilised for growth. Only a trace of growth was seen with D-xylose and D-mannitol and no growth with L-arabinose, i-inositol, rhamnose, sucrose and raffinose. The culture was active against gram-positive bacteria tested and filamentous fungi.

Strain 191. No distinctive pigments (light yellowish brown pigment on OMA and GAA) were noticed on the reverse side of colony. D-glucose was utilised for growth. Only trace of growth was seen with D-mannitol and D-fructose. No growth was seen with L-arabinose, D-xylose, i-inositol, rhamnose, sucrose or raffinose. All the other characters were similar to that of Strain 34.

Strain 73 was identified as S. gougeroti. Spore chain morphology was of Rectiflexible type (Plate 35.a). Spore chains were moderately short with 3 to 10 spores per chain, with strongly flexuous spore chains. Spore surface was of smooth type (Plate 35.b). Aerial mycelium was poorly developed on OMA and sporulating aerial mycelium was absent on GAA and ISSA. Aerial mass colour was in the white colour series on OMA. No distinctive pigments were observed on the reverse side of colony on OMA, GAA or ISSA. Melanoid pigments were not formed in TA, PYIA, or TYB. No pigment was found in the medium on OMA, GAA or ISSA. D-glucose, D-xylose and D-fructose were utilised for growth. Only a trace of growth was seen with L-arabinose, D-mannitol, raffinose, i-inositol and sucrose

Plate 33	Strain No.186			
33.a	RF spore chains	x	200	(LM)
33.b	Smooth spores	x	19,000	(EM)

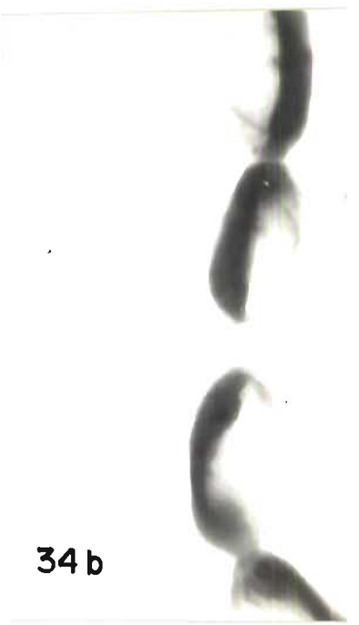
Plate 34	Strain No.191			
34.a	RF spore chains	x	200	(LM)
34.b	Smooth spores	x	19,000	(EM)



33a

33 b

34 a



34 b

were not utilised. The culture was active against gram-negative bacteria, filamentous and non-filamentous fungi tested.

Strain 91 and 108A were also identified as S. gougeroti. These 2 strains differed from 73, in carbon utilisation and antagonistic property.

Strain 91. Trace of growth was seen with L-arabinose, i-inositol and raffinose, sucrose were not utilised. Whereas other sugars tested were utilised for growth. And it was antagonistic only towards the fungi tested.

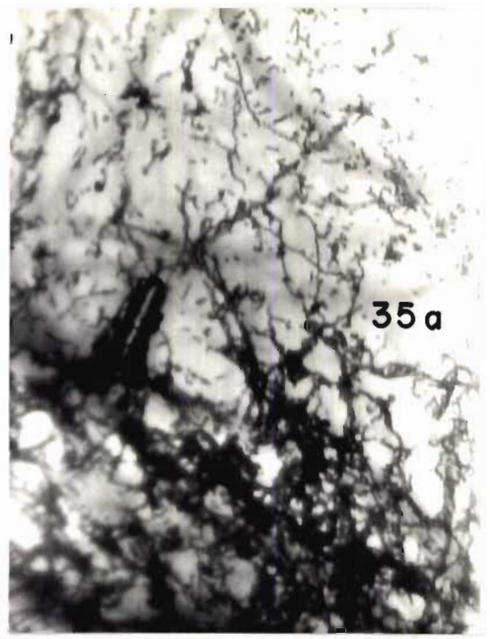
Strain 108A: D-glucose, D-xylose, D-mannitol and D-fructose were utilised for growth. Only a trace growth was seen with L-arabinose, rhamnose and raffinose and no growth with i-inositol and sucrose. It was active against gram-negative and gram-positive bacteria, filamentous and non-filamentous fungi tested.

All the other studied characters of strain 91 and 108A were similar to that of Strain 73.

Strain 103B was identified as S. griseolavendus. Spore chain morphology was of Retinaculiaperti, Rectiflexible type (Plate 38.a). Straight to flexuous, with open loops and hooks were observed. Mature spore chains were long with about 10 spores per chain. Spore surface was of smooth type (Plate 38.b). Aerial mass colour was in the red colour series (light greyish, whitish, pink) on OMA, GAA. No distinctive pigments (light yellowwish brown on OMA) was noticed on the reverse side of colony. Melanoid pigments was formed in TYB and PYIA but not on TA. A light yellow pigment was found in the medium on OMA, GAA and ISSA, the pigment was not pH sensitive when tested with 0.05 N NaOH or HCl. D-glucose

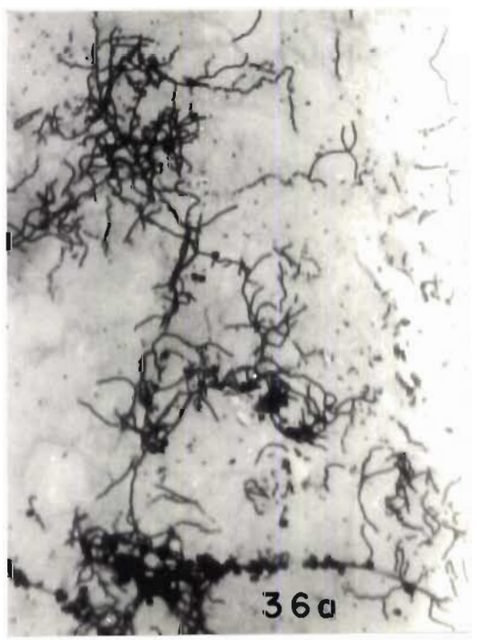
Plate 35	Strain No.73				
35.a	RF spore chains	x	200	(LM)
35.b	Smooth spores	x	15,000	(EM)

Plate 36	Strain No.91			
36.a	RF spore chains	x	200	(LM)
36.b	Smooth spores	x	19,000	(EM)



35 a

35 b



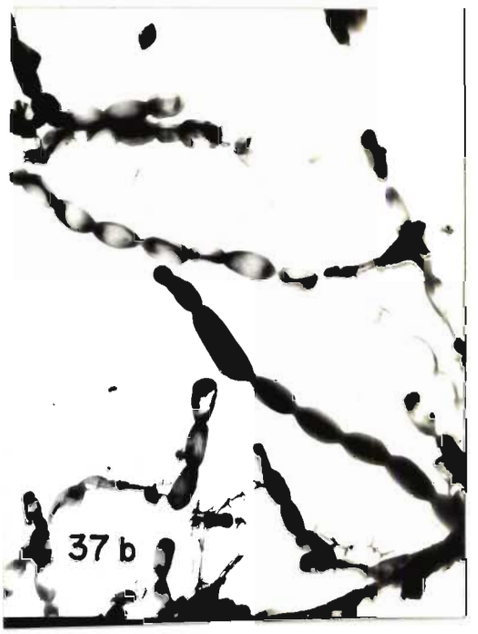
36 a

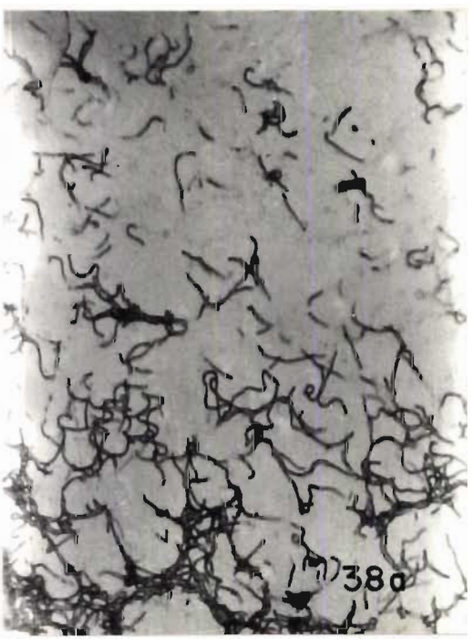
36 b

Plate 37		Strain No.108A			
	37.a	RF spore chains	x	200	(LM)
	37.b	Smooth spores	x	5,000	(EM)

Plate 38		Strain No.103B			
	38.a	RARF spore chains	x	200	(LM)
	38.b	Smooth spores	x	19,000	(EM)


37 a


37 b


38a


38b

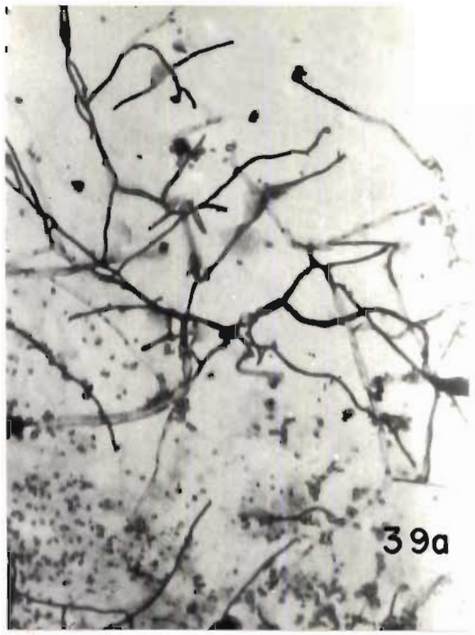
and fructose were utilised for growth. Only trace growth was seen with D-xylose, D-fructose and no growth with L-arabinose, i-inositol, D-mannitol. rhamnose, sucrose and raffinose. And the culture was active against gram-negative bacteria, gram-positive bacteria, filamentous and non-filamentous fungi tested.

Strain 106A was identified as S. indigoferus. Spore chain morphology was of Retinaculiaperti, Rectiflexible type (Plate 39.a). Mature spore chains were moderately long, with about 10 to 50 spores per chain with open loops and hooks. Spore surface was of smooth type (Plate 39.b). Aerial mass colour was in the white colour series on OMA and GAA and aerial mycelium was not found on ISSA. Light yellow colour pigment was noticed on the reverse side of colony on OMA and GAA. Melanoid pigment (green colour) was formed in TYB, but not in tyrosine agar or PYIA. Yellow pigment was found in the medium on OMA. D-glucose was utilised for growth. Only trace of growth was found with L-arabinose, D-xylose, i-inositol, D-fructose and rhamnose. No growth was seen with D-mannitol, sucrose and raffinose. The culture was active only against filamentous and non-filamentous fungi tested.

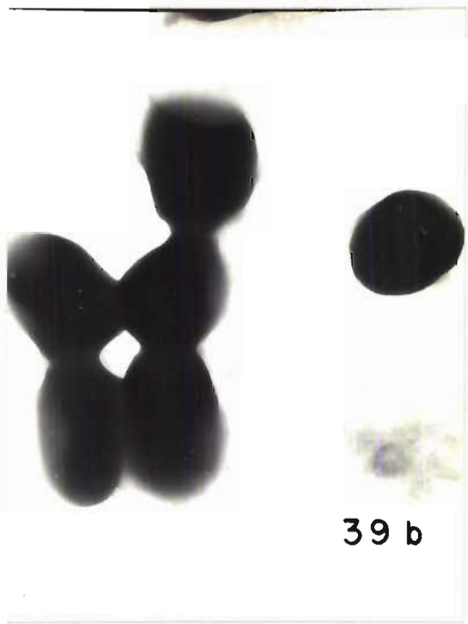
Strain 172 was identified as S. mirabilis. Spore chain morphology was found to be spirale type (Plate 40.a). Simple spirals with 1 to 4 turns with extended spirals of small diameter and open loops were also noticed. Mature spore chains were moderately long with 10 spores or more per chain. Spore surface was of smooth type (Plate 40.b). Aerial mass colour was in the grey colour series on OMA, and ISSA. Aerial mycelium was absent

Plate 39	Strain No.106A				
39.a	RA RF spore chains	x	200	(LM)
39.b	Smooth spores	x	19,000	(EM)

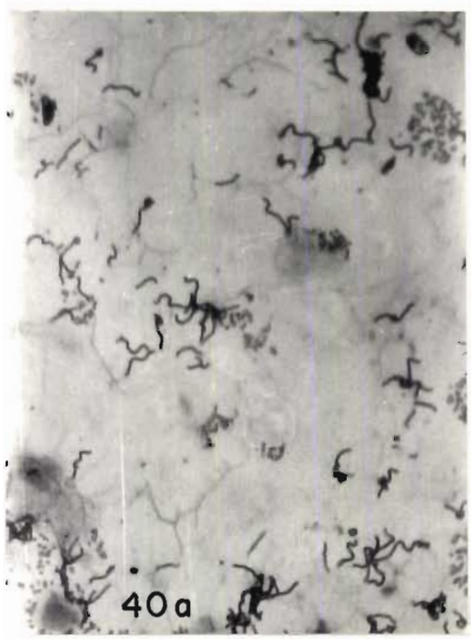
Plate 40	Strain No.172			
40.a	Spirale spore chains	x	200	(LM)
40.b	Smooth spores	x	19,000	(EM)



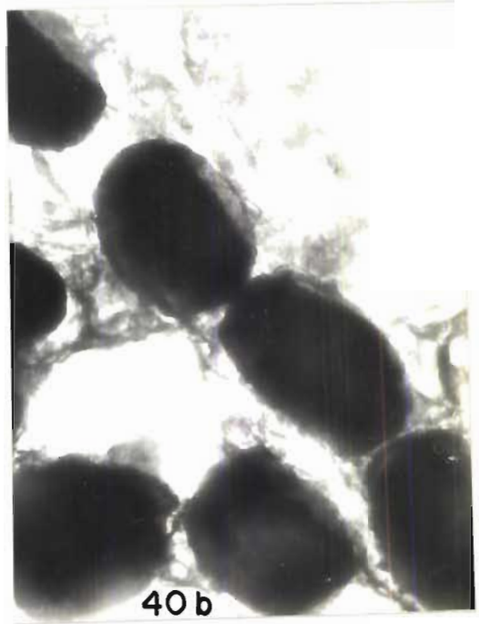
39a



39 b



40a



40b

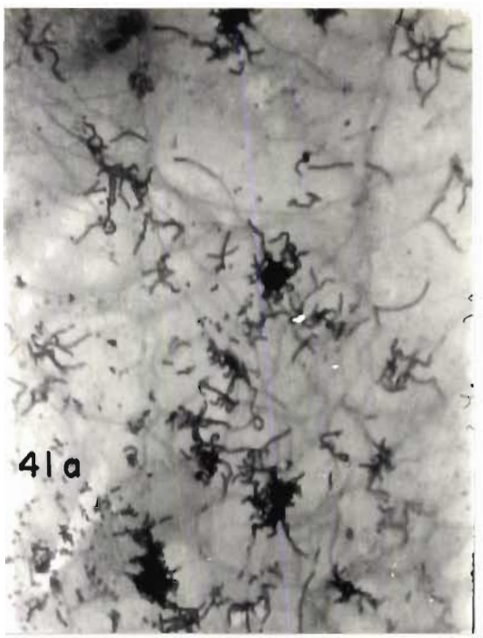
on GAA. Greyish yellow colour pigment was found on the reverse side colony on OMA, GAA and ISSA. Melanoid pigments, were formed in TA but not in PYIA or TYB. No pigment was found in the medium on OMA, GAA or ISSA. D-glucose, L-arabinose, i-inositol, D-mannitol, D-fructose and rhamnose were utilised for growth. Only trace of growth was seen with D-xylose and no growth with sucrose and raffinose. The culture was active against gram-positive and gram-negative bacteria, filamentous and non-filamentous fungi tested.

Strain 110 was identified as S. nitrosporeus. Spore chain morphology was of Rectiflexible type (Plate 41.a). Spore chain was moderately flexuous with short closed and open loops. Mature spore chains were moderately long with about 10 spores per chain. Spore surface was of smooth type (Plate 41.b). Aerial mass colour was in the grey colour series on OMA and ISSA. Aerial mycelium was absent on GAA. No distinctive pigment was found on the reverse side colony (light yellowish brown) on OMA, GAA or ISSA. Melanoid pigments were not formed in TA, PYIA or TYB. No pigment was found in the medium on OMA, GAA or ISSA. D-glucose, L-arabinose, D-xylose, D-fructose, rhamnose were utilised for growth. Only a trace of growth was seen with D-mannitol and raffinose and no growth with i-inositol or sucrose. The culture was active against gram-positive and gram-negative bacteria, filamentous and non-filamentous fungi tested.

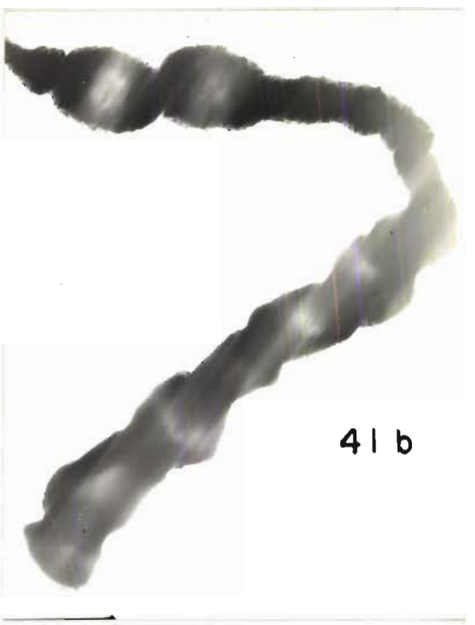
Strain 103A was identified as S. noblis. Spore chain morphology was of Rectiflexible type (Plate 42.a). Mature spore chains were flexuous with terminal open loops, moderately long with 3 to 10 spores per chain.

Plate 41	Strain No.110			
41.a	RF spore chains	x	200	(LM)
41.b	Smooth spores	x	20,000	(EM)

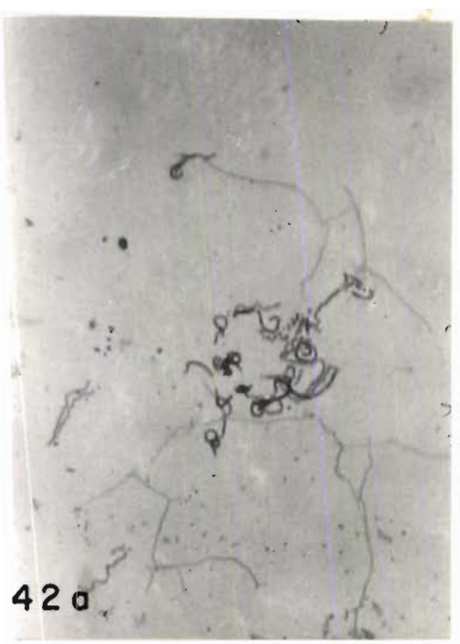
Plate 42	Strain No.103A			
42.a	RF spore chains	x	200	(LM)
42.b	Smooth spores	x	10,000	(EM)



41a



41b



42a

42b

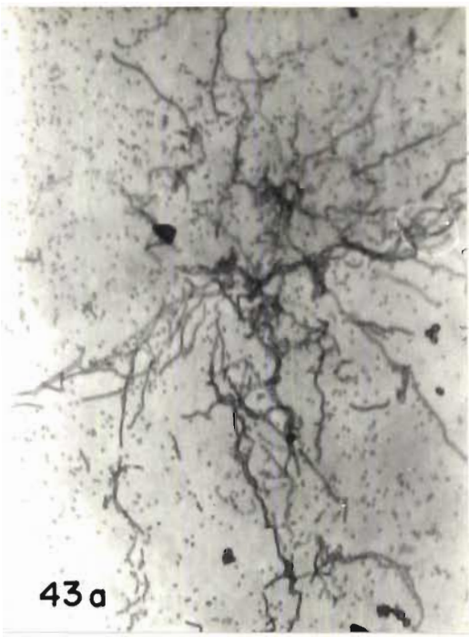
Spore surface was found to be smooth type (Plate 42.b). Aerial mass colour was in the red colour series (Whitish pink) on OMA, GAA and ISSA. Melanoid pigments were formed in PYIA, TA and TYB. No pigment was found in the medium on OMA, GAA or ISSA. D-glucose, D-mannitol, and raffinose were utilised for growth. Only trace of growth was seen with L-arabinose, D-xylose, i-inositol and D-fructose. No growth with rhamnose and sucrose. The culture was active against gram-positive and gram-negative bacteria, filamentous and non-filamentous fungi tested.

Strain 107 was identified as S. orientalis. Spore chain morphology was of Rectiflexible type (Plate 43.a), with long aerial hypae, straight spore chains and hooks. Spore surface was of smooth walled type (Plate 43.b). Aerial mass colour was in white colour series on OMA, GAA and ISSA. No distinctive pigment was noticed on OMA, GAA or ISSA. Melanoid pigments were not formed in PYIA, TA and TYB. No soluble pigment was found in the medium on OMA, GAA or ISSA. D-glucose, D-xylose and rhamnose were utilised for growth. Only a trace of growth was seen with L-arabinose, i-inositol, D-mannitol, D-fructose, sucrose and raffinose. The culture was active against gram-positive and gram-negative bacteria, filamentous and non-filamentous fungi tested.

Strain 189 was also identified as S. orientalis. Spore chains of the strain were moderately flexuous, with imperfect extended spirals ending with open loops and short hooks. D-glucose, L-arabinose, D-xylose, i-inositol, D-mannitol, D-fructose and rhamnose were utilised for growth. Only trace of growth was seen with sucrose and no growth with raffinose. The strain

Plate 43	Strain No.107				
43.a	RF spore chains	x	200	(LM)
43.b	Smooth spores	x	8,000	(EM)

Plate 44	Strain No.189			
44.a	RF spore chains	x	200	(LM)
44.b	Smooth spores	x	19,000	(EM)



43a

43b

44a

44 b

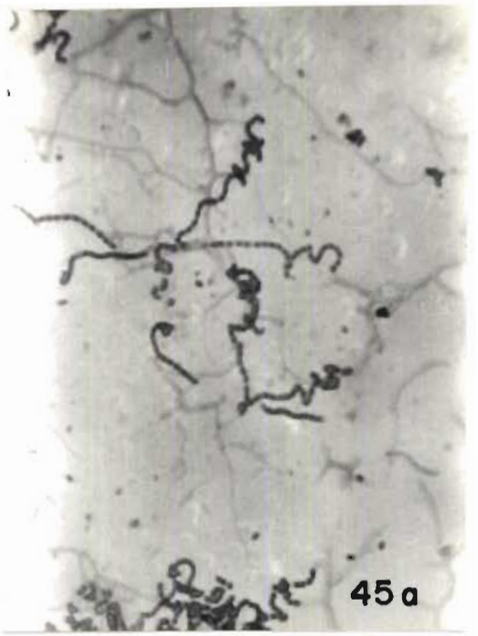
was active against filamentous and non-filamentous fungi tested. All the other characters were similar to that of Strain 107.

Strain 199 was identified as S. pyridomyceticus. Spore chain morphology was found to be RAS type (Plate 45.a). Mature spore chains were short with 3 to 10 spores per chain, with extended spirals of small diameter and hooks. Spore surface was of smooth type (Plate 45.b). Aerial mycelium poorly developed on OMA, GAA and ISSA. Aerial mass colour was in the white colour series. No distinctive pigments were found on OMA, GAA or ISSA. Melanoid pigments was not formed on TA, PYIA or TYB. No soluble pigment was found in the medium on OMA, GAA or ISSA. No growth was found with any of the sugars tested. The culture was active against gram-positive bacteria and gram-negative bacteria, filamentous and non-filamentous fungi tested.

Strain 104 was identified as S. roseus. Spore chain morphology was of RF type (Plate 46.a), with straight to flexible spore chains, with slightly closed loops and extended spirals. Mature spore chains were moderately short with 3 to 10 spores per chain. Spore surface was of smooth type (Plate 46.b). Aerial mass colour was in the red colour-series (faint yellowish to pink) on OMA, GAA and ISSA. No distinctive pigment (faint yellow to pale brown) was found on the reverse side of colony on OMA, GAA or iSSA. Substrate pigment was not an pH indicator. Melanoid pigments were not formed in TYB, TA or PYIA. No soluble pigment was found in the medium on OMA, GAA or ISSA. D-glucose was utilised for growth. Only a trace of growth with D-xylose and D-fructose was observed. No growth

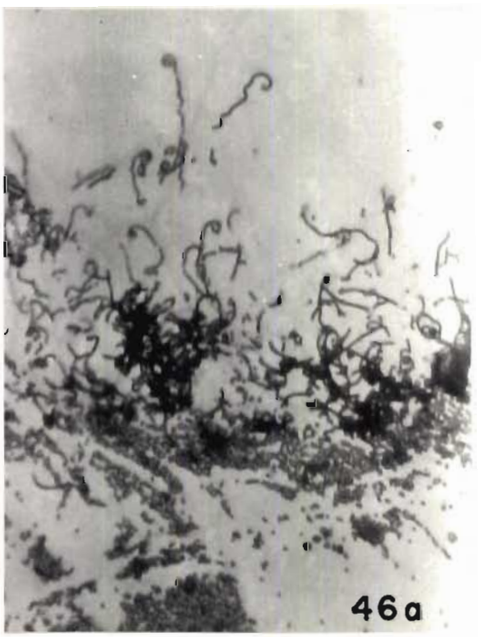
Plate 45	Strain No.199			
45.a	RAS spore chains	x	400	(LM)
45.b	Smooth spores	x	19,000	(EM)

Plate 46	Strain No.104			
46.a	RF spore chains	x	200	(LM)
46.b	Smooth spores	x	25,000	(EM)



45a

45b



46a

46b

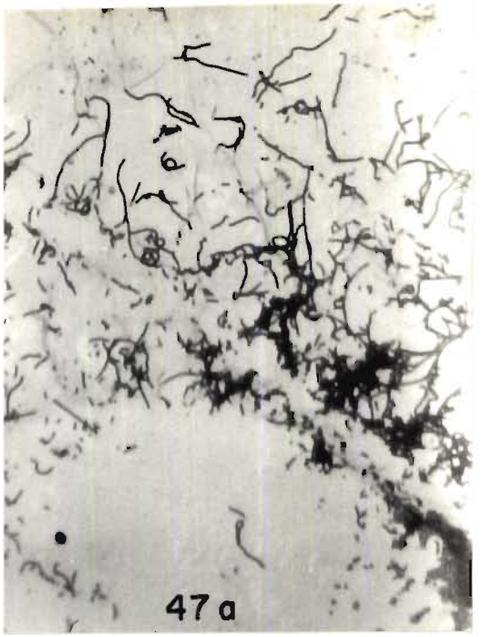
with L-arabinose, i-inositol, D-mannitol, rhamnose, sucrose or raffinose. The culture was active against gram-positive bacteria, filamentous and non-filamentous fungi tested.

