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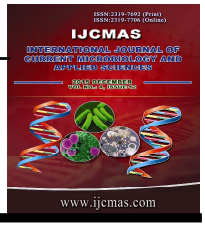

Original Research Article

A Study on Diversity of Actinomycetes Population from Industrial Area

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A B S T R A C T

Actinomycetes group are a valuable boon to the nature between the micro organsisms' communities. This group is an unexplored community where highest range of bioactive metabolites/ or antibiotics extracted and utilized for industrial and medicinal fields. This study implies on the diversity of actinomycetes group in plating industries and this area has been chosen for the stressed environment. The environment will be different from normal terrestrial area which contains high alkali/ or acidic nature which depletes the micro organisms community. But fewer of micro organisms will produce different colored/ or non colored metabolites to overcome the stressed environment for their survival between their communities. On cultivation of these microbes, they exhibit different range of activities with highest biological properties. In this current investigation we have isolated 24 isolates from Kuruvampalaiyam, Eletroplating Industry, Coimbatore. Among which 6 were pigmented while other remaining colonies were non-pigmented. Based on the media supplement the organisms produced pigments with bioactivity. This explores a vast diversity within a stipulated sampling area. Further studies will be on extraction of biometabolites from predominant species to explore its bioactivity.

Keywords

Actinomycetes, Diversity, Biometabolites, ISP media, Stressed environment

Introduction

Actinomycetes population has been identified as one of the major group of soil