Strain 22 was identified as S. rishriensis. Spore chain morphology was of RAS type (Plate 47.a), with open spirals, flexuous spore chains with terminal loops and hooks. Mature spore chains were long with about 10 to 50 spores per chain. Spore surface was of smooth type (Plate 47.b). Aerial mass colour was in grey colour series on OMA, GAA and ISSA. No distinctive pigments (pale yellow to faint pinkish red on OMA) was found on the reverse side colony on OMA, GAA and ISSA. Melanoid pigments was formed in TA, TYB and PYIA. No soluble pigment was found in the medium on OMA, GAA or ISSA. D-glucose, L-arabinose, D-xylose, i-inositol, D-fructose, rhamnose, sucrose and raffinose were utilised for growth and only a trace of growth was seen with D-mannitol. The culture was active only against filamentous and non-filamentous fungi tested.

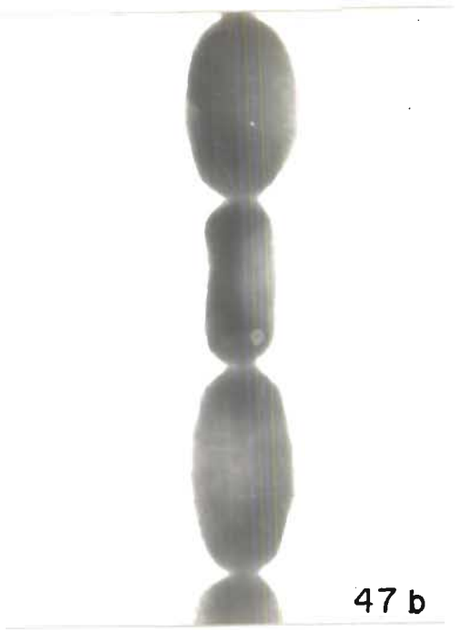
Strain 48 was identified as S. sindensis. Spore chain morphology was found to be RF type (Plate 48.a). Mature spore chains were moderately flexuous with 3 to 10 spores per chain. Spore surface was of smooth type (Plate 48.b). Aerial mycelium poorly developed on GAA and ISSA. Aerial mass colour was in the white colour series (yellowish white) on OMA. No distinctive pigment (light yellowish brown) on the reverse side of colony was noted on OMA, GAA or ISSA. Melanoid pigments were not formed in TYB, TA or PYIA. No pigment was found in the medium on OMA, GAA or ISSA. D-glucose, L-arabinose, D-xylose and D-mannitol were utilised

Plate 47	Strain No.22			
47.a	RAS spore chains	x	200	(LM)
47.b	Smooth spores	x	19,000	(EM)

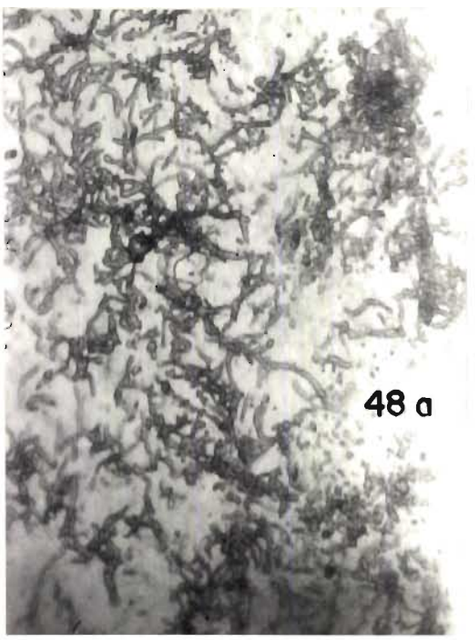
Plate 48	Strain No.48			
48.a	RF spore chains	x	200	(LM)
48.b	Smooth spores	x	5,000	(EM)



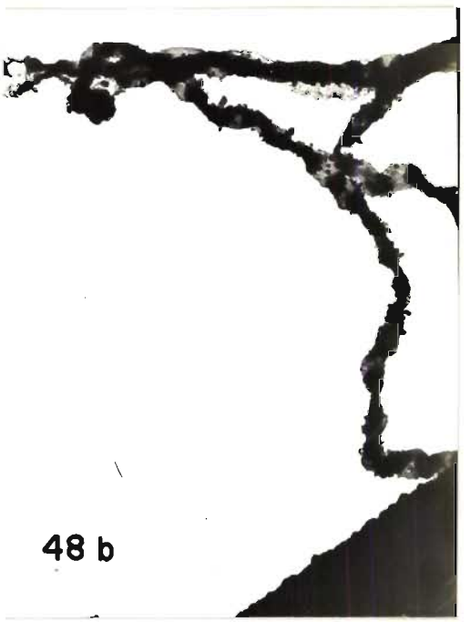
47 a



47 b



48 a



48 b

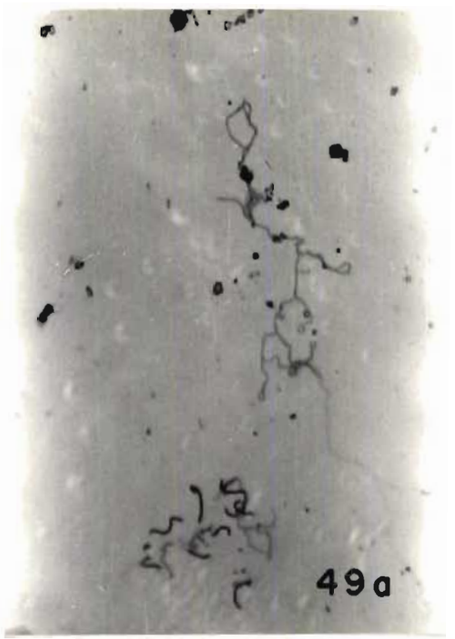
for growth. Only trace of growth was seen with i-inositol, D-fructose, and no growth was found in rhamnose, sucrose or raffinose. The culture was active only against filamentous and non-filamentous fungi tested.

Strain 93B was identified as S. sclerotialus. Spore chain morphology was found to be spirale type (Plate 49.a), with extended spirals of one or two turns, with open loops. Mature spore chains were moderately long with about 10 spores per chain. Spore surface was of smooth type (Plate 49.b). Aerial mass colour was in the white colour series on OMA, GAA and ISSA. No distinctive pigments was seen on the reverse side colony on OMA, GAA or ISSA. Melanoid pigments was not formed on TA, PYIA or on TYB. No pigment was found in the medium on OMA, GAA or ISSA. D-glucose, L-arabinose, D-xylose, i-inositol, D-mannitol, D-fructose, rhamnose, sucrose and raffinose were all utilised for growth. The strain was active against gram-positive and gram-negative bacteria, filamentous and non-filamentous fungi tested.

Strain 142 was identified as S. spadicis. Spore chain morphology was of RF type (Plate 50.a). Mature spore chains were short with about 3 spores per chain, form flexuous spore chains with open loops and extended spirals. Spore surface was of smooth type (Plate 50.b). Aerial mass colour was in the grey colour series on OMA and ISSA. Aerial mycelium was absent on GAA. Greyish yellow colour was seen on the reverse side colony on OMA and ISSA. The pigment was not pH indicator. Melanoid pigments was formed in TA and TYB. No soluble pigment was found in the medium in OMA, GAA or ISSA. D-glucose, L-arabinose, D-xylose, D-mannitol, and

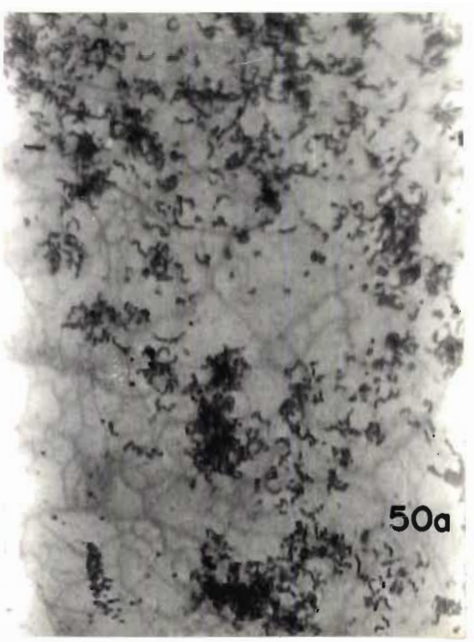
Plate 49	Strain No.93B				
49.a	Spirale spore chains	x	200	(LM)
49.b	Smooth spores	x	19,000	(EM)

Plate 50	Strain No.142			
50.a	RF spore chains	x	200	(LM)
50.b	Smooth spores	x	10,000	(EM)

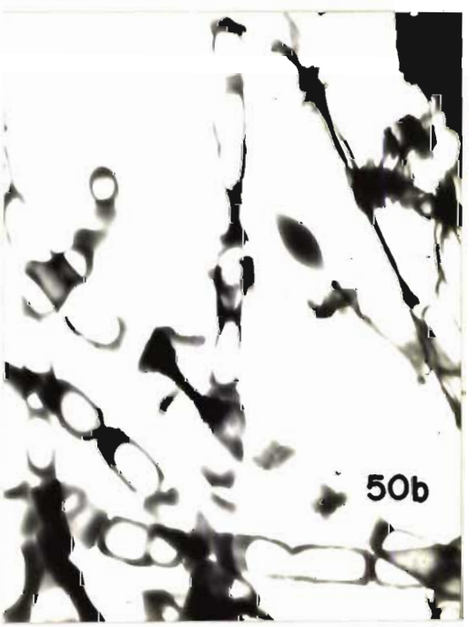


49a

49 b



50a



50b

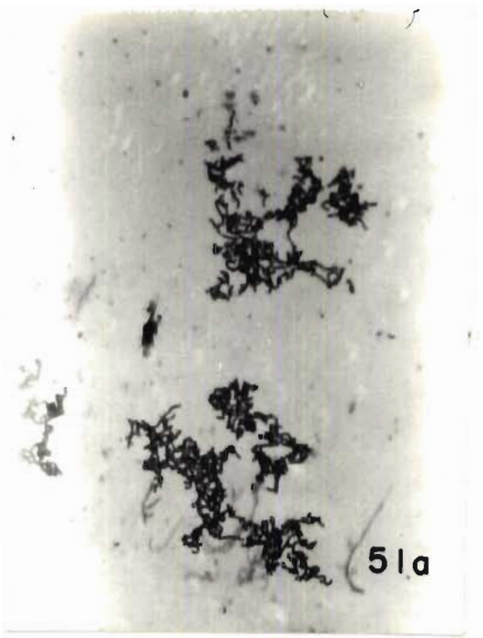
D-fructose were utilised for growth. Only a trace growth was seen with rhamnose, sucrose and raffinose. And no growth with i-inositol. The strain was active against filamentous fungi tested.

Strain 26B was identified as S. thermovulgaris. Spore chain morphology was of RAS type (Plate 51.a), with extended spirals of open loops, slightly flexuous spore chains were also seen. Spore surface was of smooth type (Plate 51.b). Aerial mass colour was in the grey colour series on OMA. No distinctive pigments were noticed on the reverse side colony on OMA, GAA or ISSA. Melanoid pigments was not formed in TA, PYIA or TYB. No soluble pigment was found in the medium on OMA, GAA or ISSA. D-glucose, L-arabinose, D-xylose, i-inositol, D-mannitol, D-fructose, rhamnose, sucrose and raffinose were utilised for growth. The strain was active against gram-negative bacteria, filamentous and non-filamentous fungi studied.

Strain 148 was identified as S. vastus. Spore chain morphology was of RAS type (Plate 52.a). Spirals of 1 to 3 turns per chain, with moderate flexuous spore chains. Spore surface was of smooth type (Plate 52.b). Aerial mass colour was in the white colour series (dull white colour on OMA, GAA or ISSA). Grey to light yellow pigment was noticed on the reverse side colony on OMA, GAA. Melanoid pigment was not formed in TA, PYIA or TYB. Soluble pigment was not found in any of the medium tested. D-glucose, L-arabinose, i-inositol, D-mannitol, D-fructose, rhamnose, sucrose and raffinose were utilised for growth. No growth was seen with D-xylose. The culture was active against gram-negative bacteria (V. parahaemolyticus) filamentous and non-filamentous fungi tested.

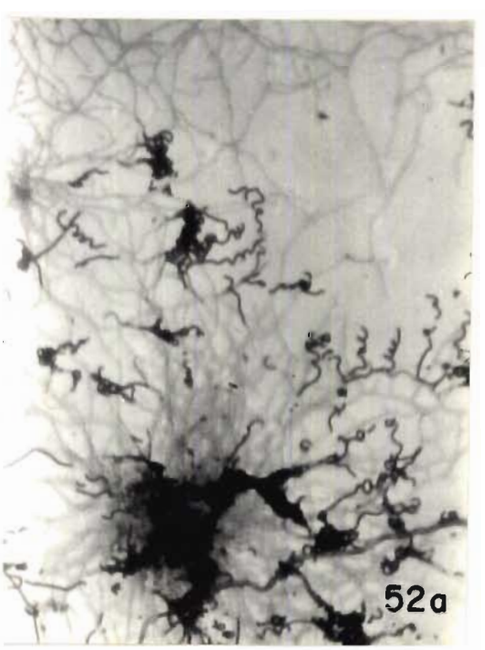
Plate 51	Strain No.26B			
51.a	RAS spore chains	x	200	(LM)
51.b	Smooth spores	x	8,000	(EM)

Plate 52	Strain No.148			
52.a	RAS spore chains	x	200	(LM)
52.b	Smooth spores	x	10,000	(EM)



51a

51 b



52a

52 b

Table 20. Morphological and physiological properties of 52 isolated actinomycetes based on ISP - Scheme (1966)

S. No.	Strain No.	Station	Month	Aerial Mass Colour	Pigment			Morphology		Carbon Utilisation									Identified as
					Melanoid Pigment	Reverse side Colony	Soluble Pigment	Sporophore	Spore	D-glucose	L-arabinose	D-xylose	i-inositol	D-mannose	D-Fructose	Rhamnose	Sucrose	Raffinose	
1.	28	III	Feb	White	+	-	-	RF	Sm	+	++	±	+	+	+	-	-	+	A.aureomonopodiales
2.	79	III	May	White	+	-	-	RF	Sm	+	+	+	+	+	+	±	±	+	A aureomonopodiales
3.	38	II	Feb	White	+	+	-	RF	Sm	+	++	+	+	+	++	++	±	+	A aureofasiculus
4.	76	II	Mar	White	+	-	-	RF	Sm	+	+	+	+	+	+	+	±	+	A aureofasiculus
5.	140	I	Jun	White	+	+	-	RF	Sm	+	+	+	+	+	+	+	-	+	A aureofasiculus
6.	78	III	May	White	-	-	-	RF	Sm	+	±	+	±	+	+	±	±	±	A aureocirculatus
7.	201	IV	Dec	White	-	-	-	RF	Sm	+	++	++	-	+	±	±	-	±	A albovinaceus
8.	8	IV	Jan	White	-	-	-	RF	Sm	+	+	+	±	+	-	±	±	±	A candidus
9.	70	III	Mar	White	-	-	-	RF	Sm	+	+	+	±	+	±	+	±	-	A candidus
10.	12	II	Jan	White	+	+	+	RAS	Sm	+	+	+	±	±	±	-	±	±	A flavescens
11.	92B	IV	Apr	White	-	-	+	RAS	Sm	+	+	±	++	++	±	+	±	+	A flavescens
12.	26A	III	Jan	Green	+	+	-	RAS	Sm	+	+	±	+	+	+	+	-	+	A griseomycini
13.	190	II	Oct	White	-	-	-	RAS	Sm	+	+	±	±	±	±	±	±	±	A flavescens
14.	92A	IV	Apr	White	-	-	-	RF	Sm	+	++	++	++	++	++	++	±	±	A aureocirculatus
15.	200	I	Nov	White	-	-	-	RAS	Sm	+	+	+	+	±	+	±	±	±	A mutabilis
16.	202	I	Nov	White	-	-	-	RAS	Sm	+	+	+	+	+	+	+	±	±	A mutabilis
17.	23A	III	Jan	Red	+	+	+	RF	Sm	+	+	+	+	+	+	+	+	+	A umbrinus
18.	23.2	III	Jan	Red	+	+	+	S	Sm	+	+	+	+	+	+	+	+	+	A vilochromogenes
19.	75	II	Mar	White	-	-	+	RF	Sm	+	+	±	+	++	-	±	±	++	S alboniger
20.	77	II	Mar	White	-	-	+	RF	Sm	+	+	±	+	++	-	-	-	±	S alboniger

S. No.	Strain No.	Station	Month	Aerial Mass Colour	Pigment			Morphology		Carbon Utilisation									Identified as
					Melanoid Pigment	Reverse side Colony	Soluble Pigment	Sporophore	Spore	D-glucose	L-arabinose	D-xylose	i-inositol	D-mannose	D-Fructose	Rhamnose	Sucrose	Raffinose	
21.	82	III	Mar	White	-	-	-	RF	Sm	+	+	+	-	+	+	±	-	-	S. albidoflavus
22.	30	III	Feb	White	-	-	-	S	SM	+	++	+	-	+	+	-	±	++	S. cacaoi
23.	187	II	Sep	White	-	-	-	S	Sm	+	+	+	±	+	+	-	-	±	S cacaoi
24.	108	I	Mar	Grey	+	+	-	RF	Sm	+	+	+	-	±	-	-	-	±	S cinereoruber
25.	171	II	Jul	Grey	-	-	+	RAS	Sm	+	±	±	-	-	-	+	-	-	S carnosus
26.	192	I	Aug	White	-	-	-	RAS	Sp	+	±	+	-	±	±	+	-	-	S craterifer
27.	193	I	Oct	White	-	-	-	RAS	Sp	+	±	+	-	-	-	+	-	-	S craterifer
28.	88	IV	Mar	White	-	-	-	RF	Sp	+	+	±	±	±	±	±	±	±	S craterifer
29.	106B	I	Mar	Grey	+	+	-	RAS	Sm	+	+	+	+	+	+	±	-	+	S echinatus
30.	135	II	Jun	White	+	-	+	RF	Sm	+	+	+	-	-	-	+	-	±	S flavochromogenes
31.	34	III	Feb	White	-	-	-	RF	Sm	+	-	-	-	-	±	-	-	±	S galtieri
32.	105	I	Mar	White	-	-	-	RF	Sm	+	-	-	-	-	±	-	-	-	S galtieri
33.	186	II	Sep	White	-	-	-	RF	Sm	+	-	±	-	±	+	-	-	-	S galtieri
34.	191	I	Aug	White	-	-	-	RF	Sm	+	-	-	-	±	±	-	-	-	S galtieri
35.	73	II	Mar	White	-	-	-	RF	Sm	+	±	+	-	±	+	+	-	±	S gougeroti
36.	91	IV	Mar	White	-	-	-	RF	Sm		±	+	±	+	+	+	-	±	S gougeroti
37.	108A	I	Mar	White	+	+		RF	Sm		±	+	-		+	±		±	S gougeroti
38.	103B	I	Mar	Red	+	-	+	RARF	Sm	-	±	-	-	-	+	-	-	-	S griseolavendus
39.	106A	I	Mar	White	+	+	-	RARF	Sm	+	±	±	±	-	±	±	-	+	S indigorerus
40.	172	II	Aug	Grey	+	+	-	S	Sm	+	+	±	+	+	+	+	-	-	S mirabilis
41.	110	I	Mar	Grey	-	-	-	RF	Sm	+	+	+	-	±	+	+	-	±	S flavochromogenes

S. No.	Strain No.	Station	Month	Aerial Mass Colour	Pigment			Morphology		Carbon Utilisation									Identified as
					Melanoid Pigment	Reverse side Colony	Soluble Pigment	Sporo-phore	Spore	D-glucose	L-arabinose	D-xylose	i-inositol	D-mannose	D-Fructose	Rhamnose	Sucrose	Raffinose	
42.	103A	I	Mar	Red	+	+	-	RF	Sm	+	±	±	±	+	±	-	-	+	S noblis
43.	107	I	Mar	White	-	-	-	RF	Sm	+	±	+	-	±	±	-	-	±	S orientalis
44.	189	II	Oct	White	-	-	-	RF	Sm	+	+	+	+	+	+	+	±	-	S orientalis
45.	199	II	Nov	White	-	-	-	RAS	Sm	+	-	-	-	-	-	-	-	-	S pyridomyceticus
46.	104	II	Mar	Red	-	-	-	RF	Sm	+	-	±	-	-	±	-	-	-	S roseus
47.	22	III	Jan	Grey	+	-	-	RAS	Sm	+	++	++	+	±	+	++	+	+	S rishiriensis
48.	48	III	Feb	White	-	-	-	RF	Sm	+	+	+	±	+	±	-	-	-	S sindensis
49.	93B	I	Mar	White	-	-	-	S	Sm	+	++	+	++	+	++	+	++	+	S sclerotialus
50.	142	IV	Jun	Grey	+	+	-	RF	Sm	+	++	++	-	++	+	±	±	±	S spadicis
51.	26B	III	Jan	Grey	-	-	-	RAS	Sm	+	+	+	+	+	+	+	+	+	S thermovulgaris
52.	148	III	Jul	White	-	+	-	RAS	Sm	+	+	-	+	++	+	+	+	++	S vastus

B. **Generic Composition**

Among 52 cultures identified it was found that Streptomycetes dominated in the mangrove sediments than that of Actinomycetes. Out of 52 cultures identified, 35 (67.31%)) represented the genus Streptomycetes and 18 (34.62%) were of Actinomycetes.

Out of 35 cultures identified as Streptomycetes, maximum 13 (37.14%) number were isolated from Mangalavana, followed by 11 (31.43%) from Narakkal, 8 (22.86%) from Puthuvyppu and only 3 (8.57%) from the light house area of Puthuvyppu. And among 18 cultures of Actinomycetes identified, 7 were (38.89%) from Puthuvyppu, 4 (22.22%) from Narakkal and 4 from light house area of Puthuvyppu only 3 (16.67%) were identified from Mangalavana (Table 21).

The cultures selected for the identification represented 4 colour series namely, white, grey, red and green. Among the 35 Streptomycetes isolated, white colour series dominated 24 (68.57%), followed by grey colour-8 numbers (22.86%) and only 3 (8.57%) were of red colour series and none were of green colour. And among the Actinomycetes 18 numbers identified, 14 (77.78%) were of white colour, 3 (16.67%) of red colour and only one (5.56%) culture was green in colour and none were of grey colour (Table 21).

Among 24 white colour Streptomycetes isolated for identification 8 (33.33%) were from Mangalavana, another 8 (33.33%) cultures were from Narakkal, 6 (25.00%) from Puthuvyppu and 2 (8.33%) from light house area of Puthuvyppu. And out of 14 Actinomycetes identified, 3 (21.43%) were

Table 21. Colour pattern of 52 identified actinomycetes in the study area

Station		Colour White	Grey	Red	Orange	Green	Total
I	A	3 (21.43%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	3 (16.66%)
	S	8 (33.33%)	3 (37.50%)	2 (66.67%)	0 (0.00%)	0 (0.00%)	13 (37.14%)
II	A	4 (28.57%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	4 (22.22%)
	S	8 (33.33%)	2 (25.00%)	1 (33.33%)	0 (0.00%)	0 (0.00%)	11 (31.43%)
III	A	3 (21.43%)	0 (0.00%)	3 (100%)	0 (0.00%)	1 (100%)	7 (38.89%)
	S	6 (25.00%)	2 (25.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	8 (22.86%)
IV	A	4 (28.57%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	4 (22.22%)
	S	2 (8.33%)	1 (12.5%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	3 (8.57%)
Ttotal	A	14 (77.78%)	0 (0.00%)	3 (16.67%)	0 (0.00%)	1 (5.56%)	18 (100%)
	S	24 (68.57%)	8 (22.86%)	3 (8.57%)	0 (0.00%)	0 (0.00%)	35 (100%)

A - Actinomycete

S - Streptomycete

isolated from Mangalavana, 4 (28.57%) from Narakkal, 3 (21.43%) from Puthuvyppu and 4 (28.57%) from light house area of Puthuvyppu (Table 21).

Out of 8 Streptomycetes of grey colour, 3 (37.50%) were from Mangalavana, 2 (25%) from Narakkal, 2 (25.00%) from Puthuvyppu and one (12.5%) from Station IV.

Three Actinomycetes of red colour series isolated for identification were from Puthuvyppu and among 3 red coloured Streptomycetes, 2 (66.67%) were from Mangalavana and one (33.33%) from Narakkal. And the only green coloured actinomycete identified was isolated from Puthuvyppu (Table 21).

C. **Sporophore Morphology**

Among 52 cultures identified 31 (59.62%) were of RF type (Rectiflexibles), 14 (26.12%) were of RAS type (both Retinaculiaperti, spirales) only 5 (9.62%) were of spirales and in 2 (3.85%) isolates both RA, RF (Retinaculiaperti, Rectiflexibles) sporphore morphology type was observed (Table 22.1).

Out of 31 isolates of RF type, 20 (64.52%) was of the genus Streptomycetes and 11 (35.48%) of Actinomycetes. RAS type sporophore morphology was observed in 8 (57.14%) Streptomycetes and 6 (42.86%) Actinomycetes. 4 (80%) Streptomycetes was of Spirale type and only one (10%) Actinomycete was of Spirale type. And 2 RARF type observed was identified as Streptomycete (Table 22.2).

Out of 31 (59.62%) isolates which was recorded as RF type, 25 (80.65%) was of white colour series, 3 (10%) were of grey colour series,

Table 22.1 Morphological properties of 52 identified actinomycetes (Sporophore morphology)

Colour / Sporophore morphology	White	Grey	Red	Orange	Green	Total
RF	25 (80.65%)	3 (10.00%)	3 (10.00%)	0 (0.00%)	0 (0.00%)	31 (59.62%)
RAS	11 (78.57%)	4 (26.66%)	0 (0.00%)	0 (0.00%)	1 (6.66%)	14 (26.12%)
S	3 (60.00%)	1 (20.00%)	1 (20.00%)	0 (0.00%)	0 (0.00%)	5 (9.62%)
RARF	1 (50.00%)	0 (0.00%)	1 (50.00%)	0 (0.00%)	0 (0.00%)	2 (3.85%)
Total	38	8	5	0	1	52

RF - Rectiflexibles
RA - Retinaculiaperti
S - Spirales

Table 22.2 Morphological properties of 52 identified actinomycetes (spore morphology)

Spore morphology		Sporophore morphology RF	RAS	S	RARF	TOTAL
Sm	A	11 (36.67%)	6 (50.00%)	1 (20.00%)	0 (0.00%)	18
	S	19 (63.33%)	6 (50.00%)	4 (80.00%)	2 (100.00%)	31
	T	30 (96.77%)	12 (85.71%)	5 (100.00%)	2 (0.00%)	49 (94.23%)
Sp	A	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	S	1 (100.00%)	2 (66.67%)	0 (0.00%)	0 (0.00%)	3
	T	1 (3.33%)	2 (14.29%)	0 (0.00%)	0 (0.00%)	3 (15.77%)
Total	A	11 (35.48%)	6 (42.86%)	1 (20.00%)	0 (0.00%)	18 (34.62%)
	S	20 (64.52%)	8 (57.14%)	4 (80.00%)	2 (100.00%)	34 (65.39%)
	T	31 (59.62%)	14 (26.12%)	5 (9.62%)	2 (3.85%)	52 (100.00%)

RF - Rectiflexibles
RA - Retinaculiaperti
S - Spirales
Sm - Smooth
Sp - Spiny

A - Actinomycetes
S - Streptomycetes

and 3 (10%) was of red colour series. Among 15 isolates recorded as RAS type, white colour series dominated 11 (78.57%) followed by grey colour series 4 (26.66%). and only one was of green colour series 1 (6.66%). 5 isolates were observed as Spirale type out of which 3 (60%) were of white colour and one (20%) of grey and one (20%) of red colour series. And only 2 was recorded as RARF type of which one (50%) was white colour series and the other of red colour series (Table 22.1).

D. **Spore Morphology**

Among 52 isolates identified, smooth type of spore morphology dominated 49 (94.23%) followed by spiny type 3 (5.77%) spore morphology (Table 22.2). Out of 49 (49.2%) isolates which were found to have smooth walled spores, 35 (71.43%) were of white aerial mass colour, 8 (16.33%) were of grey colour. and 6 (12.24%) were of red colour. Among 3 isolates with spiny spores identified all were of white colour.

E. **Sporophore and Spore Morphology**

It was found that out of 31 isolates of RF sporephore morphology, smooth walled spores dominated 30 (96.77%) and only one (3.33%) of spiny type.

Among 14 isolates identified to have RAS type of spore morphology, 12 (85.71%) isolates had smooth walled spores and only 2 (14.29%) had spiny spores. 5 (100%) isolates which showed spirale sporophore morphology were all of smooth walled spores also 2 (100%) isolates with RARF type sporephore morphology were found to harbour smooth walled spores (Table 22.2).

F. **Pigment production**

Out of 52 isolates identified only 19 (36.54%) cultures were able to produce melanoid pigment. and in 15 (28.85%) isolates reverse side pigment was observed and only 9 (17.26%) isolates produced soluble pigment. And it was found that out of 15 cultures showing reverse side pigment, 14 (26.92%) cultures were able to produce melanoid pigment. Also. out of 9 isolates producing soluble pigment. 5 (9.62%) were able to produce melanoid pigment.

G. **Carbon utilisation**

Utilisation of carbon was also used as one of the criteria in identifying the isolates. D-glucose, L-arabinose, D-xylose, i-inositol, D-mannose, D-fructose, rhamnose, sucrose and raffinose were the sugars used in the media (SWA). Among which, D-glucose was utilised by all the 52 isolates, 46 (8.85%) isolates were able to utilise L-arabinose and D-xylose, about 43 (82.7%) were able to utilise D-mannose. 38 (73.1%) were able to grow in the presence of rhamnose. and 37 (71.2%) in the presence of raffinose, 34 (65.4%) able to utilise i-inositol, and only 50% of the isolates were able to utilise sucrose.

6. ANTAGONISTIC PROPERTY OF THE ISOLATED ACTINOMYCETES

In order to find out the antagonistic property of the isolated actinomycetes, 14 test organisms were used.

A. **Antibacterial activity**

Among 104 cultures tested for antibacterial activity, 37 (35.6%) isolates showed antagonism against gram-positive bacteria. 59 (56.7%) showed

resistance against gram-negative bacteria and 33 (31.7%) of the total isolates showed antagonism towards both gram-positive and gram-negative bacteria (Table 23).

a. **Spatial distribution of the antagonistic actinomycetes exhibiting antibacterial activity**

Out of 37 isolates showing antagonism towards gram-positive bacteria 14 (37.84%) were isolated from Mangalavana, 13 (13.14%) from the light house area of Puthuvyppu, 7 (2.17%) from Narakal and only 3 (8.11%) from Puthuvyppu. Whereas, among 59 (56.7%) isolates which showed antibiotic activity towards gram-negative bacteria, maximum 20 (33.90%) cultures were isolated from the light house area of Puthuvyppu, followed by 19 (32.20%) from Mangalavana, 11 (18.64%) from Narakkal and 9 (15.25%) from Puthuvyppu. 33 isolates which showed combined activity towards both gram-positive, and negative bacteria, 14 (42.42%) were isolated from Mangalavana, from light house area. 13 (39.39%) cultures were isolated, 5 from Narakkal and only one (3.0%) from Puthuvyppu (Table 23).

b. **Seasonal distribution of the antagonistic actinomycetes exhibiting antibacterial activity**

Among 104 cultures isolated to test their antagonistic effect, 37 cultures showing antagonism towards gram-positive bacteria were isolated during pre-monsoon, monsoon, post-monsoon with 22 (59.46%), 11 (29.73%), 4 (10.81%) cultures respectively. Among 59 isolates showing antagonism towards gram-negative bacteria, maximum 28 (47.46%) cultures were isolated during pre-monsoon followed by 19 (32.20%) in monsoon, 10 (16.95%) during

Table 23. Activity (%) of 104 antagonistic actinomycetes against test pathogens in the study area

Antagonistic Activity	Station	I	II	III	IV	Total
Antibactrial	G+	14 (37.84%)	7 (18.91%)	3 (8.11%)	13 (35.14%)	37
	G-	19 (32.20%)	11 (18.64%)	9 (15.25%)	20 (33.90%)	59
	G+ and G-	14 (42.42%)	5 (15.15%)	1 (3.00%)	13 (39.39%)	33
Antifungal	F	26 (25.00%)	26 (25.00%)	27 (25.96%)	25 (24.04%)	104
	NF	23 (25.56%)	20 (22.22%)	24 (26.67%)	23 (25.56%)	90
	F and NF	23 (25.56%)	20 (22.22%)	24 (26.67%)	23 (25.56%)	90
Antibacterial and Antifungal	G+ and F	14 (37.84%)	7 (18.91%)	3 (8.11%)	13 (35.14%)	37
	G- and F	19 (32.20%)	11 (18.64%)	9 (15.25%)	20 (33.90%)	59
	G+,G- and F	14 (42.42%)	5 (15.15%)	1 (3.00%)	13 (39.39%)	33

G+ - Gram-positive, G- - Gram-negative

F - Filamentous, NF - Non-filamentous

post-monsoon season. Out of 33 cultures showing combined activity against both gram-positive and gram-negative bacteria, 20 (60.61%) were isolated during the pre-monsoon season, 11 (33.33%) in monsoon and 4 (12.12%) in post-monsoon (Table 24).

c. Distribution of antagonistic actinomycetes exhibiting antibacterial activity according to their colour series.

Out of 37 antagonistic actinomycetes showing activity against gram-positive bacteria, white colour series was dominating 17 (45.95%), followed by grey colour 13 (35.14%), red colour 7 (18.92%) and none were of orange or green colour. Among 59 actinomycetes showing antagonism towards gram-negative bacteria, 31 (52.54%) were of white colour series, 21 (35.59%) of grey colour, 4 (6.78%) of red colour, 2 (3.39%) of orange colour and only one (1.69%) was of green colour. And 33 actinomycetes showing antagonism against both gram-positive and gram-negative bacteria, 16 (48.49%) were of white colour, 13 (39.39%) of grey colour 4 (12.12%) of red colour and none were of orange or green series (Table 25).

B. **Actinomycetes showing antifungal activity**

104 cultures isolated when tested for antifungal activity, it was found that all the cultures showed antagonism towards the test organism Cladosporium. And 90 (86.5%) cultures showed antagonism towards non-filamentous fungi, and the same number of cultures showed the combined effect (ie) antagonism towards both filamentous and non-filamentous fungi (Table 23).

Table 24. Activity (%) of 104 antagonistic actinomycetes against test pathogens in different seasons.

Antagonistic Activity	Season	Pre-monsoon	Monsoon	Post-monsoon	Total
Antibacterial	G+	22 (59.46%)	11 (29.73%)	4 (10.81%)	37
	G-	28 (47.46%)	19 (32.20%)	10 (16.95%)	59
	G+ & G-	20 (60.61%)	11 (33.33%)	4 (12.12%)	33
Antifungal	F	55 (52.89%)	27 (25.96%)	22 (21.15%)	104
	NF	47 (52.22%)	23 (25.56%)	20 (22.22%)	90
	F & NF	47 (52.22%)	23 (25.56%)	20 (22.22%)	90
Antibacterial and Antifungal	G+ & AF	22 (59.46%)	11 (29.73%)	4 (10.81%)	37
	G- & AF	28 (47.46%)	19 (32.20%)	10 (16.95%)	59
	G+, & G- AF	20 (60.61%)	11 (33.33%)	4 (12.12%)	33

G+ - Gram-positive G- - Gram-negative
F - Filamentous NF - Non-filamentous

a. **Spatial distribution**

Out of 104 cultures 26 actinomycetes isolated from Mangalavana, 26 from Narakkal, 27 from Puthuvyppu and 25 from the light house area of Puthuvyppu, it was found that all these cultures showed antagonism towards filamentous fungus. And out of 90 cultures showing antagonism towards non-filamentous fungi, 23 (25.56%) were isolated from Station I, 20 (22.22%) from Station II, 24 (26.67%) from Station III and 23 (25.56%) from Station IV (Table 23).

b. **Seasonal distribution**

Out of 104 cultures isolated during the period of study and tested for their antifungal activity it was noted that, the maximum number 55 (52.89%) of isolates which showed antifungal (against filamentous fungi) activity were isolated during the period of pre-monsoon, followed by 27 (25.96%) during monsoon and 22 (21.15%) in post-monsoon. Maximum number 47 (52.22%) of isolates which showed anti-yeast activity were isolated during pre-monsoon, followed by 23 (25.56%) in monsoon and 20 (22.22%) in post-monsoon, among the 104 isolated cultures tested for anti-yeast activity. It was also found that the same cultures showing the anti-yeast activity also were antagonistic towards both filamentous and non-filamentous fungi (Table 23).

c. **Distribution of antagonistic actinomycetes exhibiting antifungal activity according to their colour series**

All the cultures isolated (with 4 different aerial mass colour) during the period of study showed antagonism towards filamentous fungi. And out of 90 isolates which exhibited antagonism towards non-filamentous fungi

Table 25. Activity (%) of 104 antagonistic actinomycetes against test pathogens in different colour series.

Antagonistic Activity \ Colour		White	Grey	Red	Orange	Green	Total
Antibacterial	G+	17 (45.95%)	13 (35.14%)	7 (18.92%)	0 (0.00%)	0 (0.00%)	37
	G-	31 (52.54%)	21 (35.59%)	4 (6.78%)	2 (3.39%)	1 (1.69%)	59
	G+ & G-	16 (48.49%)	13 (39.39%)	4 (12.12%)	0 (0.00%)	0 (0.00%)	33
Antifungal	F	61 (58.65%)	30 (28.85%)	9 (8.65%)	3 (2.89%)	1 (0.96%)	104
	NF	51 (56.67%)	28 (31.11%)	8 (8.89%)	2 (2.22%)	1 (1.11%)	90
	F & NF	51 (56.67%)	28 (31.11%)	8 (8.89%)	2 (2.22%)	1 (1.11%)	90
Antibacterial and Antifungal	G+ & AF	17 (45.95%)	13 (35.14%)	7 (18.92%)	0 (0.00%)	0 (0.00%)	37
	G- & AF	31 (52.54%)	21 (35.59%)	4 (6.78%)	2 (3.39%)	1 (1.69%)	59
	G+ G- & AF	16 (48.49%)	13 (39.39%)	4 (12.12%)	0 (0.00%)	0 (0.00%)	33

G+ - Gram-positive, G- - Gram-negative

F - Filamentous, NF - Non-filamentous

51 (56.67%) were of white colour, 28 (31.11%) were of grey colour, 8 (8.89%) of red colour, 2 (2.22%) orange colour and one (1.11%) green coloured actinomycete (Table 25).

C. **Actinomycetes showing both antibacterial and antifungal activity**

Out of 26 cultures isolated from Mangalavana, maximum number of cultures 19 (73.0%) showed activity against gram-negative bacteria and fungi, followed by 14 (54.0%) showing antagonism towards gram-positive bacteria and fungi and also same number of cultures 14 (54.0%) showed activity against both gram-negative and positive bacteria also towards fungi. Among 26 cultures isolated form Narakkal, only 7 (27.0%) showed antagonism towards gram-positive bacteria and fungi, 11 (43.3%) towards gram-negative bacteria and fungi, 5 (19.2%) against gram-positive, gram-negative bacteria and fungi. From Puthuvyppu area 27 actinomycetes were isolated, where 9 (33.3%) showed antagonism towards gram-negative bacteria and fungi, only 3 (11.0%) towards gram-positive bacteria and fungi and only one culture was able to exhibit antagonism towards all the test organisms. 25 isolates from the light house area of Puthuvyppu were tested for antagonistic property, 20 (88%) of them showed antagonism towards gram-negative bacteria and fungi, whereas only 13 (52.0%) showed activity against gram-positive bacteria and fungi, and the same number of cultures showed antagonism towards all the test organisms (Table 23).

a. **Seasonal distribution**

It was found that in all the 3 seasons, maximum number of cultures showed activity against gram-negative bacteria and fungi (as 28 (50.9%) during pre-monsoon, 19 (70.4%) during monsoon and 10 (45.5%) in post-

monsoon). And 22 (40%) cultures in pre-monsoon, 11 (40.7%) in monsoon and 4 (18.2%) in post-monsoon showed activity against gram-positive bacteria and fungi. During pre-monsoon, monsoon and post-monsoon, 20 (36.4%), 11 (40.7%) and 4 (18.2%) showed antagonism towards all the organism tested for antagonistic property respectively (Table 24).

b. **Distribution according to colour series**

Maximum number (31) of cultures which showed antagonism towards gram-negative bacteria and fungi was of white colour series, followed by grey colour series 21, red colour 4 and 2 of orange colour series and the one culture of green colour isolated showed the same type of activity. And 61 actinomycetes isolated of white colour series when tested for antagonistic effect, 16 (26.2%) of them showed activity against gram-positive bacteria and fungi and 17 (27.9%) against all the test organism. Out of 30 grey colour isolates tested, 14 (46.7%) showed activity against gram-positive bacteria and fungi and 13 (43.3%) against all the test organisms. Out of 9 red coloured actinomycete, 6 (66.7%) showed antagonism towards gram-positive bacteria and fungi and 4 (44.4%) against all the organism tested. And none of the orange or green coloured actinomycetes isolated showed either activity against gram-positive bacteria and fungi or against all the test organisms (Table 25).

D. **Antagonistic nature of the isolated actinomycetes against each test organism**

All the cultures isolated (104) were able to inhibit the growth of Cladosporium. And 86.5% of the tested cultures showed anti-yeast activity

(ie. against Rhodotorula rubra and R. marina). 9 gram-negative bacteria were used as test organism and maximum number of cultures 41 (39.4%) showed activity against E. coli, followed by 40 (38.5%) against V. parahaemolyticus. and 33 (31.7%) against V. cholerae, same number of cultures 33 (31.7%) showed antagonism towards Salmonella -I, 21 (20.2%) against V. alginoliticus 31 (30.2%) against Pseudomonas, 30 (29.8%) against Aeromonas, 25 (24.0%) against Salmonella - II and only 21 (20.2%) were able to show antagonistic effect against V. anguillarum.

And among the two gram-positive bacteria used as test organism, 33 (31.7%) were effective against Staphylococcus and 37 (35.6%) against Bacillus (Table 26).

E. **Antibiogram**

Antibiogram of 104 isolated actinomycetes from the selected area by cross streakassay method.

On the basis of the antagonistic property of the 104 isolates, the results are grouped into 10 categories as follows:

Category - I : Isolates showing inhibition zone of 1-3 mm against test pathogens.

Category - II : Isolates showing inhibition zone of 4-6 mm against test pathogens.

Category - III : Isolates showing inhibition zone of 7-9 mm against test pathogens.

Category - IV : Isolates showing inhibition zone of 10-12 mm against test pathogens.

Category V : Isolates showing inhibition zone of 13-15 mm against test pathogens.

Category VI : Isolates showing inhibition zone of 16-18 mm against test pathogens.

Category VII : Isolates showing inhibition zone of 19-21 mm against test pathogens.

Category VIII : Isolates showing inhibition zone of 22-24 mm against test pathogens.

Category IX : Isolates showing inhibition zone of 25-27 mm against test pathogens.

Category X : Isolates showing inhibition zone of 70-80 mm against test pathogens.

21 (20.2%) antagonistic actinomycetes showed inhibition to test pathogen Vibrio anguillarum, maximum zone of inhibition was recorded in (4-6 mm) Category II and X (70-80 mm), (Table 25) V. alginolitius was also inhibited by same number of actinomycetes and maximum inhibition was found in Category X. Maximum number (40) of actinomycetes showed antagonism towards V. parahaemolyticus and the highest inhibition was seen in Category X. 33 isolates exhibited inhibition towards V. cholerae, and the highest inhibition was observed in Category I. Pseudomonas and Aeromonas were inhibited by 31 and 30 antagonistic actinomycetes respectively but differed greatly in their inhibitory activity. Maximum number of isolates inhibiting Pseudomonas were found in Category I, whereas, those inhibiting Aeromonas

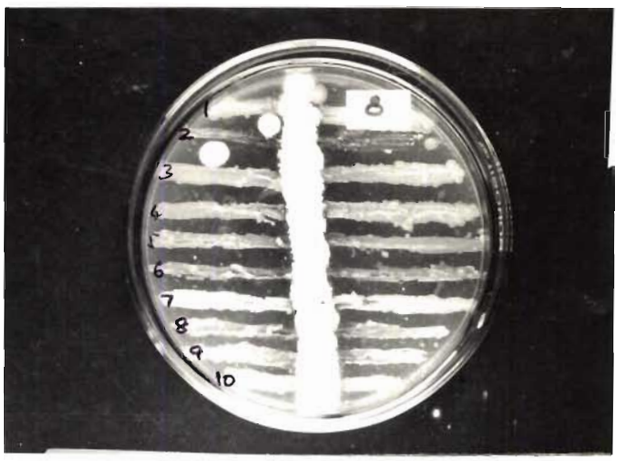
Table 26: Antibiogram of 104 isolated actinomycetes from the selected study area by Cross streak assay method

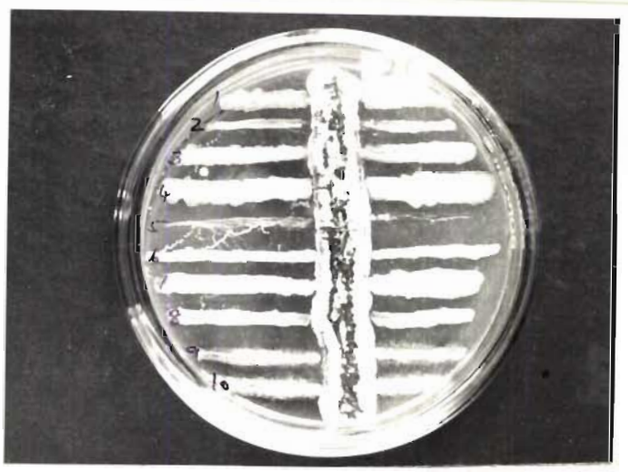
Test Pathogen	No Inhibition	Total Inhibition	Zone of inhibition in mm									
			1 - 3	4 - 6	7 - 9	10 - 12	13 - 15	16 - 18	19 - 21	22 - 24	25 - 27	70 - 80
			Category									
			I	II	III	IV	V	VI	VII	VIII	IX	X
V. anguillarum	83 (79.8%)	21 (20.2%)	5 (4.8%)	6 (5.8%)	2 (1.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (1.9%)	0 (0.0%)	0 (0.0%)	6 (5.8%)
V. cholerae	71 (68.3%)	33 (31.7%)	13 (12.5%)	8 (7.7%)	1 (1.0%)	0 (0.0%)	0 (0.0%)	1.0 (1.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	10 (9.6%)
V. alginoliticus	83 (79.8%)	21 (20.2%)	5 (4.8%)	2 (1.9%)	2 (1.9%)	1 (1.0%)	1 (1.0%)	2 (1.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	8 (7.7%)
V. parahaemolyticus	64 (61.5%)	40 (38.5%)	8 (7.7%)	8 (7.7%)	0 (0.0%)	4 (3.9%)	2 (1.9%)	0 (0.0%)	1 (1.0%)	0 (0.0%)	0 (0.0%)	17 (16.4%)
Aeromonas	74 (71.2%)	30 (28.9%)	6 (5.8%)	1 (1.0%)	1 (1.0%)	1 (1.0%)	0 (0.0%)	1 (1.0%)	1 (1.0%)	0 (0.0%)	1 (1.0%)	18 (17.3%)
Pseudomonas	73 (70.2%)	31 (29.8%)	15 (14.4%)	6 (5.8%)	0 (0.0%)	0 (0.0%)	1 (1.0%)	0 (0.0%)	1 (1.0%)	0 (0.0%)	2 (1.9%)	6 (5.8%)
Salmonella - I	71 (68.3%)	33 (31.7%)	9 (8.7%)	5 (4.8%)	2 (1.9%)	4 (3.9%)	0 (0.0%)	0 (0.0%)	3 (2.9%)	0 (0.0%)	1 (1.0%)	9 (8.7%)
Salmonella - II	79 (76.0%)	25 (24.0%)	2 (1.9%)	7 (6.7%)	1 (1.0%)	4 (3.9%)	0 (0.0%)	0 (0.0%)	2 (1.9%)	0 (0.0%)	1 (1.0%)	8 (7.7%)
E. coli	63 (60.0%)	41 (39.4%)	3 (2.9%)	7 (6.7%)	3 (2.9%)	2 (1.9%)	1 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	25 (24.0%)
Bacillus	67 (64.4%)	37 (35.6%)	11 (10.6%)	7 (6.7%)	0 (0.0%)	1 (1.0%)	1 (1.0%)	1 (1.0%)	2 (1.9%)	0 (0.0%)	2 (1.9%)	12 (11.5%)
Staphylococcus	71 (68.3%)	33 (31.7%)	6 (5.8%)	5 (4.8%)	2 (1.9%)	2 (1.9%)	1 (1.0%)	0 (0.0%)	1 (1.0%)	0 (0.0%)	0 (0.0%)	16 (15.4%)
R. rubra	14 (13.5%)	90 (86.5%)	1 (1.0%)	0 (0.0%)	1.0 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	14 (13.5%)	5 (4.8%)	2 (1.9%)	67 (64.4%)
R. marina	14 (13.5%)	90 (86.5%)	1 (1.0%)	0 (0.0%)	1.0 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	11 (10.6%)	6 (5.8%)	4 (3.9%)	67 (64.4%)
Cladosporium	0 (0.0%)	100 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	104 (100%)

Antagonistic activity of actinomycetes (cross streak assay)

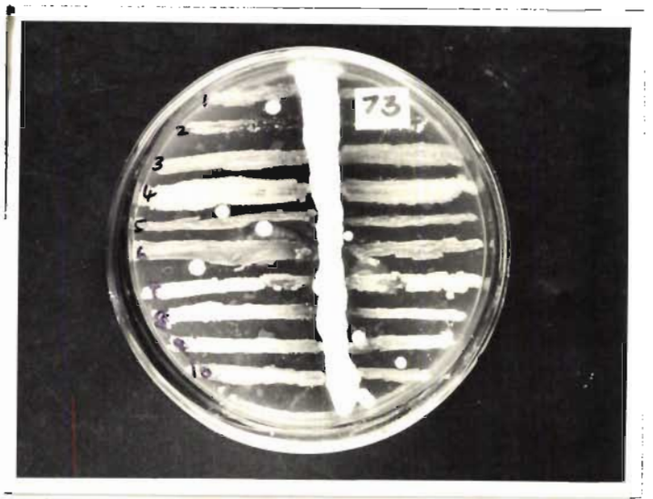
Test organisms

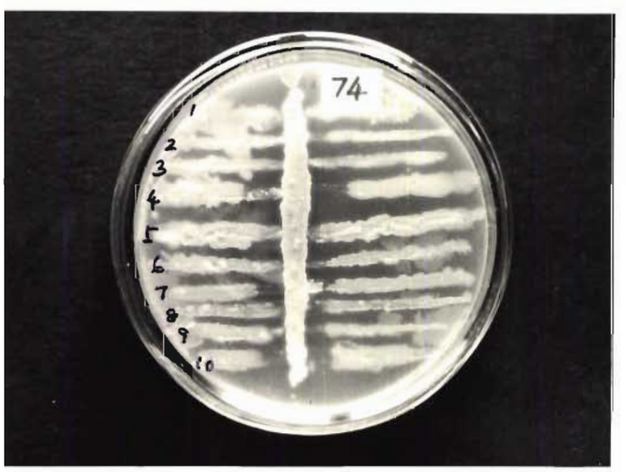
1. *V. anguillarum*
2. *V. cholerae*
3. *V. alginoliticus*
4. *V. parahaemolyticus*
5. *Aeromonas*
6. *Pseudomonas*
7. *Salmonella - I*
8. *Salmonella - II*
9. *Bacillus*
10. *Staphylococcus*


8
1
2
3
4
5
6
7
8
9
10

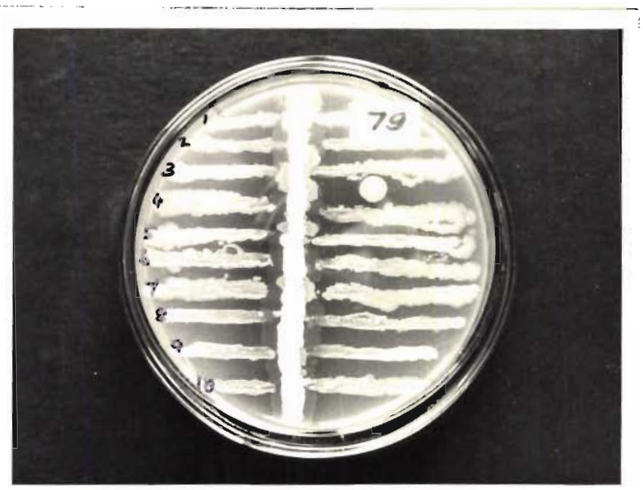

2
10

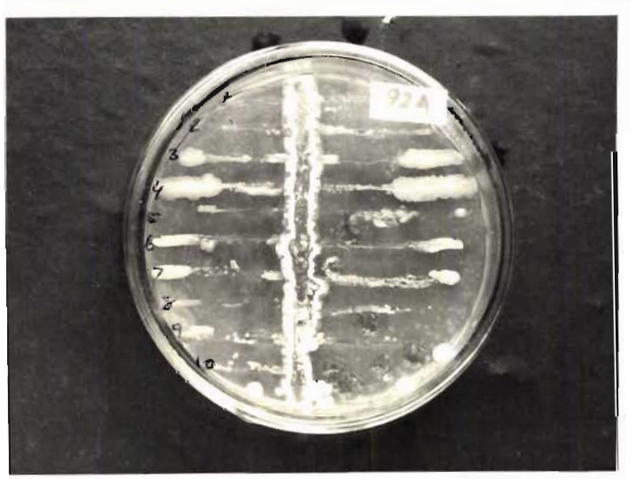

30
1
2
3
4
5
6
7
8
9
10


73
1
2
3
4
5
6
7
8
9
10


74
1
2
3
4
5
6
7
8
9
10

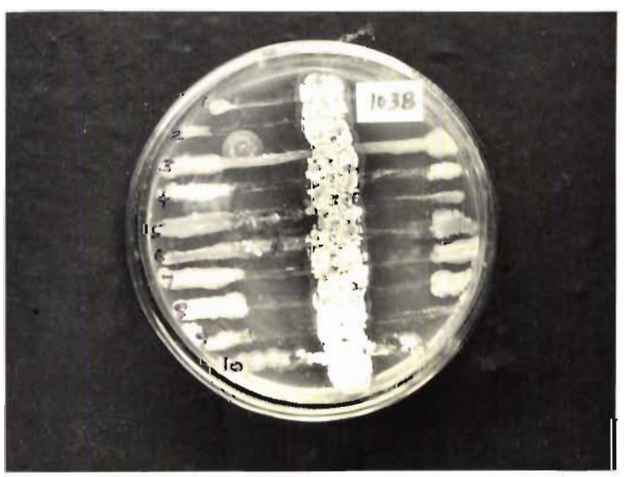

75A
1
2
3
4
5
6
7
8
9
10


79


92A


93B


103A


103B


101A
1
2
3
4
5
6
7
8
9
10


140
1
2
3
4
5
6
7
8
9
10

were recorded in the Category X. 33 isolates were able to inhibit Salmonella-I and the maximum inhibition was found in Category 1 and X. Salmonella-II was inhibited by 25 isolates and the maximum inhibition was seen in Category X. E. coli was the most sensitive bacteria inhibited by 41 antagonistic actinomycetes and maximum inhibition was found in Category X. 37 antagonistic actinomycetes inhibited Bacillus and maximum inhibition zone falls under Category X. Staphylococcus was inhibited by 33 antagonistic actinomycetes and maximum inhibition zone falls in Category X. Rhodotorula rubra and R. marina were found to be inhibited by 90 isolates and maximum inhibition occured in the zone of 70-80 mm. Cladosporium was inhibited by all the isolates and maximum inhibition was found in Category X. The overall picture of cross streak assay showed that only 2 extreme zones of inhibition patterns was exhibited by most of the antagonistic actinomycetes (Table 26).

7. ANTAGONISTIC ACTIVITY OF CRUDE ANTIBIOTIC EXTRACT FROM SELECTED ACTINOMYCETE CULTURES

Out of the 104 cultures isolated 6 cultures giving different type of inhibition were selected. Six of the following cultures (3 actinomycetes and 3 streptomycetes) were selected according to their nature of activity.

(1) Active against gram-positive bacteria, gram-negative bacteria, filamentous and non-filamentous fungi (Strain-103A, 92A).

(2) Active against gram-positive bacteria, filamentous and non-filamentous fungi (Strain-104).

(3) Active against gram-negative bacteria, filamentous and non-filamentous fungi (Strain-187).

Antagonistic activity of the antibiotic extracts of actinomycetes

(Agar difusion method)

C - Chloroform

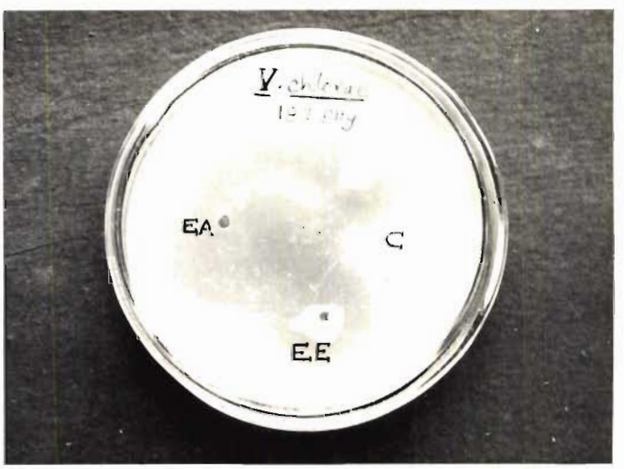
EE - Ethyl Ether

EA - Ethyl Acetate


92A
EE
GA
92A
EE
EA


92A
C
EA
92A
EA
EE


V. anguillarum
187 Phg
EA
C
EE


V. cholerae
EA
C
EE


Staphylococcus
EE
C
EA

(4) Active against filamentous and non-filamentous fungi (Strain-202).

(5) Active only against filamentous fungi (Strain-190).

These 6 cultures were mass cultured and extracted the extracellular antibiotics using non-polar solvents viz. chloroform, ethyl ether and ethyl acetate at pH 4.0, 7.0 and 9.0. The antagonistic activity of these antibiotic extracts are given in the figure 8-13.

Antagonistic activity of crude antibiotic strain 103A against test pathogens showed highest activity against V. parahaemolyticus at pH 4.0 and 7.0 in ethyl acetate, and the original strain also showed antagonistic activity towards V. parahaemolyticus. Staphylococcus was the next actively inhibited test pathogen at pH 7.0 in ethyl acetate. No inhibition was found at pH 7.0 in ethyl acetate against V. alginoliticus, Pseudomonas and E. coli. In ethyl ether also pH 7.0 was found to be optimum and highest activity was found for V. parahaemolyticus. While using chloroform as the extraction solvent, peak activity was obtained for V. parahaemolyticus, less intense activity was obtained for Salmonella-I and Bacillus, but original culture showed no antagonism (Fig.8).

Antagonistic activity of crude antibiotic extract of strain 92A against test pathogens showed highest activity against V. parahaemolyticus at pH 7.0 in ethyl acetate. Original strain also showed antagonistic activity towards V. parahaemolyticus., V. cholerae, Aeromonas and Bacillus were the next actively inhibited test pathogens at pH 9.0 in ethyl acetate. When the antibiotic was extracted at pH 4.0 in ethyl acetate, none of the test pathogens were inhibited except minimal activity was found towards Aeromonas,

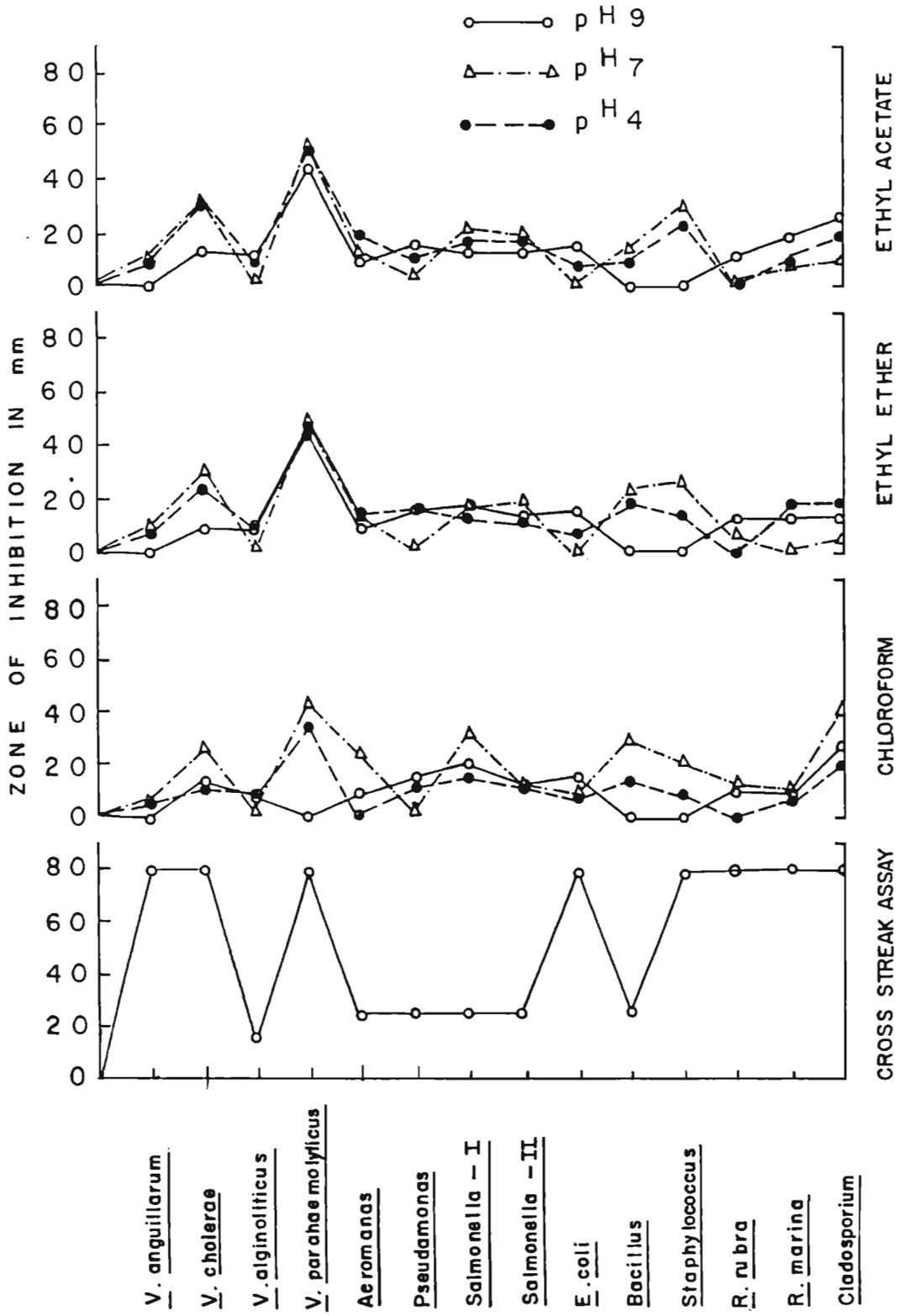


Fig. 8. Antagonistic activity of the antibiotic extract of Strain No.103A against test pathogens.

R. marina and cladosporium. Antibiotic extract of ethyl ether at all pH tested showed poor inhibition except for cladosporium at pH 7.0. While using chloroform as the extraction solvent maximum activity was obtained for V. cholerae, V. parahaemolyticus, Bacillus, Staphylococcus and Cladosporium at pH 7.0, whereas the original strain showed maximum activity against V. cholerae, V. parahaemolyticus, Aeromonas, Salmonella-I, Salmonella-II, Staphylococcus and against Cladosporium (Fig.9).

Antagonistic activity of strain 104 was only towards gram-positive bacteria Bacillus, Staphylococcus, non-filamentous fungi R. rubra, R. marina and towards filamentous fungi Cladosporium. Whereas the crude antibiotic were even active against gram-negative bacteria. Ethyl acetate gave the best result at pH 7 where all the test pathogens were inhibited. When antibiotic was extracted at pH 4.0 with ethyl acetate gave better results than at pH 7.0. Whereas when ethyl ether was used as the solvent for the extraction of antibiotic, pH 4.0 and pH 7.0 gave better results then at pH 9.0. When chloroform was used all the test pathogens were inhibited at pH 4.0 and the activity being in the zone of inhibition between 10 to 40 mm, whereas at pH 7.0 also all the test pathogens were inhibited except for V. cholerae and Aeromonas. But only Staphylococcus was inhibited at pH 4.0 with the chloroform extract (Fig.10).

Strain 187 which was identified as S. cacaoi inhibited all the tested gram-negative bacteria, filamentous and non-filamentous fungi except the gram-positive bacteria. Antagonistic activity of crude antibiotic of the Strain 187 showed highest activity towards V. cholerae only at pH 9.0 in

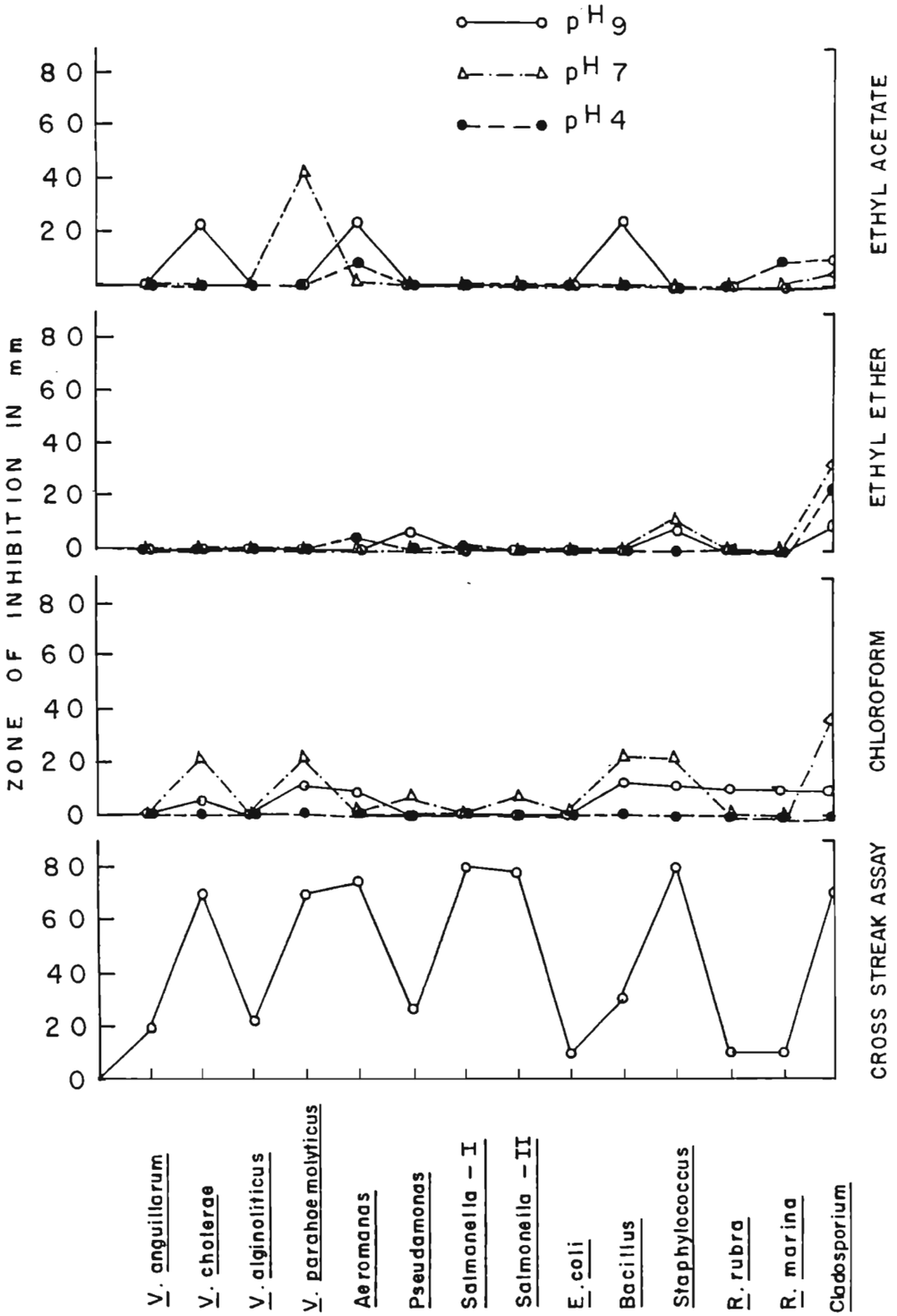


Fig.9. Antagonistic activity of the antibiotic extract of Strain No.92A against test pathogens.

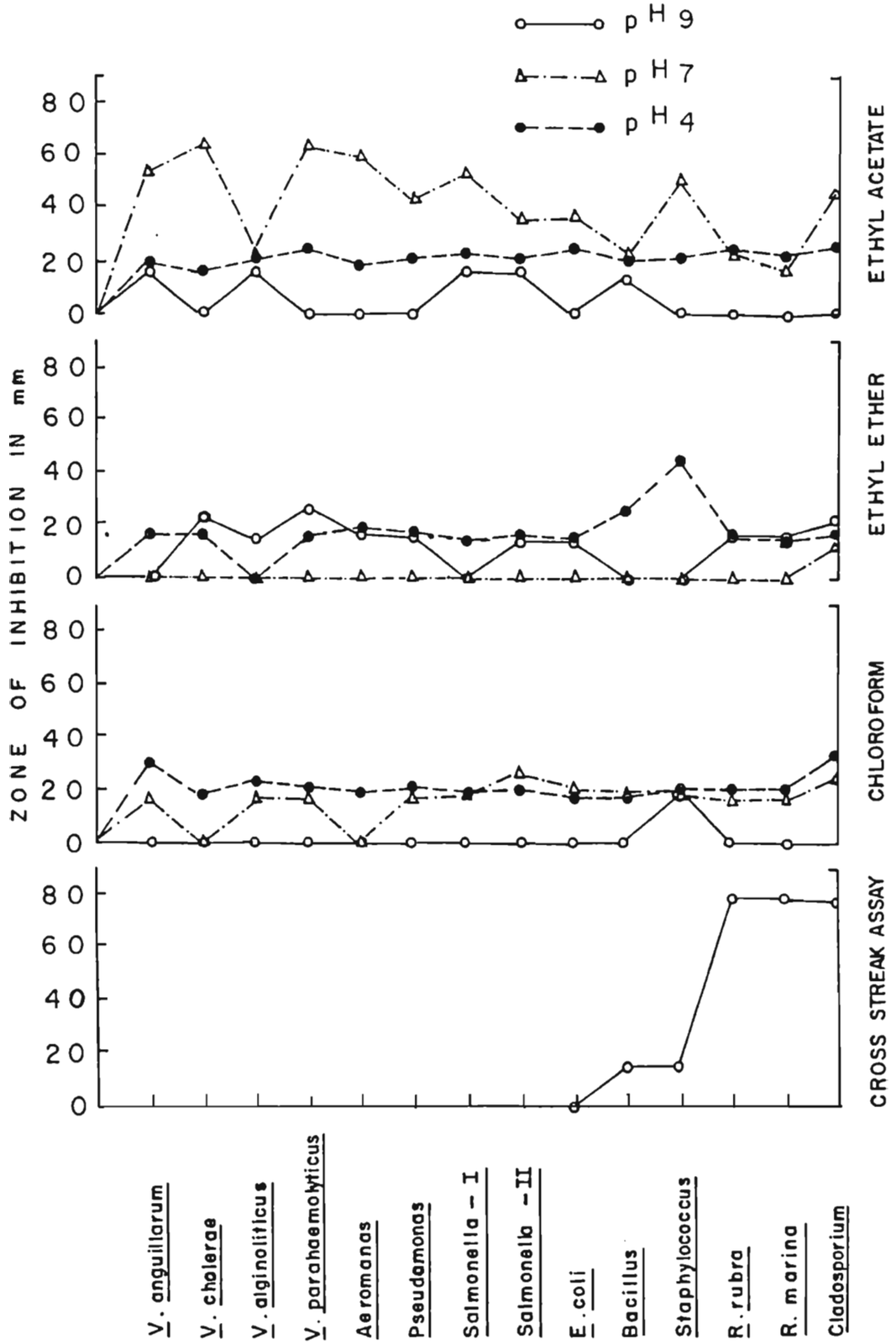


Fig. 10. Antagonistic activity of the antibiotic extract of Strain No. 104 against test pathogens.

ethyl acetate whereas pH 7.0 showed the highest activity towards V. parahaemolyticus and Cladosporium. In ethyl ether pH 7.0 was found to be optimum and highest activity was found towards V. parahaemolyticus. Whereas ethyl ether in pH 9.0 showed maximum activity towards V. anguillarum and V. cholerae. In chloroform also pH 7.0 was found to be optimum and showed maximum activity towards V. parahaemolyticus and V. cholerae, V. anguillarum, V. alginoliticus, Aeromonas, Pseudomonas, Salmonella-II, E. coli, Bacillus, Staphylococcus, R. rubra, R. marina and Cladosporium were also inhibited. Chloroform at pH 9.0 and 7.0 were also able to inhibit most of the test pathogens but only with minimal inhibitory activity (Fig.11).

Strain 202 which was identified as A. mutabilis was able to inhibit only filamentous and non-filamentous fungi, whereas the crude antibiotic extracts showed activity towards most of the test pathogens. Maximum activity (80 mm) was shown when chloroform was used at pH 4.0 towards Salmonella-II whereas inhibition towards other test pathogens was only below 20 mm except for Cladosporium (above 30 mm). When ethyl ether was used, pH 7.0 was found to be the best which inhibited all the test organisms except Bacillus and R. rubra. And pH 9.0 showed better result than pH 4.0 where none of the test organisms were inhibited. Also pH 4.0 in ethyl acetate did not inhibit any of the test organisms whereas pH 7.0 showed better result where Staphylococcus, R. marina and Cladosporium were inhibited to maximum than other test organisms, at pH 4.0 most of the test organisms were inhibited but with minimal inhibitory action (below 20 mm) (Fig.12).

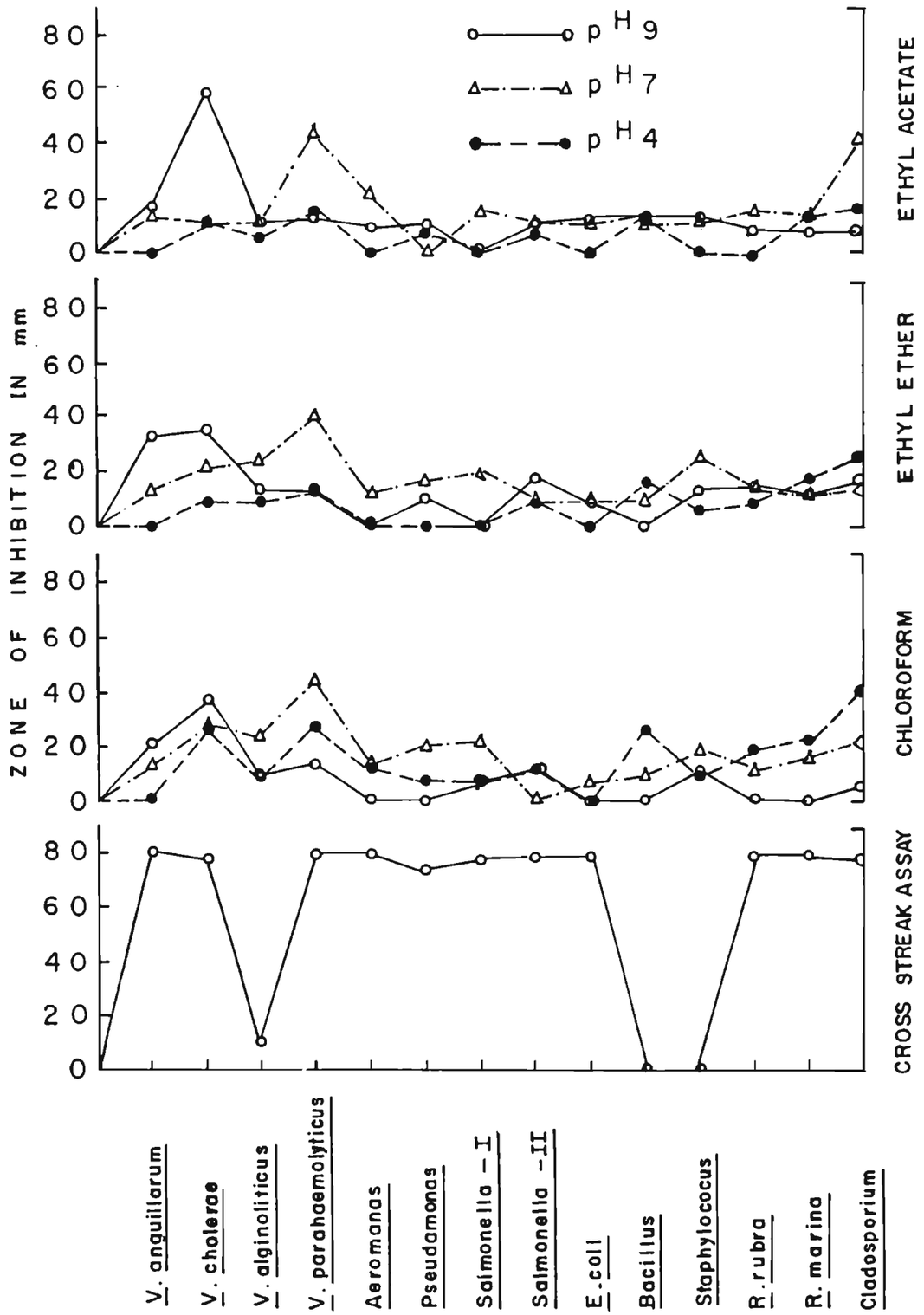


Fig.11. Antagonistic activity of the antibiotic extract of Strain No.187 against test pathogens

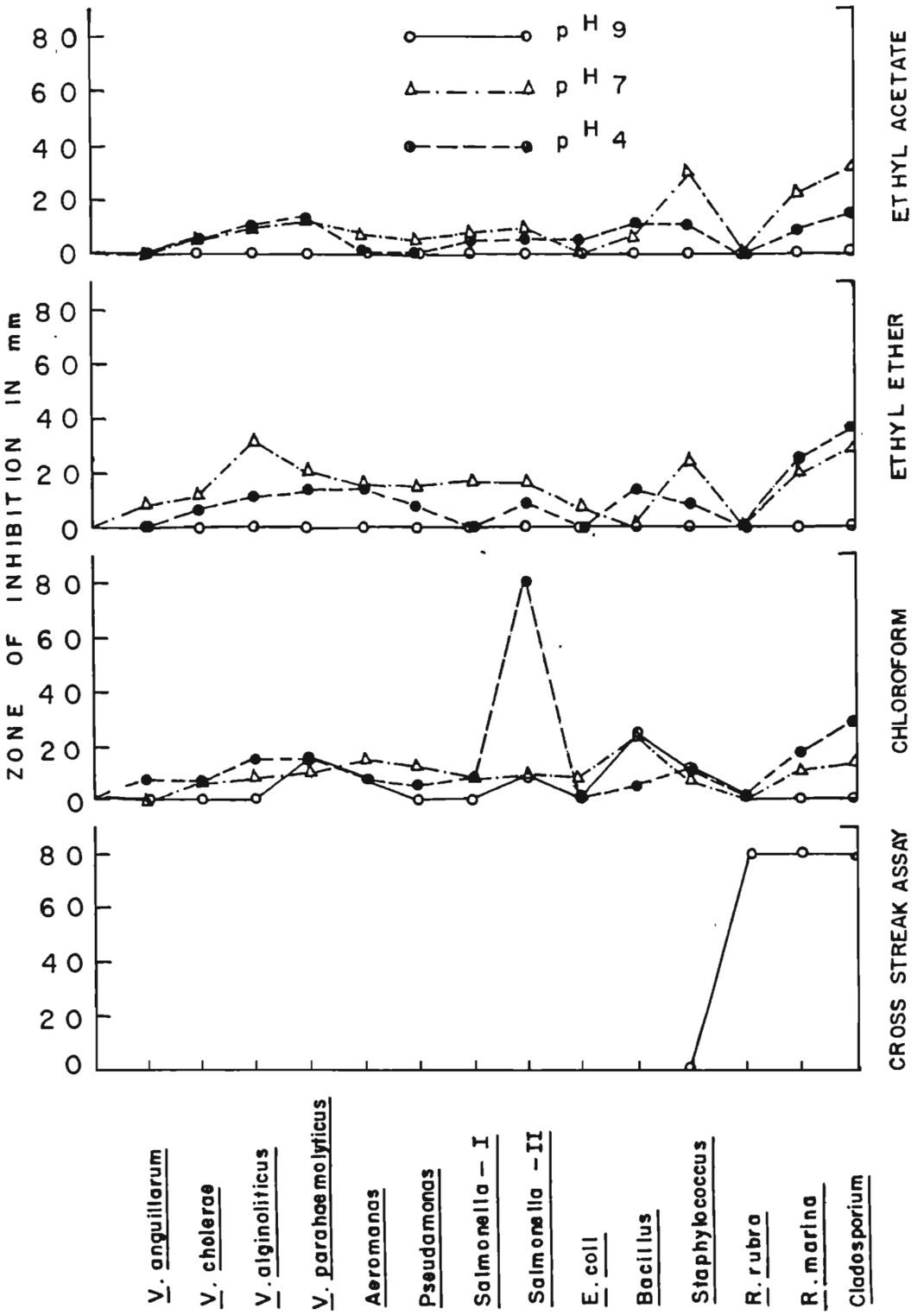


Fig.12 Antagonistic activity of the antibiotic extract of Strain No.202 against test pathogens.

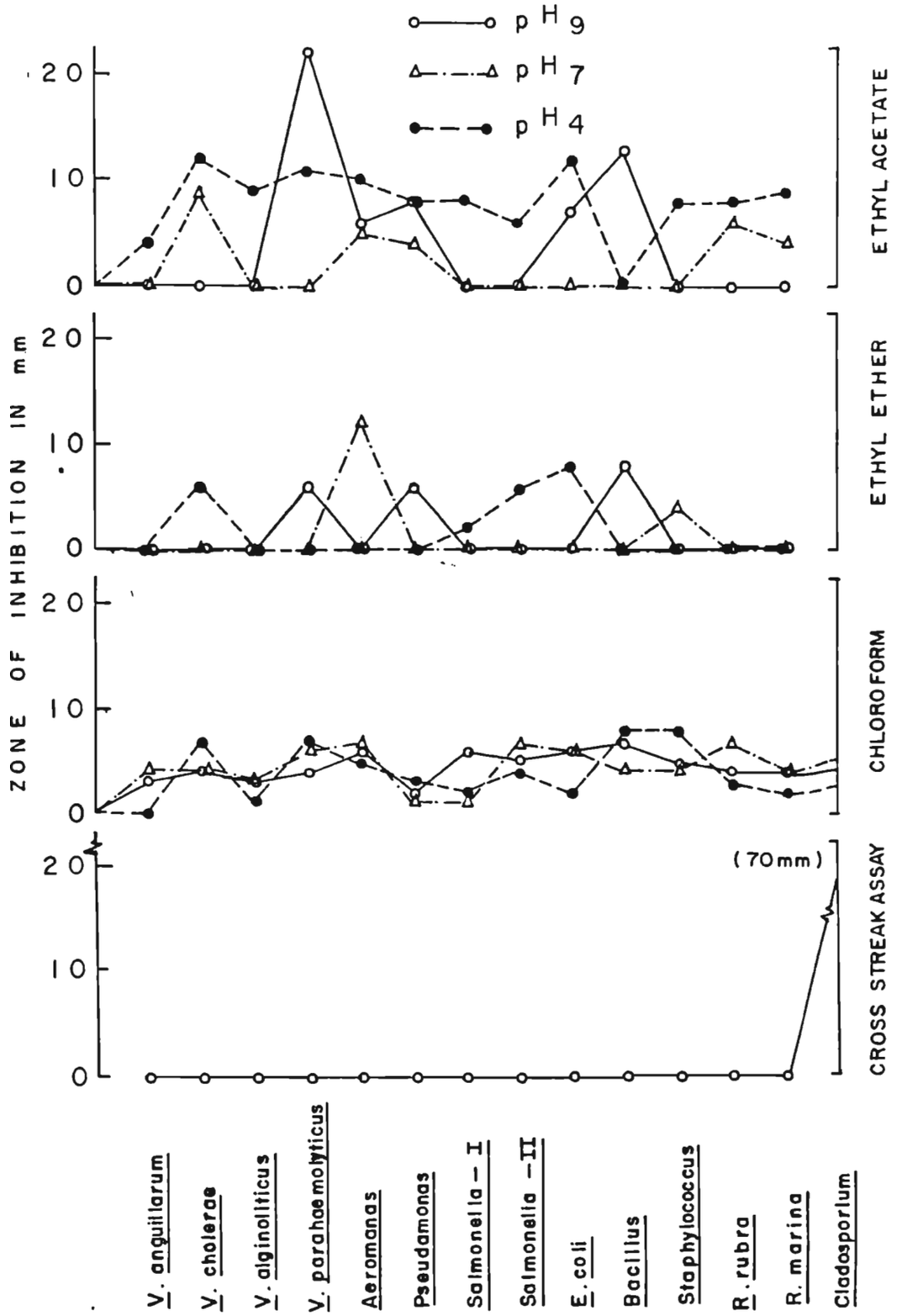


Fig.13. Antagonistic activity of the antibiotic extract of Strain No.190 against test pathogens.

Strain 190 which was identified as A. flavescens was able to inhibit only Cladosporium. Whereas the crude antibiotic extracts were able to inhibit most of the test organisms but only with minimal inhibitory action ie. below 20 mm, none of the antibiotic extracts showed activity more than 20 mm. Here, maximum inhibition was obtained towards V. parahaemolyticus with ethyl acetate at pH 9.0, followed by Bacillus, E. coli, Pseudomonas, and Aeromonas and pH 4.0 was found to be the next best optimum pH of ethyl acetate, where all the test organisms were inhibited except Bacillus, pH 7.0 in ethyl acetate also inhibited V. cholerae Aeromonas, Pseudomonas, R. rubra, R. marina and Cladosporium. In ethyl ether at pH 7.0 only Aeromonas and Staphylococcus were inhibited, at pH 4.0 V. cholerae, Salmonella-I, Salmonella-II, E. coli were inhibited at pH 9.0, V. parahaemolyticus, Pseudomonas and Bacillus were inhibited. In all the 3 pH selected, chloroform showed activity towards most of the test pathogens but with minimum inhibition below 10 mm (Fig.13).

8. INVITRO EVALUATION OF pH ON SOLVENT IN TESTING THE ANTIMICROBIAL ACTIVITY OF SELECTED ISOLATES (TABLE 27)

Strain 103A identified as S. mobilis showed the same activity in culture as well as in extracted crude antibiotic form. Ethyl acetate was found to be the best solvent at pH 4.0 (Fig.8). The Strain 92A was identified as A. aureocirculatus showed effective antagonistic activity towards all the test pathogens whereas the crude antibiotic extracted from this strain was effective against only gram-positive bacteria, gram-negative bacteria and filamentous fungi. Chloroform was found to be the best suitable solvent at pH 7.0 (Fig.9). Strain 104 identified as S. roseus was inhibiting gram-

Table 27. Invitro evaluation of pH on solvents in testing antimicrobial activity of the antibiotic extracts of selected isolates.

Strain	Identified as	Antibiotic Activity		Suitable	Optimum
		Original strain (C S A)	Crude antibiotic extract (A D A)	Solvent	pH
103 A	S. noblis	Gp, Gn, F, NF	Gp, Gn, F, NF	Ethyl acetate	4
92 A	A. aureocirculatus	Gp, Gn, F, NF	Gp, Gn, F	Chloroform	7
104	S. roseus	Gp, F, NF	Gp, Gn, F, NF	Ethyl acetate	7 and 4
187	S. cacaoi	Gn, F, NF	Gp, Gn, F, NF	Ethyl ether	7
202	A. mutabilis	F, NF	Gp, Gn, F, NF	Ethyl ether	7
190	A. flavescens	F	Gp, Gn, F, NF	Ethyl acetate	4

C S A - Cross Streak Assay; A D A - Agar Diffusion Assay
Gp - Gram-positive bacteria; Gn - Gram-negative bacteria
F - Filamentous fungi; NF - Non-filamentous fungi

positive bacteria, filamentous and non-filamentous fungi whereas extracted antibiotic by this strain exhibited antagonism towards all the test pathogens. pH 4.0 and 7.0 gave good results in ethyl acetate (Fig.10). Strain 187 identified as S. cacaoi was effective against gram-negative bacteria, filamentous and non-filamentous fungi. Ethyl ether was found to be the best solvent at pH 7.0 to produce crude antibiotic, which was found to be effective against all the test pathogens (Fig.11). Strain 202 was identified as A. mutabilis which was inhibiting only filamentous and non-filamentous fungi, but after extraction of the crude antibiotic from this strain with ethyl ether at pH 7.0 the efficiency was found to increase by showing the activity against all the test pathogens (Fig.12). Strain 190 was identified as A. flavascens which gave very poor antagonistic activity when extracted with ethyl ether at pH 4.0 the activity was turned to broad-spectrum inhibiting gram-positive bacteria, gram-negative bacteria, filamentous and non-filamentous fungi (Fig.13) (Table 27).

Table 28. Antibiotics produced by the isolated actinomycetes (Ref: Umezawa*. 1967; Pridham and Tresner**, 1974)

Actinomycetes	Antibiotic produced	Active on
1. A. aureomonopodiales	-	Antibacterial Antifungal**
2. A. aureofasiculus	-	-
3. A. aureocirculatus	-	-
4. A. albovinaceus	-	-
5. A. candidus	Lemonomycin*	Gp & Gn
6. A. flavescens	-	-
7. A. griseomycini	-	-
8. A. mutabilis	-	Antibacterial Antifungal**
9. A. umbrinus	-	Antibacterial**
10. A. vilochromogenes	-	-
11. S. alboniger	Achromycin* Puromycin	Gp, Pr Gp,Gn,T,Pr
12. S. albidoflavus	-	Antibacterial Antifungal**
13. S. cacaoi	Cacaomycetin	F,Gp,Gn
14. S. cinereoruber	Rhodomycin AfB*	B. subtilis
15. S. carnosus	-	-
16. S. craterifer	-	-
17. S. echinatus	Echanomycin* Levomycin** Quinomycin*	Gp,Gn,m Pr, r Gp

Actinomycetes	Antibiotic produced	Active on
18. S. flavochromogenes	Amidinomycin**	B. subtilis
	Amaromycin*	Gp, Gn
	Shinocomycin AfB*	Gp
	Vulgarin*	
19. S. galtieri	-	-
20. S. gougeroti	Gougerotin*	Gp, Gn
	Kabicidin*	Y, F
21. S. griseolavendus	-	-
22. S. indigoferus	-	-
23. S. mirabilis	Miramycin*	Gp, V
24. S. nitrosporeus	Nitrosporin*	Gp
25. S. noblis	-	-
26. S. orientalis	Vamcomycin*	Gp, M
27. S. pyridomyceticus	-	-
28. S. roseus	GB/229*	Gp
	Sulfactin*	Gp, Gn
	Arsimycin*	-
29. S. rishiriensis	Coumermycin** (A, A2, BfD)	-
30. S. sindenesis	Allomycin*	Gp, M
31. S. sclerotialus	-	-
32. S. spadicis	-	-
33. S. thermovulgaris	-	-
34. S. vastus	-	Slight** antibacterial activity

Table 29. Identified strains and their antagonistic activity

Sl. No.	Strain No.	Identified as	Antagonistic activity of the present study	Antagonistic activity referred in the literature
			Active against	
1.	28	A. aureomonopodiales	F, NF	AB, AF
2.	79	A. aureomonopodiales	F, NF	AB, AF
3.	38	A. aureofasiculus	F	-
4.	76	A. aureofasiculus	F, NF	-
5.	140	A. aureofasiculus	Gn, F, NF	-
6.	78	A. aureocirculatus	F, NF	-
7.	92A	A. aureocirculatus	Gp, Gn, F, NF	-
8.	201	A. albovinaceus	F, NF	-
9.	8	A. candidus	Gn, F, NF	Gp, Gn
10.	70	A. candidus	F, NF	Gp, Gn
11.	12	A. flavescens	F, NF	-
12.	92B	A. flavescens	Gp, Gn, F, NF	-
13.	190	A. flavescens	F	-
14.	26A	A. griseomycini	Gn, F, NF	-
15.	200	A. mutabilis	F, NF	AB, AF
16.	202	A. mutabilis	F, NF	AB, AF
17.	23A	A. umbrinus	Gn, F, NF	AB
18.	23.2	A. vilochromogenes	F, NF	-
19.	75	S. alboniger	Gn, F	Gp, Gn, T, Pr
20.	77	S. alboniger	Gn, F, NF	Gp,Gn,T,Pr
21.	82	S. albidoflavus	F, NF	AB, AF
22.	30	S. cacaoi	Gn, F, NF	Gp, Gn, F
23.	187	S. cacaoi	Gn, F, NF	
24.	108	S. cinereorubes	Gn, F, NF	B. subtilis
25.	171	S. carnosus	Gn, F, NF	-
26.	192	S. craterifer	Gp,Gn,F,NF	-
27.	193	S. craterifer	Gp,Gn,F,NF	-
28.	88	S. craterifer	Gp, Gn,F,NF	-

Sl. No.	Strain No.	Identified as	Antagonistic activity of the present study	Antagonistic activity referred in the literature
29.	106B	S. echinatus	Gn,F,NF	Gp,Gn,Pr,r,m
30.	135	S. flavochromogenes	F, NF	Gp,Gn,B. subtilis
31.	34	S. galtieri	Gn,F,NF	-
32.	105	S. galtieri	F, NF	-
33.	186	S. galtieri	Gp	-
34.	191	S. galtieri	Gn, P, NF	-
35.	73	S. gougeroti	Gn, F, NF	Gp,Gn,Y,F
36.	91	S. gougeroti	F, NF	Gp,Gn,Y,F
37.	108A	S.gougeroti	Gn,Gp,F,NF	Gp,Gn,Y,F
38.	103B	S. griseolavendus	Gn,GP,F,NF	-
39.	106A	S. indigoferus	F, NF	-
40.	172	S. mirabilis	Gp,Gn,F,NF	Gp, V
41.	110	S. nitrosporeus	Gp,GN,F,NF	Gp
42.	103A	S. noblis	Gp,Gn,F,NF	-
43.	107	S. orientalis	Gp,Gn,F,NF	Gp,m
44.	189	S. orientalis	F, NF	Gp,m
45.	199	S. pyridomyceticus	Gp,Gn,F,NF	-
46.	104	S. roseus	Gp,F,NF	Gp,Gn
47.	22	S. rishiriensis	F, NF	-
48.	48	S. sindensis	F, NF	Gp,m
49.	93B	S. sclerotialus	Gp,Gn,F,NF	-
50.	142	S. spadicis	F	-
51.	26B	S. thermovulgaris	F, NF	-
52.	148	S. vastus	Gn,F,NF	AB

AB - Antibacterial; AF - Antifungal; Gn - Gram-positive bacteria; Gn - Gram-negative bacteria; F - filamentous fungi; NF - non-filamentous fungi; Y - yeast, Pr - Protozova; V - virus

M - Mycobacteria

IV DISCUSSION

There is a growing awareness in the study of antagonistic compounds derived from mangrove microorganisms that may be of medical value. We are still in great need of useful drugs that can inhibit viral infection and neoplastic diseases. Studies of terrestrial organisms have proved to be fruitful and hundreds of useful drugs with antibiotic, antitumour cardiotropic and neurotropic activities have been added to our pharmacopia. As mangrove ecosystem is an unexplored area for antagonistic compounds from actinomycetes, a study on "Microbial production of antibiotics from mangrove ecosystem" was initiated and regular collections of mangrove sediments was made from fixed stations for a period of one year from January to December 1991. A total of 1591 actinomycetes were screened during the sampling period. Based on morphological and colour variations, 104 cultures were isolated and tested for their antagonistic activity against 14 (fish) test pathogens. 52 cultures representing 4 colour series were subjected to identification tests of the International Streptomycetes Project (ISP). Out of 104 cultures showing antagonistic activity against one or more test pathogens, 6 isolates were selected based on their nature of activity.

(1) Active against gram-positive bacteria, gram-negative bacteria, filamentous and non-filamentous fungi (Strain, 103A and 92A).

(2) Active against gram-positive bacteria, filamentous and non-filamentous fungi (Strain 104).

(3) Active against gram-negative bacteria, filamentous and non-filamentous fungi (Strain 187).

(4) Active against filamentous and non-filamentous fungi (Strain 202).

(5) Active only against filamentous fungi (Strain 190).

The 6 cultures selected were mass cultured in order to extract the crude antibiotic by extraction procedure. These extracted crude antibiotics were tested against the test pathogens.

The discussion is presented under the following headings:

Quantitative and qualitative distribution of microbial flora and their inter-relationship.

Relationship of environmental parameters with microflora.

Antagonistic property of the isolated actinomycetes and the extracted crude antibiotics from six selected actinomycete strains.

Quantitative and qualitative distribution of microflora and their inter-relationship.

Though much emphasis has been given to antagonistic actinomycetes in the present study, an attempt was also made to understand the distribution patterns of other microflora in the sediments, as other microorganisms will have an influence in the distribution of antibiotic producers. Totally 24 collections were made for a period of one year to understand the seasonal distribution of microbial flora.

In the present study, samples were plated in four different selective media apart from seawater agar (SWA) as mentioned in the results for the enumeration of actinomycete population. It is always better to employ more than one medium to assess the antagonistic actinomycetes than by employing a single media .Grein and Meyers (1968); Aranson (1970) Okazaki

and Okami (1972) Walker and Colwell (1975). However, for estimation of bacteria only seawater Agar medium was used. Out of 4 selective media used for isolation of actinomycetes, OMA (oat meal agar) gave the best results for the estimation of fungal population. Of the 5 media employed SWA and glucose asparagine agar (GAA) provided the better recovery of actinomycetes, but SWA was found best suited to retrieve maximum number of actinomycetes on a routine way and also as maintenance medium for actinomycetes. This finding differed from earlier observation made by Rangaswami et al. (1967), Walker and Colwell (1975) as OMA was found best for the maintenance of cultures during the identification period. As the sediment samples are from mangrove ecosystem, 50% seawater was employed for the preparation of media.

Maximum bacterial population was observed during the period of monsoon. The primary environmental factors influencing the sediment bacteria include moisture, temperature, acidity, organic matter and inorganic nutrients supplied. The influence of season occurs only from the combination of primary determinants. In the present observation, highest count obtained during monsoon may be due to environmental parameters other than temperature, as temperature was recorded low during monsoon than other seasons.

Among the 4 stations sampled, Narakkal recorded highest bacterial count during monsoon. The number and types of bacteria is governed by mangrove sediment type also. In the present observation, Narakkal sediment was of sandy silt type, rich in organic matter which influenced the highest

count. Alexander (1978) found that bacterial density is always influenced to a large extent by the organic matter content in the habitat. Stationwise variations were evident regarding total microbial flora. Narakkal (Station II) recorded maximum number of actinomycetes during the study period which may be due to tidal effect. Whenever maximum number of actinomycetes occurred variety decreased, in all the three microbes especially during monsoon. During pre-monsoon season number of actinomycetes decreased and number of genera increased (Fig.2).

Bacterial population was dominating in all the sediment samples throughout the period of study, except in very few cases where actinomycetes dominated.

No significant difference in the distribution of bacteria was observed between the seasons or between stations sampled during the study period (Table 2). All stations recorded positive correlation between distribution of bacteria and B/F, B/A ratio which indicated powerful enzyme potential in bacteria competing for nutrients in their natural habitat.

Only 2 to 3 types of fungi were observed in the culture plates throughout the study period. It has been found out by Alexander (1978), that the dominance of one or more group is frequently related to the vegetation. The three types of fungi isolated during the study period may be associated with the mangrove plants at the study area viz. Avicennia sp. at Station I and II and Acanthus sp. at Station III and IV.

Fungal population in all the stations, recorded highest count during monsoon which showed adaptation of fungi in the mangrove sediment, which

is again governed by environmental factors. The major environmental influences imposed on the distribution of fungi are organic matter, pH, moisture, temperature, season of the year and composition of the vegetation (Alexander, 1978). In the present study also the highest count recorded during monsoon may be due to lowest temperature. Matondkar et al. (1980) also stated that in monsoon, when the salinity is low and leaf content is high, the microbial flora consisted of a large number of fungi.

Total number of fungi was always low when compared to bacteria and actinomycete throughout the study period. Hydrogen ion concentration is another major factor in controlling the activity and composition of fungi. Their capacity to grow readily at acidic pH values is well known. This is not the result of the fungi finding their optimum conditions for growth but instead it is a consequence of lack of microbiological competition for their nutrients as bacteria and actinomycete are uncommon in acid habitats.

Analysis of variance did not show any significant difference between season or stations. In all the four stations fungal flora had a positive relationship with F/A ratio whereas in the light house area of Puthuvyppu the fungal distribution had an inverse relationship with B/F and B/A ratio (Table 5. 1-4).

Like bacteria and fungi, maximum population of actinomycetes was recorded in the late monsoon period and the highest count was recorded at Narakkal in the month of August which may be due to the high dissolved oxygen content. Alexander (1978) is of view that actinomycetes are second only to the bacteria and the viable count are some time recorded equal

in soil. Alkaline pH will harbour more number of total actinomycetes. In the present study also actinomycete recorded was next to bacteria in abundance. Jensen (1930) recorded an increase in number of actinomycete with decrease in acidity. The size of the actinomycete community is also dependent upon the soil type, physical characteristics, organic matter content and pH of the environment. A direct relationship was observed between the actinomycete population and pH of sediment and water organic matter (Fig.5). Waksman (1950) has also stated that, there is a close correlation between the abundance of actinomycetes in the soil and the amount and extent of decomposition of available organic matter.

An inverse relationship was found between the distribution of actinomycetes and B/A and F/A at Mangalavana which may be due to higher actinomycete counts compared to bacteria and fungi encountered in this station (Table 5.1). At the light house area of Puthuvyppu total actinomycete counts showed a direct relationship with the total bacteria. The counts of bacteria and actinomycete also had a direct relationship because they are gradient organisms.

Totally 1591 actinomycetes were screened from the 4 fixed mangrove stations, out of which 104 actinomycetes were isolated and sub-cultured. Roach and Silvey (1959) isolated 75 marine actinomycetes from 39 sampling stations in Texas Gulf Coast Sediments. Contrary of this Kriss et al. (1967) could find actinomycetes only occassionally in sea and they were assumed to be from terrestrial habitat. Weyland (1969, 1970) routinely isolated marine actinomycetes from the sediment of Weser estuary and German

Bight. He also isolated 23 to 2909 actinomycetes per cm^2 from 107 sediment samples of North Sea between 1967 to 1969. Nine out of twelve sediment samples in Atlantic Ocean of West Africa yielded actinomycetes. The occurrence of actinomycetes in ocean sediments suggest their autochthonous nature.

Weyland (1986) suggested that areas little affected by terrestrial run off and dust exhibited a high proportion of Nocardio forms. In open sea, remote from land the Micromonosporae dominated, whereas in North sea and especially in mangrove streptomycetes were frequent. The data of open sea areas investigated showed that 50% of the isolates formed Nocardioforms followed by the Micromonosporae 39% whereas streptomycetes were found relatively rare (11%). Most population estimated of actinomycete numbers represent in reality almost only the Streptomycetes (Alexander, 1978).

Relative frequency of actinomycetes with which representatives of each genus observed in the marine environment.

	Actinomycetales			
Family	Marine Water (Zobell, 1946)		Shallow Water (Weyland, 1986*)	
Mycobactereceae	Mycobacterium	++	Mycobacterium	++
Actinomyceteceae	Actinomyces	+	Actinomyces (Present study)	++
	Nocardia	++	Nocardia*	-
Streptomyceteceae	Streptomyces	-	Streptomyces* (Present study)	++
	Micromonospora	++	Micromonospora*	-

An attempt was made to identify 52 isolates using ISP procedures. The following families are recognised under order Actinomycetales. Actinomycetaceae, Mycobacteriacae, Frankiaceae, Actinoplanaceae, Dermatophilaceae, Nocardiaceae, Micromonosporaceae and Streptomycetaceae. Out of which only 2 families were recorded in the present study. 34.62% was found to be actinomycetes. As such no true mycelium was found in this group, usually strictly to facultatively anaerobic. 65.39% was found to be streptomycetes. In this hyphae usually do not fragment. Extensive aerial mycelium and chains of spores with 5 to 50 or more conidia per chain. Antagonistic property of the identified strains when compared with the available literature, slightly differed in their activity. Possible antibiotics produced by the identified strains are referred and tabulated along with their antibiotic nature (Table 27). The antagonistic activity exhibited by the identified isolates in the present study are compared with previous literature and tabulated (Table 28). Actinomycetes from other family was absent in the present survey.

One of the primary factory in the identification of actinomycetes is the colour of the aerial mycelium (Waksman 1957, 1959, 1961) and Pridham and Tresner (1974). Aerial mass mycelial colour is greatly influenced by the composition of medium (Conn and Conn, 1941) and as such identification based aerial mass colour are subjected to reservation. ISP has recommended examining the aerial mass colour on 4 different media and the same procedure was followed in the present study. Totally 5 colour series of actinomycetes was encountered (white, grey, red, orange and green), out of which the grey colour series was found predominant (Table 7). The dominance of

grey colour streptomycetes was reported in soil by Rangaswami et al. (1967), Davies and Williams (1970), and in river sediments Batra (1972). In grey colour series 27.5% was identified as streptomycetes and could be closely related to one or more known species Vanajakumar (1979). Likewise white series was also identified upto species level 36.8%, 63.5% was identified as actinomycetes and streptomycetes respectively. The distribution of red colour series has been illustrated in the (Table 13 and 20). Out of 10 isolated red series 5 were identified of which 2 were actinomycetes and 3 were of streptomycetes. In the present study one green colour isolate was identified as actinomycete, Lakshmanaperumalsamy (1978) also identified only one green colour actinomycete but it was not recorded by Vanaja Kumar (1979).

Gottlieb and Shirling (1970) used four characters (aerial mass colour, sporophore morphology, spore surface and the ability to produce melanin like substances) for the fundamental arrangement into "primary groups". They used the formation of soluble pigments and carbon utilisation patterns in forming "secondary groups" of strains that were placed together on the basis of the four prime characters. In the case of spore-chain morphology, which was defined in terms of the morphological sections of Pridham et al. (1958), the results obtained showed that the definition of section Retinaculiaperti (RA) given by Shirling and Gottlieb (1966) is inadequate. According to Cross and Good fellow (1973), most strains can be easily placed in one of the ISP morphological groups (RF, RA or S), providing the organism is grown on agar media which support good sporulation.

Pridham and Lyons (1965) stated that the best way to handle streptomycete classification, nomenclature and identification is through application of a genus-species-sub-species concept. To establish a species, principle criteria are morphology of chains of spores and nature of spore-wall surfaces. Sub species can be differentiated one from another by other criteria, such as chromogenicity, colours of sporulating aerial mycelium and of vegetative mycelium, carbon utilization patterns and assessment of qualitative production of antibiotics and sensitivity and resistance to antibacterial antibiotics.

The concept of smooth, warty, spiny and hairy spore surfaces was sufficiently established by Cross and Maclver (1966) and Shirling and Gottlieb (1966) listed this as one of the criteria to be used in characterising species.

In the present study sporophore morphology of the majority of the isolates were of Rectiflexible type 57.69% (Table 21.1). A definite relationship was seen between sporophore pattern and aerial mass colour, where 80.00% of the RF type actinomycetes were of white colour series. Lakshmanaperumalsamy (1978) found that sediment isolates of Porto Novo coastal regions are the same as in the present study, being RF type mostly associated with white colour series. In the present study 60.00% of spiral type was of white colour series, whereas, 63.4% of the spiral type was of grey colour series reported by Vanaja Kumar (1979) from molluscs. 92.31% of the identified actinomycetes was of smooth walled type Tresner et al. (1961) stated that in streptomycetes spore mass colour was yellow, cream, buff or white shade and only smooth spores were found. In the present study

also, more than 95% of the isolates were of smooth walled type, which confirms that smooth type spores are very common in actinomycetes. In the present study Rectiflexible was found to be predominant both in actinomycete and streptomycete which was of smooth walled type. Whereas in Porto Novo sediments several species of actinomycetes with spiral sporophores had smooth spores (Lakshmanaperumalsamy, 1978). Actinomycetes isolated from molluscs also had spiral sporophore with smooth walled spores (Vanaja Kumar, 1979). Tresner et al. (1961) could record straight to flexuous sporophores having other than smooth spores. Strains having Retinaculiaperti, spiral type harboured only spiny spores in the present study.

36.54% of the total isolates produced melanoid pigment, and 26.92% of the cultures showing reverse side pigments and 9.62% of soluble pigment producers were able to produce melanoid pigment. In molluscs of Porto-Novo out of 67 isolates only 25.4% were able to produce melanoid pigment. Shirling and Gottlieb (1966) found that melanin pigment was produced by 83 of the 457 ISP strains. Szabo and Marton (1976) reported melanin production was doubtful in the case of 12 and 31 isolated strains on peptone-yeast-iron-agar and tyrosine agar respectively. 27 strains were unable to produce melanin production on tyrosine agar.

From the present study it is evident that in mangrove sediment streptomycetes dominate than actinomycetes. Among 17 actinomycete identified 10 species were recorded namely A. aureomonopodiales, A.aureofasiculus, A. aureocirculatus, A. albovinaceus, A. candidus, A. flavescens, A.griseomycini, A. mutabilis, A. umbrinus, and A. vilochromogenes. Among 35 streptomycetes

identified 24 species were recorded viz. S. alboniger, S. albiodoflavus, S. cacaoi, S. cinereoruber, S. carnosus, S. craterifer, S. echinatus, S. flavochromogenes, S. galtieri, S. gongeroti, S. griseolavendus, S. indigoferus, S. mirabilis, S. nitrosporeus, S. noblis, S. orientalis, S. pyridomyceticus, S. roseus, S. rishiriensis, S. sindensis, S. sclerotialus, S. spadicis, S. thermovulgaris and S. vastus.

Sodium chloride tolerance test is used as a criteria for the identification of streptomycetes. As the actinomycetes were isolated from mangrove sediments, the sodium chloride tolerance test was studied to know the origin of the isolates and the results are given in Table 19. 50.96% of the isolates showed good growth at 0.5% of sodium chloride and 25.96% of the isolates were able to tolerate upto 3.0% level and 16.35% of the isolates exhibited good growth at 4.5% sodium chloride concentration and only 7 (6.73%) isolates were able to tolerate sodium chloride upto 7%. All the isolates exhibited growth even when sodium chloride was not added in the medium and 53.85% of the isolates exhibited growth even when sodium chloride and sea water was omitted in the medium where only distilled water was added in the medium. Eliah and Reddy (1987) found that in the absence of sodium chloride or sea water 34 isolates showed poor to moderate growth but all the isolates were able to grow in the presence of 3% sodium chloride. Salt requirement or tolerance data suggested that the actinomycetes isolated from marine sediments of Visakapatnam, East Coast of India might be terrestrial form that have adapted to the salinity of marine sediments.

Tresner et al. (1968) tested 313 species of streptomycetes at 4 sodium chloride concentrations (4, 7, 10 and 13%) 1.8% could not tolerate 4% sodium chloride 26.9% of the organisms could grow at a maximum of 4%, nearly half of the isolates (49.7%) could tolerate a maximum of 7%, 18.8% could grow at a maximum of 10% and only a few 2.8% could tolerate 13%. Weyland (1981b) found that streptomycetes were able to develop better in 0.5% and 3.0% concentration of sodium chloride and also stated that there is only low response to increased salt concentration, but the completion of the life cycle is favoured by the addition of salt.

In the present study all the isolates subjected to sodium chloride test were able to grow in different concentrations of salt incorporated in the media. This aspect shows that they are indegenious mangrove flora. Since the samples were taken from mangrove ecosystem, they are more likely to be exotic terrestrial organisms that were carried to the mangrove ecosystem through rain water inflow and get adapted to the salinity of the mangrove ecosystem. Vegiga et al. (1983) found that actinomycetes isolated from littoral zone were able to grow in media containing 3.5% sodium chloride and isolates were more likely to be land organisms. Tresner et al. (1968) found, higher tolerance was statistically associated with the yellow and white spored streptomycetes. Whereas the red spored series tended to have lesser tolerance. Higher tolerance was also indicated for spiny spored species than for smooth spored forms.

Actinomycetes are heterotrophic in nutrition and their distribution is controlled by the availability of organic stubstrates. Utilisable carbon sources include simple and highly complex molecules from organic acids

and sugars to the polysaccharides, lipids, proteins and aliphatic hydrocarbons. In the present study sea water agar was found to be the best for isolation and maintenance which was deficient in carbon source. Isolates which grow well deficient in carbon are termed as oligocarbophilic micro-organisms (Alexander, 1978). After the survey of actinomycetes from the selected mangrove ecosystem it was concluded that both actinomycetes and streptomycetes are oligocarbophilic microorganism in nutrition.

Krassilnikov (1960) stated that more significance should be given to the assimilation manifestations of organisms, ie., to their ability to assimilate certain sources of carbon or nitrogen. Studies have shown that related actinomycetes react differently with certain carbon compounds (Kurosawa, 1951; Benedict et al., 1955; Lahner and Ettlinger, 1957). Krassilnikov (1960) also stated that some organisms assimilate rhamnose and raffinose well, others rhamnose but not raffinose or vice versa. Certain cultures freely assimilate fructose, mannitol and xylose, whereas others cannot utilise these compounds. It was also noted that, when these isolates were subjected to carbon utilisation with various sugars viz-D-glucose, L-arabinose, D-xylose, i-inositol, D-mannose, D-fructose, rhamnose, sucrose and raffinose, D-glucose was utilised by all the isolates, L-arabinose and L-xylose was utilised by most (88.5%) of the isolates, D-mannose and D-fructose was utilised by nearly 82.7% of the isolates, rhamhose and raffinose was utilised by 73.0% and 71.2% of the isolates respectively, i-inositol by 65.4% and the least utilised carbon source was found to be sucrose by 50.0% of isolates.

Relationship of environmental parameters with microflora.

In qualitative and quantitative terms, the actinomycete flora is governed by the surrounding habitat. The stage of the life cycle predomi-

nates, the size of the community, its biochemical transformations and the genera and species found are determined by the environmental factors. For the actinomycetes, the primary ecological influences include the organic matter status, pH, moisture and the temperature.

Production of organic matter in mangrove is mainly by mangrove plants. Only a small group of photo and chemoautotrophic microorganisms are regarded as primary producers, they further require sufficient light and suitable hydrogendonors. Hydrogen sulfide and other organic compounds are required for photosyntheticaly active organisms. All these requirements do not frequently coincide in all the ecosystems, but where they do as in some eutrophic ponds or mangroves there may be massive growth of photosynthetic bacteria accompanied by production of organic substances.

Functions of actinomycetes and bacteria area remineralisation and decomposition of organic substrates produced. Complete mineralisation can be attained only in the presence of oxygen (ie) in the aerobic environment. Under microaerophilic and anaerobic conditions like mangrove, the breakdown often remains incomplete. Substances which are easily decomposed like protein, sugar are decomposed by endo oxidations but the more resistant substances such as fats, cellulose and lignin accumulate and contribute to the detrital humus.

The rate of decomposition is again determined by constituents and the environmental conditions. At higher temperatures usually degradation takes place very rapidly. In the present study organic carbon showed a negative relationship with sediment temperature at Puthuvyppu (Table 5-3)

and also at light house area of Puthuvyppu (Table 5-4), with pH of water and pH of sediment at Mangalavana (Table 5-1). A positive relationship between organic carbon and total actinomycetes was obtained at Narakkal (Table 5-2). Waksman (1950) also found a close correlation between the abundance of actinomycetes in the soil and the amount and extent of decomposition of available organic matter.

Hydrogen-ion concentration affects the growth and reproduction of actinomycetes in mangrove ecosystem. The pH range in the present observation was 4.3 to 9.7 in sediment and 5.1 to 8.4 in water. The optimum range for most of the aquatic microorganisms is between pH 6.5 and 8.5. In the present observation there was only very little deviation from the varying magnitude of pH. Large fluctuations (ie) between 7.01 and 10.0 will naturally affect the composition of the actinomycetes by affecting the morphological and physicological functions. Water pH had a direct relationship with pH of sediment and pH had an inverse relationship with salinity at Narakkal (Table 5-2), Puthuvyppu (Table 5-3), light house area of Puthuvyppu (Table 5-4) and indirect relationship with sediment pH and total actinomycetes was observed at Narakkal (Table 5-2). Temperature had an indirect relationship with sediment pH at Puthuvyppu (Table 5-3) and light house area of Puthuvyppu (Table 5-4). Sediment pH also showed a direct relationship with organic carbon at Mangalavana (Table 5-1) with total actinomycetes at Narakkal (Table 5-2). Sediment pH also had an inverse relationship with salinity at Puthuvyppu (Table 5-3) and light house area of Puthuvyppu (Table 5-4). Rangaswami et al. (1967), Lakshmanaperumalswamy, 1978 and Vanaja Kumar, 1979 stated that pH did not show any

correlation with actinomycetes. But in the present observation pH was found to be actively influencing the distribution of actinomycetes.

Temperature may cause various morphological changes in various microorganisms including actinomycetes and their life cycle is temperature dependent. A rise in temperature results in increased activity and reduction of the generation time, but at the same time toxic effects are also increased and all enzymatic process are accelerated including autolysis. Both water and sediment temperature was found to be maximum in the post-monsoon season. The seasonal temperature fluctuations cause a change in population as microorganisms multiply vigorously at higher temperature, but others for which the higher temperature is unfavourable, will quickly perish at lower temperature on the other hand all metabolic activities are slowed down so that the time of survival is prolonged. In the present study, atmospheric temperature showed a negative correlation with organic carbon at Puthuvyppu (Table 5-3) and F/A ratio at the light house area of Puthuvyppu (Table 5-4). Water temperature showed a positive correlation with dissolved oxygen at Narakkal (Table 5-2) and Puthuvyppu (Table 5-3) and negative correlation with organic carbon at Puthuvyppu (Table 5-3). Sediment temperature showed a positive correlation with dissolved oxygen at Narakkal (Table 5-2), Puthuvyppu (Table 5-3) and light house area of Puthuvyppu (Table 5-4). Negative correlation was found to exist between sediment temperature and organic carbon at Puthuvyppu (Table 5-3) and between sediment temperature and organic carbon, total bacterial count at light house area of Puthuvyppu (Table 5-4).

The broad range in salinity obtained in the present study showed that the actinomycetes are highly salt tolerant and can thrive at higher salinity. The high variation in sodium chloride concentration in the mangrove might have led to the development of physiologically different fresh water and marine actinomycetes. Only few living creatures including bacteria, fungi and actinomycetes can thrive both in fresh water and sea. Accordingly most microorganisms in lakes and rivers are more or less halophilic and cannot under natural conditions grow with more than one percent salt. The decisive factor is not the higher osmotic value but the sodium ions which are vital necessity for most marine creatures and some, in addition, Cl ions (Mac Leod, 1965; 1968). In the present study salinity showed a positive correlation with dissolved oxygen at light house area of Puthuvyppu (Table 5-4) with water pH at Narakkal (Table 5-2). A negative correlation was found to exist between salinity and pH of water and sediment at Puthuvyppu (Table 5-3) and light house area of Puthuvyppu (Table 5-4).

Majority of acquatic actinomycetes are facultative anaerobes. Obligate anaerobes also play an important role in deep sediment. Obligate aerobes need oxygen as terminal hydrogen acceptor in respiration. Fluctuations in oxygen content (0.4 ml/l to 17.3 ml/l) within a wide range has not affected the actinomycetes considerably. The development of obligate aerobes are impaired only at very low oxygen tension although there are differences regarding the utilisation of small oxygen concentrations. Sometimes, small fluctuations may lead to important population changes (Rheinheimer, 1985). Dissolved oxygen showed a positive correlation with atmospheric temperature at Puthuvyppu (Table 5-3), with water temperature at Narakkal (Table 5-2) and Puthuvyppu (Table 5-4), with sediment temperature at Puthuvyppu

(Table 5-2) and with light house area of Puthuvyppu (Table 5-4) and with salinity at Narakkal (Table 5-2) which indicated the influence of oxygen in the distribution of actinomycetes.

Antagonistic property of the isolated actinomycetes and the extracted crude antibiotics from 6 selected actinomycete strains.

Very few reports are available regarding the antagonistic actinomycetes from the mangrove environment. Postmaster and Freitas (1975) isolated an antibiotic producer from marshy land. Mangrove sediment form the important medium in nature for the growth, multiplication, survival and other activities of actinomycetes. Actinomycetes exists in soil in complex association with environment and among them various forms of antagonistic relationships are noted. The presence of abundance of antagonistic organism in sediment have been studied by Waksman et al. (1942) and Burkholder (1946). Bioactive substances from marine actinomycetes from Sagami Bay Japan showed new and unique spectrum of antibiotic activity (Okazaki and Okami, 1972). Varieties of microorganism reported were more in marine sediments compared to soil samples, thus showing every possibility of getting new bioactive substances.

Seasonal effect was found in the antagonistic activity of the actinomycetes during different seasons. Pre-monsoon recorded a high antifungal activity, especially in filamentous fungal inhibition of different test organisms, whereas antibacterial and both antibacterial and antifungal showed same intensity of inhibition towards test organisms by the antagonistic actinomycetes. Vanaja Kumar (1979) also found that pre-monsoon was the favourable

season for isolating maximum number of antagonistic actinomycetes. Monsoon showed same pattern but with less intensity. Post-monsoon recorded same type of activity but the activity was still less, which may be due to 100% inhibition of the filamentous fungi, also the isolates antagonistic towards bacteria also inhibited the filamentous fungi.

Isolates belonging to the white colour series dominated in the antagonistic activity, maximum activity was obtained against Gram-negative bacteria followed by Gram-positive bacteria and both Gram-negative and Gram-positive bacteria. Grey colour series was next in order exhibiting same intensity of activity. Combined antibacterial and antifungal activity was exhibited by more number of white coloured cultures than other pigmented ones. Except orange and green all the colour series showed activity. against Gram-negative bacteria. The actinomycetes from molluscs of Porto-Novo coastal region also predominated in white colour series exhibiting activity against Gram-positive bacteria (14.5%). More activity exhibited by mangrove isolates against test pathogens showed the antibiotic potential of mangrove actinomycetes. This was also stressed by Rangaswami and Oblisami (1967) who found that there was considerable variation in the potential of actinomycete from different ecosystem. Thus mangrove proved to be a potential source of antagonistic actinomycetes, as evidenced by the present study.

Qualitative studies on the antagonistic properties of actinomycetes against test pathogens are presented in Table 22. Out of 104 actinomycetes 37 showed antagonism towards Gram-positive bacteria, and 59 was found to be antagonistic towards Gram-negative bacteria, 33 against Gram-positive and negative bacteria. Karssilnikov et al. (1953) found that Gram-positive

bacteria were the most susceptible against the antagonists from soil. In the present study Gram-negative bacterial inhibition was more (52.54%), where as isolates of Porto Novo region from mollusc indicated very little activity against Gram-negative bacteria. White colour series dominated in Gram-negative bacterial activity followed by grey, red, orange and green. Combined antibacterial and antifungal activity was exhibited by more number of grey colour actinomycetes (20.3%) than white coloured ones (19.0%) isolated from molluscs (Vanaja Kumar, 1979). In all the colour series isolates with combined filamentous and non-filamentous activity were found to be the most common forms.

All the isolates exhibited antibacterial or antifungal activities. Antagonistic activity recorded were more towards Gram-negative bacteria (59) when compared to both Gram-positive and Gram-negative bacteria (33) Table 23. Whereas, the actinomycete cultures isolated from mollusc showed more antagonism towards Gram-positive bacteria than Gram-negative bacteria (Vanaja Kumar, 1979). Rangaswami et al. (1967) isolated actinomycete which was antagonistic to Gram-positive and Gram-negative bacteria except E. coli and E. corotovora were least inhibited. And it was reported by Okazaki and Okami (1972), that there is a possibility of strains active against Gram-positive and Gram-negative bacteria from marine sediments. In marine sediments (Velankar, 1955; Matonkar, 1980) Gram-negative bacteria predominated and only 5% of the total formed Gram-positive forms. This may be the reason why the mangrove actinomycetes have produced antagonistic compounds inhibiting mostly Gram-negative bacteria. Actinomycetes

isolated from mollusc produced more antagonistic compounds to inhibit the indigenous Gram-positive forms. From these observation it is concluded that the native actinomycetes in each and every niche has a decisive factor in determining the antagonistic compounds to be produced by them.

Cladosporium, filamentous fungi was inhibited by all the (100%) antagonistic actinomycetes. 90% antagonistic actinomycete showed antagonism towards yeast, non-filamentous fungi. A high rate of inhibition was obtained with 90% actinomycete cultures towards filamentous and non-filamentous fungi. Ramaswami and Obliswami (1967) found that S. cerevisiae was inhibited by a higher percentage of actinomycetes by all the samples and among the filamentous fungi H. orazyae, A. niger were better inhibited. It is very evident that actinomycetes inhibiting filamentous fungi, yeast and bacteria in order, which showed that actinomycetes isolated from mangrove sediments was having highly inhibiting principle especially for filamentous fungi when compared to other 2 forms. There were considerable variations in the nature of inhibition of different test organisms for the actinomycetes from different stations.

Antibiogram of 104 isolated actinomycetes from the selected area showed the nature of inhibition of test cultures (Table 23). Out of 104 antagonistic actinomycetes 100% of the isolates were able to inhibit filamentous fungi. Nearly 86.5% inhibited non-filamentous fungi. Generally Pseudomonas and Aeromonas were reported to be more resistant to the existing antibiotics. Nearly 29.8% and 28.9% were able to inhibit Pseudomonas and Aeromonas respectively. 35.6% inhibited Bacillus 31.7% inhibited Staphylococcus, 39.4% inhibited E. coli, 38.5% inhibited V. parahaemolyticus

31.7% inhibited V. cholerae and 31.7% inhibited Salmonella I, 24.0% inhibited Salmonella II. Very poor activity (20.2%) was exhibited against V. anguillarum and V. alginoliticus, which showed the virullence. In general, activity against filamentous fungi and Gram-negative rods was highest followed by non-filamentous fungi and Gram-positive rods.

On the basis of antagonistic qualities, Lakshmanaperumalswamy (1978) grouped his isolates into 3 basic categories. Category I : Cultures showing an inhibition zone of 1-10 mm against test organisms. Category II : Cultures showing an inhibition zone of 11-30 mm against test organisms. Category III : Cultures showing an inhibition zone of 31 mm and above against the test organisms.

The same classification was also followed by Vanaja Kumar (1979), whereas in the present study, the results were grouped into 10 categories as mentioned in the Chapter III.

Number of antagonistic compounds inhibiting the test pathogens Pseudomonas and V. cholerae showed maximum activity only in the minimum inhibitory zone. It may be worthwhile to mention here that none of the sediment isolates from Portonovo coastal region could inhibit P. aeruginosa (LakshmanaperumaIswamy, 1978), Vanaja Kumar (1979) found that 10% of the actinomycete associated with mollusc was found to produce antibiotics against P. aeruginosa. Out of which 16.6% showed 1-10 mm inhibition against Pseudomonas. The presence of actinomycetes against Pseudomonas in molluscs and in mangrove sediment suggest that very good prospect exist for isolating newer antibiotics from the antagonistic actinomycetes from these two sources

for effectively employing them in combating this challenging bacteria. The presence of active principle against Pseudomonas in mangrove sediments and their paucity in marine sediments (Lakshmanaperumalswamy, 1978) suggest that mangrove sediment may be a potential source of newer antibiotics.

All the other Gram-negative rods subjected to cross streak assay showed maximum activity in the inhibition zone of 70-80 mm in Category X (Table 23), as 53% of the actinomycete culture were active against Gram-negative bacteria. In contrary to this marine sediments reported by Grien and Meyer's (1958), Okazaki and Okami (1972), Solovieva (1972), were active against Gram-positive forms. Postmaster and Frietas (1975) isolated an antibiotic producer from marsh sediment which was also found active against Gram-positive forms only. From mangrove environment, cultures active against Gram-negative bacteria were more frequently encountered than those exhibiting the combined activity. These observations suggest that mangrove cultures may be different from marine and terrestrial counterparts regarding their antagonistic properties. Thus mangrove sediments proves to be potential source for newer antibiotics. Antagonistic actinomycetes isolated from mollusc of Portonovo coastal region was predominant in having active principle against Gram-positive forms. In general animals harbour more Gram-positive forms which may be the reason why antagonistics inhibiting Gram-positive is more in mollusc. Among the terrestrial isolates those antagonistic to both Gram-positive and Gram-negative bacteria were more common in anti-bacterial groups (Rangaswami et al., 1967). However, in marine sediments, strains acting on both Gram-positive and Gram-negative bacteria were less in number when compared to those active against Gram-positive alone (Okazaki

and Okami, 1972). Vanaja Kumar (1972) isolated cultures against Gram-positive bacteria more frequently than those exhibiting combined activity. The dominance of Gram-negative bacteria over Gram-positive bacteria (Murchelano and Bishop, 1969; Murchelano and Brown, 1970) is only because of the antagonistic principles are active against Gram-positive bacteria in marine environment.

Maximum number of isolates that inhibited Bacillus was found in the Category X and the same pattern of inhibition was also found for Staphylococcus (31.7%) Table 23. Vanaja Kumar (1979) also found 61.7% of the actinomycete culture were active against B. circulans, in the inhibition zone of 1-10 mm and 11-30 mm. Among bacteria, Gram-positive groups seems to be more susceptible to various antagonists. In the present study more number of antagonists was active against Gram-negative forms.

Out of 104 isolates tested for their antibiotic activity 90 of them were able to exhibit antagonism towards Rhodotorula marina and R. rubra, and most of the isolates showing anti-yeast activity was recorded in the maximum inhibition zone (ie) 70-80 mm - Category X. And all the antagonistic actinomycetes were able to inhibit the filamentous fungi Cladosporium. Actinomycetes and fungal distribution is determined by the availability of oxidised carbonaceous substrates, so there will be definitely competition for nutrients between fungi and actinomycetes. The dominance of one or another group is frequently related to the type off vegetative cover. Certain microorganisms are associated with definite plant community while others seem to be unaffected by the kind of vegetation. Here, as the actinomycete and fungi had favourable nutrients for their growth, in order to compete

for their nutrients and to supress the fungi, antagonism is very essential, that may be the reason why, more occurrence of antagonistic actinomycetes towards fungi from mangrove sediments in the present study.

An attempt was also made to understand the nature of antibiotics produced by six selected strains of actinomycetes. These six cultures were selected based on their nature of antagonistic property (as stated in the earlier part of the discussion) and mass cultured. For the extraction of active principle from the culture filtrate three solvents (chloroform, ethyl ether and ethyl acetate) at 3 different pH (4.0, 6.0 and 9.0) were employed. Burkholder et al. (1966) used methanol, ethyl ether and chloroform to extract the inhibitory compound from Pseudomonas bromoutilis.

Strain 103A, S. mobilis showed similar antagonistic activity in culture as well as in extracted crude antibiotic. Ethyl acetate was found to be the suitable solvent at pH 4.0 (Fig.8) for S. mobilis. Anderson et al. (1974) used ethyl acetate for extraction of autotoxic antibiotic from a marine purple pigmented Chromobacterium designated strain 1-L-33 from sea water in the North Pacific. Oka et al. (1991) also used ethyl acetate to extract a new topoisomerase inhibitor from streptomycete Strain BA 10988 isolated from a soil sample collected in Takatsuki, Osaka Prefecture, Japan. A new napthoquinone antibiotic was extracted from the culture filtrate of Streptomyces sp. using ethyl acetate isolated from sample collected in Strathgordon, Australia (Henkel and Zeeck, 1990).

Strain 92A A. aureocirculatus showed effective antagonistic activity towards all the test pathogens whereas the crude antibiotic extracted from this strain was effective against only Gram-positive bacteria, Gram-negative

bacteria and filamentous fungi. Chloroform was found to be the best suitable solvent at pH 7.0 (Fig.9) Strain 104 S. roseus was inhibiting Gram-positive bacteria, filamentous and non-filamentous fungi whereas extracted antibiotic by this strain exhibited antagonism towards all the test pathogens. pH 4.0 and 7.0 gave good results in ethyl acetate (Fig.10). Strain 187 S. cacocoi was effective against Gram-negative bacteria, filamentous and non-filamentous fungi. Ethyl ether was found to be suitable at pH 7.0 to produce crude antibiotic, which was found to be effective against all the test pathogens (Fig.11). Strain 202 was A. mutabilis which was inhibiting only filamentous and non-filamentous fungi, but after extraction of the crude antibiotic from this strain with ethyl ether at pH 7.0, the efficiency was found to increase by exhibiting the activity against all the test pathogens (Fig.12). Strain 190 A. flavascens gave very poor antagonistic activity when extracted with ethyl ether at pH 4.0, the activity was turned to a broad-spectrum inhibiting Gram-positive bacteria, Gram-negative bacteria, filamentous and non-filamentous fungi (Fig.13). It has been found out by Hiremath et al. (1993) that higher concentrations of petroleum ether, chloroform and ethanol extracts of Acalypha indica revealed marked antibacterial and antifungal activity.

pH 4.0 was found suitable when ethyl acetate was used as a solvent for the extraction of antibiotics and pH 7.0 was found to be optimum when ethyl ether and chloroform were used, (Table 27). It is found, that there is a considerable influence of pH in enhancing the activity of antagonistic compounds.

V SUMMARY

1. The present study on "Microbial production of antibiotics from mangrove ecosystem" was carried out for a period of one year in four selected stations, Mangalavana, Narakkal, Puthuvyppu and light house area of Puthuvyppu (9°55' - 10°10'N and 76°10' - 76°20'E) from January to December 1991. Though much emphasis has been given to occurrence and distribution of actinomycetes, an attempt was also made to understand the distribution patterns of other microflora in the sediments. Data on physico-chemical parameters were also collected to find out their relationship if any with the microflora.

2. Seasonal distribution of actinomycetes showed that monsoon recorded the highest number of actinomycetes. The actinomycetes ranged between 1.0 to 243.0×10^4/gm in monsoon, $1.0-111.0 \times 10^4$/gm in post-monsoon and $1.0-13.0 \times 10^4$/gm in pre-monsoon.

3. Whenever number of actinomycetes increased, number of genera of actinomycetes decreased. It was also found that whenever number decreased type of genera increased.

4. Streptomycetes was always found predominant in all the selected stations sampled especially during pre-monsoon season.

5. The isolates usually had broad salinity and temperature spectra.

6. Analysis of variance of the microflora studied (bacteria, fungi and actinomycetes) did not show any significant difference between the seasons or stations.

7. Distribution of bacteria showed a positive correlation with B/F and B/A ratio in all the four mangrove stations studied.

8. Distribution of fungi showed a positive relationship with F/A ratio at all the four stations and at the light house area of Puthuvyppu the fungal distribution had a negative correlation with B/F and B/A ratio.

9. Negative correlation was found between the distribution of actinomycetes and B/A, F/A, ratio at mangalavana. Distribution of actinomycetes at Narakkal showed a positive correlation with pH of water, pH of sediment, organic carbon and a negative correlation with B/A. At Puthuvyppu distribution of actinomycetes did not show any relation with any of the parameters studied. Positive correlation existed between total actinomycete and total bacteria at the light house area of Puthuvyppu.

10. Positive correlation at 5% level was found between bacteria and actinomycete at Puthuvyppu and light house area of Puthuvyppu in the post-monsoon season. The pooled data for all the season showed positive relationship with 5% significant difference at light house area of Puthuvyppu for total counts of bacteria and actinomycetes.

11. Direct relationship was found between fungal and actinomycete counts at 5% level in the pre-monsoon season at Mangalavana and Narakkal and during monsoon at Narakkal, Puthuvyppu and light house area of Puthuvyppu.

12. 1591 actinomycetes were retrieved from the four fixed mangrove stations. Maximum number [427 (44.71%)] of actinomycetes were obtained from Narakkal during monsoon. In the post-monsoon season maximum number of actinomycetes 183 (33.83%) were recorded at Narakkal and 35 (36.84%) was the maximum number of actinomycetes recorded during pre-monsoon at Mangalavana.

13. Among 1591 actinomycetes encountered, five colour series were recorded viz., white (42.50%), grey (52.80%), red (4.40%), orange (0.25%) and green (0.06%). Grey colour series dominated followed by white colour series in abundance. 62.62% of grey coloured actinomycetes were encountered during the period of monsoon. Only one green coloured actinomycete was observed throughout the period of study.

14. Five selective media were used to retrieve actinomycetes viz. oat meal agar, Grein and Meyer's agar, glucose asparagine agar, Kuster's agar and sea water agar. Out of these 5 media used sea water agar was found to be best for isolation and maintenance of actinomycetes.

15. Out of 1591 actinomycetes encountered 104 cultures were isolated and maintained in sea water agar. Among 104 actinomycetes isolated 26 were from Mangalavana, 26 from Narakkal, 27 from Puthuvyppu and 25 were isolated from light house area of Puthuvyppu.

16. Maximum number of actinomycetes were isolated during pre-monsoon 55 (52.89%) followed by 27 (25.96%) during monsoon and 22 (21.15%) actinomycetes were isolated during the period of post-monsoon.

17. White colour series dominated among the isolates 60 (57.69%) followed by grey coloured series 29 (27.89%), red colour 10 (9.62%), orange colour series 4 (3.85%) and only one (0.96%) green coloured actinomycete was isolated.

18. 50% of the isolated actinomycetes were subjected to characterization according to the methods recommended by ISP and identified upto species level. Among 18 Actinomycetes identified 10 species were recorded, and 24 species were identified out of 35 Streptomyces cultures.

19. In order to characterize the actinomycetes, aerial mass colour, spore and sporophore morphology, pigment production and carbon utilisation were carried out (Table 20) and the species description of 52 actinomycetes are outlined.

20. White, grey and red were the predominant colour series observed in the isolated actinomycetes. Rectiflexible (RF); Retinaculiaperti, Spiral (RAS); Spiral (S) and RARF were the four types of sporophore morphology recorded among which (57.69%) RF type dominated. Spore-morphology of actinomycetes can be grouped into smooth, spiny, warty, hairy and rough type, however only smooth and spiny type were recorded during the present study.

21. D-glucose, L-arabinose, D-xylose, i-inositol, D-mannose, D-fructose, rhamnose, sucrose and raffinose, were selected to study the carbon utilisation of the actinomycetes in order to identify them upto species level. Only glucose was utilised by all the actinomycetes identified. Most of the isolates were not able to utilise either, L-arabinose, D-xylose or D-fructose. Only few were not able to utilise D-mannose. About 30% of the isolates were not able to utilise either i-inositol, rhamnose or raffinose. Only 50% of the isolates were able to utilise sucrose.

22. Sodium chloride tolerance test was carried out to know the origin of the isolates. It was found that most of the isolates (46.15%) were able to tolerate sodiumchloride upto 3% level. It was also noted that, most of the actinomycetes studied exhibited (53.85%) good growth even when sodiumchloride and sea water was omitted in the medium.

23. The antagonistic activity was tested using 14 test pathogens viz - Vibrio anguillarum, V. cholerae, V. alginoliticus, V. parahaemolyticus, Aeromonas, Pseudomonas, Salmonella-I, Salmonella-II, E. coli, Bacillus, Staphylococcus, Rhodotorula rubra, R. marina and Cladosporium. All the cultures tested showed antagonistic activity towards one or more of the test pathogens.

24. Out of 104 actinomycetes tested for their antimicrobial activity, about 56% exhibited antagonistic effect towards Gram-negative bacteria, 35.6% towards Gram-positive bacteria. 100% of the isolates were able to inhibit the growth of the filamentous fungi (Cladosporium) and 90% of the isolates were antagonistic towards non-filamentous fungi (R. marina and R. rubra).

25. Antibiogram of the actinomycetes against test pathogens showed that two extreme zones (Category I and Category X) of inhibition was exhibited by most of the antagonistic actinomycetes.

26. Six isolates exhibiting different antimicrobial activity were selected and mass cultured for the extraction of crude antibiotics.

27. It was found that few test pathogens which were resistant to original strain were sensitive to their antibiotic extracts.

28. pH 4.0 was found to be suitable when ethyl acetate was used as a solvent for the extraction of antibiotics and pH 7.0 was found to be optimum when ethyl ether and chloroform were used.

29. From the present study it was found out that, most of the isolates (56%) were able to inhibit Gram-negative bacteria and all the isolates were able to inhibit Cladosporium the filamentous fungi.

30. Extracted antagonistic compounds were able to inhibit most of the pathogens tested exhibiting the broad-spectrum antibiotic activity. These studies considerably enhances our knowledge of distribution of antagonistic actinomycetes in mangrove environment of Cochin which play a significant role in the economy of the sea. Further studies in the isolated antagonistic compounds are needed in order to produce new antibiotics effective against fish pathogens.

VI REFERENCES

ABBAS, A. and **C. EDWARDS**, 1990. Effects of metals on a range of Streptomycetes species. Appl. Env. Microbiol., **55**: 2030-2035.

ABDALLAH, N.M. and **S.A. FATHY**, 1976. On the biochemical activities of S. flavohelwanesis. Indian J. Microbiol., **16**: 13-19.

ABRAHAM, T.A. and **L.J. HERR.**, 1964. Activity of actinomycetes from rhizosphere and non-rhizosphere soil of corn and soybean in four physiological tests. Can. J. Microbiol., **10**: 281-285.

ALEXANDER, M. 1978. II Ed. Introduction to Soil Microbiology. Wiley Eastern Limited, pp. 37-51.

ALI, S.S. and **M.G. ROYMON**, 1984. Antibiosis and biodegradability of some Streptomyces species from Raipur. Indian J. Microbiol., **24**: 124-126.

ANDERSON, R.J., M.S. WOLFE and **D.J. FAULKNER**, 1974. Autotoxic antibiotic production by a marine Chromobacterium. Marine Biol., **27**: 281-285.

ARANSON, S., 1970. Experimental Microbial Ecology. Academic Press, London, 70 pp.

ARONSON, J.D. 1926. Spontaneous tuberculosis in Salt Water fish. J. Infect. Dis., **39**: 315-320.

ATTWELL, R.W. and **T. CROSS**, 1972. Germination of Thermoactinomyces vulgaris. J. Gen. Microbiol., **73**: 471-481.

BAAM, R.B., N.M. GANDHI and **Y.M. FREITAS,** 1966. Antibiotic activity of marine microorganisms. Helgo - laender Wiss. Meeresunters., **13:** 181-187.

* **BALDACCI, E.** and **A. GRIEN,** 1955. Esame della from delle spore di attinomiceti al microscopia ellettronico cloro valutazione al fini una Classificazione. Giorn. Microbiol., **1:** 28-38.

BALL, A.S., W.B. BETTS and **A.J. Mc CARTHY,** 1989. Degradation of lignin - related compounds by actinomycetes. Appl. Env. Microbiol., **55:** 1642-1644.

BALTZ, R.H. 1978. Genetic recombination in S. fradiae by protoplast fusion and cell regeneration. J. Gen. Microbiol., **107:** 93-102.

BARTEL, P.L., N.C. CONNORS and **W.R. STROHL,** 1990. Biosynthesis of anthracyclines: analysis of mutants of Streptomyces sp. Strain C5 blocked in daunomycin biosynthesis. J. Gen. Microbiol., **136:** 1877-1886.

BATRA, S.K., H.V. SAHNI and **M. SRIVASTAVA,** 1972. Distribution pattern of Streptomycetes from flooded Ganges water. Indian. J. Exp. Biol., **10:** 439-441.

BECKER, B., M.P. LECHEVALIER, R.E. GORDON and **H.A. LECHEVALIER,** 1974. Rapid differentiation between Nocardia and Streptomyces by paper chromatography of whole-cell hydrolysates. Appl. Microbiol., **12:** 421-423.

BECKER, B., M.P. LECHEVALIER and **H.A. LECHEVALIER,** 1965. Chemical composition of cell-wall preparations from strains of various form Genera of aerobic actinomycetes. Appl. Microbiol., **13:** 236-243.

BENEDICT, R.G., T.G. PRIDHAM, L.A. LINDENFELSER, H.H. HALL and **R.W. JACKSON,** 1955. Further studies in the evaluation of carbohydrate utilization test as aids in the differentiation of species of Streptomyces. Appl. Microbiol., **3:** 1-6.

BERD, D. 1973. Laboratory identification of clinically important aerobic actinomycetes. Appl. Microbiol., **25:** 665-681.

BLOOM, R.A., B.C. MULLIN and **R.L. TATE,** 1989. DNA restriction patterns and DNA-DNA solution hybridization studies of Frankia isolates from Mycicapensylvanica (Bayberry). Appl. Env. Microbiol., **55:** 2155-2160.

BOONE, C.J. and **L. PINE,** 1968. Rapid method for characterization of actinomycetes by cell-wall composition. Appl. Microbiol., **2:** 279-284.

BRADLEY, S.G. 1956. Genetics in applied microbiology. Adv. Appl. Microbiol. **8:** 29-59.

***BRINGMAN, O.** 1951. Electronenmikroskopische und lichtmikroskopische studien zur Morphologie und Entwicklungvon Streptomyces griseus Krainsky. Zentralbl. Bakteriol. Abt. I. Orig., **157:** 349-355.

BROOKS, P.C. and **S.P.P.Mc.GRATH,** 1986. Effects of heavy metal accumulation in field soils treated with sewage-sludge on soil microbial process and soil fertility. FEMS Symposium., **13:** 327-343.

BURKHOLDER, P.R. 1946. Studies on the antibiotic activity of actinomycetes. J. Bacteriol., **52**: 503-504.

BURKHOLDER, P.R., R.M. PFISTER and **F.H. LIETZ,** 1966. Production of a pyrrole antibiotic by marine bacterium. Appl. Microbiol., **14**: 649-653.

CARAVAJAL, F. 1946. Studies on the structure of S. griseus. Mycologia, **38**: 587-595.

CASIDA, L.E. 1968. Industrial Microbiology, Wiley Eastern Limited, 105 pp.

CHAHAL, D.S. and **S.K. NANDA,** 1975. A screening of some actinomycetes for protease production. Proc. Indian Natl. Sci. Acad. Part B. (Biol. Sci) 41-B(**5**): 427-434.

CHANDRAMOHAN, D., S. RAMU and **R. NATARAJAN,** 1972. Cellulolytic activity of marine streptomycetes. Current Science, **41**: 245.

CHANDRIKA, V., P.V.R. NAIR and **L.R. KHAMBHADKAR,** 1985. Distribution of phototrophic thionic bacteria in the anaerobic and micro aerophilic strata of mangrove ecosystem of Cochin. Abstract EN. P-3-26th Annual Conference of the Association of Microbiologist of India.

CHARY, C.V.K., S. RAMBHAV, G. VENKATESWERLU and **L.K. RAMACHANDRAN,** 1989. Possible precursors for thiostrepton in S. azureus. Indian. J. Microbiol., **29**: 191-198.

CHATER, K.F. 1974. Rifampicin - resistant mutants of S. coelicolor A3(2). J. Gen. Microbiol., **80**: 277-290.

CHUNG, S.A., W.S. RIGGSBY and **B.C. MULLIN**, 1985. Relationships of Frankia isolates based on deoxyribonucleic acid homology studies. Int. J. Syst. Bacteriol., **35**: 140-146.

CLAUDE, E., D. BOY and **V.K. PILLAI**, 1984. Water quality management in aquaculture. CMFRI Special Publication. No.**22**: pp 77-80.

CONN, H.J. and **J.E. CONN**, 1941. Value of pigmentation in classifying actinomycetes. A preliminary note. J. Bacteriol., **42**: 791-800.

COYKENDALL, A.L. and **A.J. MUNZENMAIER**, 1979. Deoxyribonucleic acid hybridization among strains of A. viscosus and A. naeslundii. Int. J. Syst. Bacteriol., **29**: 234-240.

CRAMERI, R., G. HINTERMANN and **R. HUTTER**, 1983. Deoxyribonucleic acid restriction endonuclease fingerprint characterization of actinomycete strains. Int. J. Syst. Bacteriol., **44**: 293-300.

***CRIBB, A.B.** and **J.W. CRIBB**, 1956a. Marine fungi from Queens land. Id Univ. Queensl. pap., Dep. Bot., **3**: 97-105.

CROSS, T., M.P. LECHEVALIER and **H. LECHEVALIER**, 1963. A new genus of the Actinomycetales Microellobosporia gen. nov., J. Gen. Microbiol., **31**: 421-429.

CROSS, T. and **A.M. MACLVER**, 1966. An alternative approach to the identification of Streptomyces sp. a working system. pp. 103-110. In identification methods for microbiologists. Part A., The society for Applied Microbiology, Technical Series. Academic Press. Inc. London.

*CROSS, T. and **M. GOOD FELLOW**, 1973. Taxonomy and classification of the actinomycetes, pp. 11-112. In: G. Sykes and Skinner, F.A. (ed.), Actinomycetales: Characteristics and practical importance. Academic press. Inc., NY.

CUMMINS, C.S. and **H. HARRIS,** 1958. Studies on the cell-wall composition and taxonomy of Actinomycetales and related groups. J. Gen. Microbiol., **18:** 173-189.

DAGINAWALA, H.F. and **B.J. WADHER,** 1985. B lactam antibiotic from Streptomyces sp. JA13. Indian J. Microbiol., **25:** 93-96.

DAVIES, F.L. and **S.T. WILLIAMS,** 1970. Studies on the ecology of Actinomycetes in soil. I. The occurrence and distribution of actinomycetes in a pine forest soil, Soil. Biol. Biochem., **2:** 227-238.

DEJONG, P.J. 1972. L. Asparaginase production by Streptomyces griseus., Appl. Microbiol., **23:** 1163-1164.

DEKLEVA, M.L., J.A. TITUS and **W.R. STROHL,** 1985. Nutrient effects on anthracycline production by S. peucetius in a defined medium. Can. J. Microbiol., **31:** 287-294.

DESAI, A.J. and **S.M. BETRABET,** 1972. Cellulase activity of microorganisms isolated from cotton deteriorated during storage. Indian J. Biochem. and Biophysics. **9:** 212-214.

DEWEESE, M.S., M.A. GERENCSER and **J.M. SLACK,** 1968. Quantitative analysis of Actinomyces Cell Walls. Appl. Microbiol., **16:** 1713-1718.

DIAB, A. and **M.Y. GOUNAIM,** 1982. S. spinoverrucosus, a New species from the air of Kuwait. Int. J. Syst. Bact., **32:** 327-331.

DIETZ, A. and **J. MATHEWS,** 1962. Taxonomy by carbon replication. 1. An examination of S. hygroscopicus. Appl. Microbiol., **10:** 258-263.

DIETZ, A. and **J. MATHEWS,** 1968. Taxonomy by carbon replication II. Examination of 8 additional cultures of S. hygroscopicus. Appl. Microbiol., **16:** 935-941.

DIETZ, A. and **J. MATHEWS,** 1971. Classification of Streptomyces spore surfaces into 5 groups. Appl. Microbiol., **21:** 527-533.

DIWIVEDI, S.N., A.M. PARULEKAR, S.C. GOSWAMI and **A.G. UNTAWALE,** 1973. Proc. Inter. Sym. Biol. Mang. Man, Honolulu Hawaii, Vol.1. Ed. Walsh. G.E., S.C. Snedakar and H.J. Teas. (Univ. Florida) 115 pp.

DOUGLAS, H.W. 1970. A study of the electrokinetic properties of some actinomycete spores. J. Gen. Microbiol., **63:** 289-295.

EGOROV, N.S. 1985. Formation of antibiotics in nature and their biological role. pp. 63. In antibiotics a scientific approach. MIR Publishers, Moscow.

ELLAIAH, P. and **A.P.C. REDDY,** 1987. Isolation of actinomycetes from marine sediments of Visakhapatnam, East Coast of India. Indian. J. Mar. Sci., **16:** 134-135.

EL NAKEEB, M.A. and **H.A. LECHEVALIER,** 1963. Selective isolation of aerobic actinomycetes. Appl. Microbiol., **11:** 75-77.

FEITELSON, J.S., F. MALPARTIDA and **D.A. HOPWOOD,** 1985. Genetic and biochemical characterization of the red gene cluster of S. coelicolor A 3(2). J. Gen. Microbiol., **131:** 2431-2441.

FERNANDEZ, M.P., H. MEUGNIER, P.A.D. GRIMONT and **R. BARDIN.** 1989. Deoxyribonucleic acid relatedness among members of the genus Frankia. Int. J. Syst. Bacteriol., **39:** 424-429.

FLAIG, W., H. BEUTELSPACHEN, E. KUSTER and **G.S. HOLZWEISSIG,** 1952. Beitrag zur physiologie und morphologie der Streptomyceten. Plant Soil., **4:** 118-127.

FRAZIER, W.C. 1958. Food Microbiology, II Ed, TATA McGraw-Hill publishing Company Ltd. 421 pp.

FREITAS, Y.M. and **J.V. BHAT,** 1954. Microorganisms associated with the deterioration of fishnets and cordage J. Univ. Bombay, **28:** 53-59.

GERALD, E. and **WALSH,** 1974. Mangroves: A Review. In: (ed.), Reimold. R. and W. Queen. Ecology of Halophytes. Academic Press INC. NY. 63 pp.

GLEDHILL, W.E. and **L.E. CASIDA,** 1969. Predominant catalase-negative soil bacteria II. Occurrence and characterization of Actinomyces humiferus, sp. nov. Appl. Microbiol., **18:** 114-121.

GOMES, H.R., and **S. MANINKURVE,** 1982. Studies on mangrove swamps of Goa: 2. Microorganisms degrading phenolic compounds. Mahasagar, **15:** 111-115.

GORDON, R.E., D.A. BARNETT, J.E., HANDERHAN and **C.H. PANG,** 1974. Nocardia coeliaca, N. autotrophica and the Nocardia strain. Int. J. Syst. Bacteriol., **24:** 54-63.

GOTTLIEB, D. 1961. An evaluation of criteria and procedures used in the description and characterization of Streptomycetes. A cooperative study. Appl. Microbiol, **9:** 55-65.

*__GOTTLIEB, D.__ and **E.B. SHIRLING,** 1970. An analysis of species groups among Streptomyces, pp. 67-77. In H. Prauser (ed.), The Actinomycetales. VEB G. Fischer Verlag, Jena.

GOTTLIEB, D., 1974. Actinomycetales. In Bergy's Manual of determinative Microbiology. Buchanan, R.E. and N.E. Gibbons (ed.), The Williams and Wilkins Company/Baltimore. 657 pp.

GRABLEY, S., H. HAMMANN, K. HUTTER, H. KLUDGE, R. THIERICKE and **J. WINK.** 1991. Secondary metabolites by chemical screening. Part 19. SM 196A and B, novel biologically active angucyclinones from Streptomyces sp. J. Antibiotics, **44:** 620-673.

GREIN, A. and **S.P. MEYERS,** 1958. Growth Characteristics and antibiotic production of actinomycetes isolated from littoral sediments and materials suspended in seawater. J. Bacteriol., **76:** 457-463.

GUTSELL, J. 1946. Sulfa drugs and the treatment of furunculosis in trout. Science, **104:** 85-86.

HEALD, E.J. and **W.E. ODUM,** 1962. The contribution of mangrove swamps of Florida fisheries. Proc. Gulf. Caribb. Fish. Inst. 22 Ann. Sess: 130-135.

HENKEL, T. and **A. ZEECK,** 1991. Secondary metabolites by chemical screening. Structure and absolute configuration of Napthomevalin, A new dihydro-Napthoquinone antibiotic from Streptomyces sp. J. Antibiot., **44**: 665-669.

HENSENS, O.D., R.P. BORRIS, L.R. KOUPAL, C.G. CALDWELL, S.A.CURRIE, A.A. HAIDRI, C.F. HOMNICK, S.S. HONEY CUTT, S.M.L. MAYER, C.D. SCHWARTZ, B.A. WEISSBERGER, H.B. WOODRUFF, D.L. ZINK, L. ZITANO, J.M. FIELDHOUSE, T. ROLLINS, M.S. SPRINGER and **J.P. SPRINGER,** 1991. L-156, 602, AC5a antagonist with a novel cyclic hexadepsipeptide structure from Streptomyces sp. MA 6348. Fermentation, isolation and structure determination. J. Antibiot., **44**: 249-253.

HERNANDEZ, N.M.R. and **O. COTOPEREZ,** 1978. Antimicrobial properties of extracts from Rhizophora mangle. Rev. Cub. Med. Trop., **30**: 181-187.

HIREMATH, S.P., S. BADAMI, H.K.S. SWAMY and **J.S. BIRADAR,** 1993. Antimicrobial activity of various extracts of Acalypha indica (Euphorbiaceae) Indian. J. Microbiol., **33**: 75-77.

HOPWOOD, D.A. 1960. Phase-contrast observations on Streptomyces coelicolor J. Gen. Microbiol., **22**: 295-302.

HOPWOOD, D.A. and A.M. GLAUERT, 1961. Electron microscope observations on the surface structures of S. violaceoruber. J. Gen. Microbiol, **26**: 325-330.

HORAN, A.C. and **B.C. BRODSKY**, 1982. A novel antibiotic producing Actinomadura, Actinomadura kijaniata sp. nov. Int. J. Syst. Bacteriol., **32**: 195-200.

HUMNADKAR, V.S. and **A.D. AGATE,** 1985. Ecology of estuarine mangrove ecosystem with reference of autotrophic bacteria. Abstract - E.No.7. 26th Annual Conference of the Association of Microbiologists of India.

IBRAHIM, A.R. and **Y.J.A. HAJJ,** 1989. Aromatic hydroxylation and sulfation of 5-hydroxyflavone by Streptomyces fulvissimus. Appl. Env. Microbiol., **55**: 3140-3142.

IMAI, S., A. SHIMAZU, K. FURIHATA, Y. HAYAKAWA and **H. SETO,** 1990. Isolation and structure of a new phenoxazine antibiotic, Ex foliazone, produced by Streptomyces exfoliatus. J. Antibiot., XLIII: 1606-1607.

IWASAKI, A., H. ITOH and **T. MORI,** 1981. Streptomyces sannanensis sp. nov. Int. J. Syst. Bacteriol., **31**: 280-284.

JENSEN, H.L. 1930. Actinomycetes in Danish soils. Soil Sci., **30**: 59-77.

KAWATO, M. and **R. SHINOBU,** 1959. On S. herbaricolor nov. sp. Supplement: A simple technique for the microscopical observation. Mem. Osaka Univ. lib. Arts Educ., **8**: 114 pp.

KHANNA, S.S. and **D.V. YADAV,** 1979. Practical manual for introductory courses in soil. Haryana Agricultural University. Hissar. 33-43.

KLONTZ, G.W. 1970. Mariculture Medicine. Proc. World Maricult. Soc., **1**: 129-131.

KOJIRI, K., H. KONDO, T. YOSHINARI, H. ARAKAWA, S. NAKAJMA, F. SATOH, K. KAWAMURA, A. OKURA, H. SUDA and **M. OKANISHI,** 1991. A new antitumour substance, BE-13793C, produced by a Streptomycete Taxonomy, fermentation, isolation, structure determination and biological activity. J. Antibiot., **44**: 723-727.

KRASSILNIKOV, N.A., A.I. KORENIAKO and **O.I. ARTAMONAVA,** 1953. The distribution of actinomycete antagonist in soil. Microbiologia, **23**: 3-10.

KRASSILNIKOV, N.A. 1960. Taxonomic principles in the Actinomycetes. Rules for the classification of antibiotic producing Actinomycetes. J. Bacteriol., **79**: 75-80.

KRASSILNIKOV, N.A., 1962. Antibiotic properties of microorganisms isolated from various depths of World Oceans. Microbiology, **30**: 545-550.

KRISS, A.E., E.A. RUKINA and **B.M. ISAEV,** 1945. Electron microscopy studies on the structure of actinomycetes. Mikrobiologia, **14**: 172-176.

KRISS, A.E., N. MITSKEVICH, I.E. MISHUSTINA and **E.V. ZEMTSOVA,** 1967. Microbiol populations of Oceans and Seas. Annold, London.

KUROSAWA, H. 1951. Mycological characters of antagonistic Streptomyces 1. On the correlation between Pridham's classification method and antibiotic characters. J. Antibiot., (Japan), **4**: 183-193.

KUSTER, E. and **S.T. WILLIAMS,** 1964. Selection of media for isolation of Streptomyces. Nature, **202:** 928-929.

LABEDA, D.P., R.T. TESTA, M.P. LECHAVALIER, 1984. Saccharothrix of a new genus of the Actinomycetales related to Nocardiopsis Int. J. Syst. Bacteriol., **34:** 426-431.

LACEY, J. 1971. Thermoactinomyces sacchari sp. no., a thermophilic actinomycete causing bagossosis. J. Gen. Microbiol., **66:** 327-338.

*__LAKSMANAPERUMALSAMY, P.__ 1978. Studies on actinomycetes with special reference to antagonistic Streptomycetes from sediments of Portonovo Coastal zone. Ph.D. Thesis. Annamalai, University.

LECHEVALIER, M.P. and **H. LECHEVALIER,** 1957. A new genus of the Actinomycetales. Waksmania gen. nov. J. Gen. Microbiol., 104-111.

LECHEVALIER, H.A., M. SOCOTOROVSKY and **C.I. McDURMONT,** 1961. A new genus of the Actinomycetales. Micropolyspora gen. nov. J. Gen. Microbiol., **26:** 11-18.

LECHEVALIER, H.A., M.P. LECHEVALIER and **P.E. HOLBERT,** 1966. Electron microscopic observation of the sporangial structure of strains of Actinoplanaceae. J. Bacteriol., **92:** 1228-1234.

LECHEVALIER, M.P., H. PRAUSER, D.P. LABEDA and **J.S. RUAN,** 1986. Two new genera of Nocardia form Actinomycetes; Amycolata gen. nov. and Amycolatopsis gen. nov. Int. J. Syst. Bacteriol., **36:** 29-37.

LECHEVALIER, H.A. and **L. PINE,** 1989. The Actinomycetales. pp. 151-161. In practical hand book of Microbiology. Leary, W.M.O. (ed.), CRC Press Inc. Boca. Raton. Florida.

LINGAPPA, Y. and **J.L. LOCKWOOD,** 1961. Chitin medium for isolation, growth and maintenance of actinomycetes. Nature, **189:** 158-159.

LLOYD, A.B. 1969. Behaviour of streptomycetes in soil. J. Gen. Microbiol., **56:** 165-170.

LOKABHARATHI, P.A., S. OAK and **D. CHANDRA MOHAN,** 1991. Sulphate reducing bacteria from mangrove swamps 2. Their ecology and physiology, Oceanol Acta., **14:** 163-171.

MACLEOD, R.A. 1965. The question of the existence of specific marine bacteria. Bacteriol. Rev., **29:** 9-23.

MACLEOD, R.A. 1968. On the role of inorganic ions in the physiology of marine bacteria. pp. 95-126. In: Advances in Microbiology of the Sea . Droop and Wood (ed.). London and New York. Academic Press.

MACNAE, W. 1968. A general account of the flora and fauna of mangrove swamps and forests in Indo-West Pacific region. Advances in Marine Biol., **6:** 73-270.

MASAYUKI, S. and **A. KAJI,** 1980. Exopolygalacturonatelyase produced by S. massasporeus. Agric. Biol. Chem., **44:** 717-772.

MATONDKAR, S.G.P., S. MAHTANI and **S. MAVINKURVE,** 1980b. Fungal flora of mangrove swamps of Goa. Mahasagar Bulletin of the National Institute of Oceanography, **13:** 281-283.

MATONDKAR, S.G.P., S. MAHTANI and **S. MAVINKURVE,** 1981. Studies on mangrove swamps of Goa. 1. Heterotrophic bacterial flora from mangrove swamps. Mahasagar-Bulletin of the National Institute of Oceanography. **14:** 325-327.

MAYER, J. 1976. Nocardiopsis, a New genus of the order Actinomycetales. Int. J. Syst. Bacteriol., **26:** 483-487.

MCVITTIE, A., H. WILDERMUTH and **D.A. HOPWOOD,** 1972. Fine structure and surface topography of endospores of Thermoactinomyces vulgaris. J. Gen. Microbiol., **71:** 367-381.

MERRICK, M.J. 1976. A morphological and genetic mapping study of bald colony mutants of S. coelicolor. J. Gen. Microbiol., **96:** 299-315.

MINI RAMAN, 1986. Studies on Rhizophere microflora of Acanthus ilicifolius. M.Sc., Dissertation submitted to the Cochin University of Science and Technology.

MINNIKIN, D.E., P.V. PATEL, L. ALSHAMAONY and **M. GOOD FELLOW,** 1977. Polar lipid composition in the classification of Nocardia and related bacteria. Int. J. Syst. Bacteriol., **27:** 104-117.

MIYASHITA, K., Y. MIKAMI and **TADASHIARAI,** 1984. Alkalophilic actinomycete Nocardiopsis dassonvillei sub sp. nov., isolated from soil., Int. J. Syst. Bacteriol., **34:** 405-409.

MURCHELANO, R.A. and **J.L. BISHOP,** 1969. Bacteriological study of laboratory reared juvenile American oysters (Crassostrea virginica). J. Invertebrate Pathology. **14:** 321-327.

MURCHELANO, R.A. and **C. BROWN,** 1970. Heterotrophic bacteria in Long Island Sound. Int. J. on Life in Oceans and coastal waters. **7**(1): 1-6.

NAIR, L.N. and **V.S. NAIR,** 1986. Studies on two species of Streptomyces. Indian J. Microbiol., **26:** 101-104.

NAKJIMA, M., K. ITOI, Y. TAKAMATSU, T. KINOSHITA, T. OKAZAKI, K. KAWAKUBO, M. SHINDO, T. HONMA and **M. TOHJIGAMORI,** 1991. Hydantocidin. A new compound with herbicidal activity S. hygroscopicus. J. Antibiot., **44:** 293-300.

NATARAJAN, R., M. ABRAHAM and **G. BALAKRISHNANNAIR,** 1979. Distribution of Vibrio parahaemolyticus and allied vibrios in backwater and mangrove biotopes at Porto Novo. Indian J. Mar. Sci., **8:** 286-289.

ODUM, W.E. and **E.J. HEALD,** 1975. The detritus-based food web of an estuarine mangrove community. pp. 265-286. In: Estuarine research, L.E. Cronin (ed.), Academic Press, New York, **1:**

OKA, H., T. YOSHINARI, T. MURAI, K. KAWAMURA, F. SATOH, K. FUNAISHI, A. OKURA, H. SUDA, M. OKANISHI and **Y. SHIZURI,** 1991. A new topoiromerase-II inhibitor, BE-10988, Produced by a Streptomycete. J. Antibiot., **44:** 486-491.

OKANISHI, M., K. SUZUKI and **H. UMEZAWA,** 1974. Formation and reversion of streptomycete protoplasts: Cultural condition and morphological study. J. Gen. Microbiol., **80:** 389-400.

OKAZAKI, T. and **Y. OKAMI,** 1972. Studies on marine microorganisms. 11. Actinomycetes in Sagami Bay and their antibiotic substances. J. Antibiot (Japan)., **25:** 461-466.

OVERMAN, J.R. and **L. PINE,** 1963. Electron microscopy of cytoplasmic structures in faculative and anaerobic Actinomyces J. Bacteriol. **86:** 657-665.

PELCZAR, M.J., R.D. REID and **E.C.S. CHAN,** 1977. Microbiology. TATA McGraw Hill publishing Company Ltd. ND. 259 pp.

PORTER, J.N., J.J. WILHELM and **H.D. TRENSER,** 1960. Method for the preferential isolation of actinomycetes from soils. Appl. Microbiol., **8:** 174-178.

POSTMASTER, C. and **Y.M. FREITAS,** 1975. An antibiotic producer from marsh sediments. Hindustan Antibiot. Bull., **17:** 118-120.

PRIDHAM, T.G. and **D. GOTTLIEB,** 1948. The utilisation of carbon compounds by some Actinomycetales as an aid for species determination. J. Bacteriol., **56:** 107-114.

PRIDHAM, T.G., C.W. HESSELTINE and **R.G. BENEDICT,** 1958. A guide for the classification of streptomyces according to selected groups: Placement of strains in morphological sections. Appl. Microbiol. **6:** 52-79.

PRIDHAM, T.G. and **A.J. LYONS**, 1965. Further taxonomic studies on straight to flexuous Streptomycetes. J. Bacteriol. **89**: 331 pp.

PRIDHAM, T.G. and **H.D. TRESNER,** 1974. Actinomycetales In: Buchanan,R.E. and N.E. Gibbons Bergey's Manual of determinative bacteriology. (Eighth edition). The Williams and Wilkins Co., Baltimore 657 pp.

RAMACHANDRA, M., D.L. CRAWFORD and **A.L. POMETTO,** 1987. Extracellular enzyme activities during lignocellulose degradation by Streptomyces sp. : A comparative study of wild-type and genetically manipulated strains. Appl. Env. Microbiol., **53**: 2754-2760.

RAMADHAS, V., A. RAJENDRAN and **V.K. VENUGOPALAN,** 1975. Studies on trace elements in Pichavaram mangroves. Proc. Int. Sym. Biol. and Mang of Mang. Walsh, Snedakar and Teas (ed.), vol.**1**: 96-114.

RAMAMURTHY, T., R.M. RAJU and **R. NATARAJAN**, 1990. Distribution and ecology of methanogenic bacteria in mangrove sediments of Pitchavaram, East coast of India, Indian J. Mari. Sci., **19**: 269-273.

RANCOURT, M. and **H.A. LECHEVALIER,** 1963. Electron microscopic observation of the sporangial structure of an actinomycete, Microellobosporia flavea. J. Gen. Microbiol., **31**: 495-498.

RANCOURT, M.W. and **H.A. LECHEVALIER,** 1964. Electron microscopic study of the formation of spiny conidia in species of Streptomyces. Can. J. Microbiol., **10**: 311-316.

RANGASWAMI, G. and **G. OBLISAMI,** 1967. A study on the correlation between the antagonistic actinomycetes and the physical and chemical properties of some soils of South India. Indian pytopathology, **20**: 280-290.

RANGASWAMI, G., G. OBLISAMI and **R. SWAMINATHAN,** 1967. Antagonistic actinomycetes in the soils of South India. Univ. Agrl. Sci. Bangalore and USDA P.L. 480 (FG. In-129).

REDDY, T.K.K., A. RAJASEKHAR, B. JAYASUNDERAMMA and **R. RAMAMURTHI.,** 1991. Studies on marine bioactive substances from the Bay of Bengal. Bio active substances from the latex of the mangrove plant Excoecaria agallocha L. Antimicrobial activity and degradation. In: Bioactive compounds from marine organisms. An Indo United States Symposium (ed.), Thompson, M.F., R. Sarojini and R. Nagabushanam. Oxford and IBH Publishing Co. Pvt. Ltd. pp. 75-78.

REYNES, J.P., T. CALMELS, D. DROCOURT and **G. TIRABY,** 1988. Cloning, expression in E. coli and nucleotide sequence of a tetracycline resistance gene from S. rimosus., J. Gen. Microbiol., **134:** 585-598.

RHEINHEIMER, G. 1985. Acquatic Microbiology, John Wiley and Sons. pp. 115-117.

RIVIERE, J. 1977. Antibiotics. In: Industrial application of Microbiology, Survey Univ. Press. pp. 197-218.

ROACH, A.W. and **J.K.G. SILVEY,** 1959. The occurrence of marine actinomycetes in Texas Gulf coast substrates. Ann. Midl. Nat., **62:** 482-499.

RODINA, A.G., 1972. Methods in aquatic microbiology. Univ. Park. Press. London. pp. 373-380.

RUDD, B.A.M. and **D.A. HOPWOOD,** 1980. A pigmented mycelial antibiotic in S. coeliocolor: Control by a chromosomal gene cluster. J. Gen. Microbiol., **119:** 333-340.

SAXENA, D., P.A. LOKABHARATHI and **D. CHANDRAMOHAN,** 1988. Sulphate reducing bacteria from mangrove swamps of Goa, Central West Coast of India. Indian. J. Mar. Sci., **17:** 153-157.

SCHUSTER, W.H. 1952. Fish culture in brackish water ponds of Java. Indo Pacific Fish. Counc. Specl. Publ. No.1: pp 1-143.

SENGUPTA, A. and **S. CHAUDHURI,** 1990. Halotolerant Rhizobium strains from mangrove swamps of the Ganges river delta. Indian J. Microbiol. **30:** 483-484.

SHARPLES, G.P. and **S.T. WILLIAMS,** 1976. Fine structure of spore germination of actinomycetes. J. Gen. Microbiol., **96:** 323-332.

SHEARER, M.C., P.M. COLMAN and **C.H. NASH,** 1983. Nocardiopsis mutabilis, a New species of Nocardioform bacteria isolated from soil. Int. J. Syst. Bacteriol., **33:** 369-374.

SHINOBU, R. 1958. Physiological and cultural study for the identification of soil actinomycetes species. Mem. Osaka Univ., Ser. B, **7:** 1-76.

SHIRLING, E.B. and **D. GOTTLIEB,** 1966. Methods for characterization of Streptomyces species. Int. J. Syst. Bacteriol., **16:** 313-340.

SHIRLING, E.B. and **D. GOTTLIEB,** 1968a. Cooperative descriptions of type cultures of Streptomyces II. Species descriptions from first study. Int. J. Syst. Bacteriol. **18:** 69-189.

SHIRLING, E.B. and **D. GOTTLIEB,** 1968b. Cooperative descriptions of type cultures of Streptomyces III. Additional species descriptions from first and second studies. Int. J. Syst. Bacteriol., **18:** 279-392.

SHIRLING, E.B. and **D. GOTTLIEB,** 1969. Cooperative descriptions of type cultures of Streptomyces IV. Species description from the second, third and fourth studies. Int. J. Syst. Bacteriol., **19:** 313-340.

SHIRLING, E.B. and **D. GOTTLIEB,** 1972. Cooperative description of type strains of Streptomyces V. Additional descriptions. Int. J. Syst. Bacteriol., **22:** 265-394.

SIEBURTH, J.M., 1979. Aerobic gram-positive Epibacteria. In Sea Microbes. Oxford Univ. Press. NY. pp. 317-319.

SOHLER, A., A.H. ROMANO and **W.J. NICKERSON,** 1958. Biochemistry of the Actinomycetales. III. Cell-wall composition and the action of lysozyme upon cells and cell walls of Actinomycetales. J. Bacteriol., **75:** 283-290.

SOLOVIEVA, N.K. 1972. Actinomycetes of littoral and sublittoral zones of the white sea. Antibiotiki, **17:** 387-392.

STANECK, J.L. and **G.D. ROBERTS,** 1974. Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. Appl. Microbiol., **28:** 226-231.

STRICKLAND, J.D.H. and **T.R. PARSONS,** 1968. A practical hand book of seawater analysis, Bull. Fish. Res. Bd. Can., **167:** pp. 2-311.

SURENDRAN, V. 1985. Studies on heterotrophic bacteria in the mangrove ecosystem near Cochin. M.Sc., Dissertation submitted to the Cochin University of Science and Technology.

SWART, H.J. 1958. An investigation of the mycoflora in the soil of some mangrove swamps. Acta. Bot. Neerl., **7:** 741-768.

SZABO, M. and **M. MARTON,** 1976. Evaluation of criteria used in the ISP cooperative description of type strains of Streptomyces and Streptoverticillium species. Int. J. Syst. Bacteriol., **26:** 105-110.

TOMITA, K., Y. NAKAKITA, Y. HOSHINO, K. NUMATA and **H. KAWAGUCHI,** 1987. New genus of the Actinomycetales : Streptoalloteichus hindustanus gen. nov., nom rev., Sp., Nov., nom. rev. Int. J. Syst. Bacteriol. **37:** 211-213.

TRESNER, H.H., E.J. BACKUS and **M.C. DAVIES,** 1961. Electron microscopy of Streptomyces spore morphology and its role in species differentiation. J. Bacteriol., **81:** 70-80.

TRESNER, H.D., M.C. DAVIES and **M.E. ENGLERT,** 1966. Morphological subtype of the smooth spored streptomycetes. J. Bacteriol. **91:** 1998-2005.

TRESNER, H.D., E.J. BACKUS and **J.A. HAYNES,** 1968. Differential tolerance of streptomycetes to sodium chloride as a taxonomic aid. Appl. Microbiol., **16:** 1134-1136.

UMEZAWA, H. 1967. Index of antibiotics from actinomycetes. Univ. of Tokyo Press, Tokyo. pp. 2-81.

UNTAWALE, A.G., S.N. DWIVEDI and **S.Y.S. SINGBAL,** 1973. Ecology of mangrove in Mandovi and Zuari estuaries and the interconnecting Cambarjua Canal of Goa. Indian J. Mar. Sci., **2:** 47-53.

UNTAWALE, A.G., T. BALASUBRAMANIAN, M.V.M. WAFAR, 1977. Structure and production in a detritus rich estuarine mangrove swamp. Mahasagar Bulletin of National Institute of Oceanography, **10:** 173-177.

VANAJA KUMAR, 1979. Studies on actinomycetes associated with molluscs from Portonovo coastal waters. Ph.D. Thesis, Annamalai University.

VANAJA KUMAR, 1991. Antagonistic properties of actinomycetes isolated from molluscs of the Porto Novo region, South India. In: Bioactive compounds from marine organisms. An Indo-United States Symposium. Thompson, M.F., R. Sarojini and R. Nagabushanam. Oxford and IBH Publishing Co., Pvt. Ltd. pp. 75-78.

VEIGA, M., A. ESPARIS and **J. FABREGAS,** 1983. Isolation of cellulolytic actinomycetes from marine sediments. App. Env. Microbiol., **48:** 286-287.

VELANKAR, N.K. 1955. Bacteria in the inshore environment at Mandapam. Indian. J. Fish. **2:** 96 pp.

VENKATESAN, V. and **V.D. RAMAMURTHY,** 1971. Marine microbiological studies of mangrove swamps of Killai backwaters. Tour of Occ. Soc. Japan. **27:** 51-55.

VILCHES, C., C. MENDEZ, C. HARDISSON and **J.A. SALAS,** 1990. Biosynthesis of oleandomycin by Streptomyces antibioticus, influence of nutritional conditions and development of resistance. J. Antibiot., **136:** 1447-1454.

WACHINGER, G., K. BRONNENMEIER, W.L. STAUDENBAUER and **H. SCHREMPF,** 1989. Indentification of mycelium associated cellulase from S. reticuli. Appl. Env. Microbiol., **55:** 2653-2657.

WAKSMAN, S.A. 1919. Studies in the metabolism of actinomycetes. J. Bacteriol., **4:** 189-216.

WAKSMAN, S.A., E.S. HORNING, M. WELCH and **H.B. WOODRUFF,** 1942. Distribution of antagonistic actinomycetes in nature. Soil Sci. **54:** 291-296.

WAKSMAN, S.A. 1950. The Actinomycetes. Nature, Occurrence, Activities and Importance, Waltman, Mass: Chronica Botanica Co. 106 pp.

WAKSMAN, S.A. 1957. Species concept among the actinomycetes with special reference to the genus Streptomyces. Bacteriological Reviews., 21.

WAKSMAN, S.A. 1959. The Actinomycetes: Vol.I. Occurrence and activities. Williams and Wilkims Co., Baltimore, U.S.A.

WAKSMAN, S.A. 1961. The Actinomycetes: Vol.II. Classification identification and description of genera and species. Williams and Wilkins Co., Baltimore, U.S.A.

WAKSMAN, S.A. 1963. The actinomycetes and their antibiotics. pp. 235-293. In: Advances in applied microbiology. Ed. Umbreit, W.W., Vol.5., Academic Press. NY.

WAKSMAN, S.A. 1967. The Actinomycetes. A summary of current knowledge. Ronald Press Co., N.Y. 245 pp.

WALKER, J.D. and **R.R. COLWELL,** 1975. Factors affecting enumeration and isolation of actinomycetes from Chesapeake Bay and South eastern Atlantic Ocean sediments. Marine Biology, **30:** 193-202.

WATSON, E.T. and **S.T. WILLIAMS,** 1974. Studies on the ecology of actinomycetes in soil-VII. Actinomycetes in a coastal sand belt. Soil. Biol. Biochem., **6:** 43-52.

WEYLAND, H. 1969. Actinomycetes in North Sea and Atlantic Ocean sediments. Nature, **223:** 858 pp.

WEYLAND, H. 1970. Studies on Actinomycetes of the sea benthos. Presented at the Ocean world joint Oceanographic Assembly, Tokyo. **8:** pp. 495-497.

WEYLAND, H. 1981b. Characteristics of actinomycetes isolated from marine sediments. Zentralbl. Bakteriol. Prasistenkd. Infektionskr. Hyg. Abt. 1. Suppl., **11:** 309-314.

WEYLAND, H. 1986. Actinomycetes of the bottom sediments of various seas. GERBAM-Deuxieme Colloque International de Bacteriologie marine-CNRS, Brest, 1-5 October, 1984. IFREMER, Actes de Colloques., **3:** 73-79.

WILLIAMS, S.T. and **F.L. DAVIES,** 1967. Use of SEM for the examination of actinomycetes. J. Gen. Microbiol., **48:** 171-177.

WILDERMUTH, H. 1970. Development and organization of the aerial mycelium in Streptomyces coelicolor. J. Gen. Microbiol., **60:** 43-50.

WILDERMUTH, H. and **D.A. HOPWOOD,** 1970. Septation during sporulation in Streptomyces coelicolor. J. Gen. Microbiol., **60:** 51-59.

WILLIAMS, S.T. and **G.P. SHARPLES,** 1976. Streptosporangium corrugation sp. nov., an Actinomycete with some unusual morphological features. Int. J. Syst. Bacteriol., **26:** 45-52.

WILLONGHBY, L.G. 1966. A conidial Actinoplanes isolate from Bielham Tara. J. Gen. Microbiol., **44:** 69-72.

WOOD, E.J.F. 1967. Microbiology of Oceans and Estuaries. Elsevier publishing Company, Amsterdam, 319 pp.

YAGI, S., S. KITAI and **T. KIMURA**, 1971. Simulative effect of elemental sulphur on siomycin production by Streptomyces sioyaensis. Appl. Microbiol., **22**: 153-156.

YOSHIDA, M., M. HARA, Y. SAITOH and **H. SANO**, 1990. The Kapurimycins, New antitumour antibiotics produced by Streptomyces-physico-chemical properties and structure determination. J. Antibiot., XLIII: 1519-1523.

***ZAHNER, H.** and **L. ETTINGER,** 1957. Zur systematik det Actinomyceten. 3. Die verwertung ver schiedener Kohlensto ffquellenals Hilfsmittel der Artbestimmung innerhalb der Gattung Streptomyces. Arch. Microbiol., **26**: 307-328.

***ZHICHENG, Z., Z. MEIYING, Y. BINGRIN,** 1989. Composition and biological activity of actinomycetes in mangrove Rhizosphere. J. Xiamen. Univ., **28**: 306-310.

ZOBELL, C.E. 1946. Marine Microbiology. Chronica Botanica, Waltham, Massachusetts. 126 pp.

---

* Not referred to original

Effect of different media for isolation, growth and maintenance of Actinomycetes from mangrove sediments

R Rathna Kala & V Chandrika

Central Marine Fisheries Research Institute, Post Bag No.2704

Cochin 682 031, Kerala, India

Received 13 October 1992, revised .....

Out of many recommended media for selective isolation of actinomycetes from soil, glucose asparagine agar, Grein and Meyer's agar, oat meal agar and Kuster's agar were found suitable for isolation of actinomycetes from mangrove sediments. The best media allowing good development of actinomycetes, while suppressing bacterial growth, were those containing starch or glucose as the carbon source with casein, and asparagine or nitrate as the nitrogen source. Seawater-agar was also tried for isolation and maintenance and found extremely good as a maintenance medium. Bacteriostatic and fungistatic compounds such as calcium carbonate, phenol, lactic acid and acetic acid were used for selective isolation of actinomycetes.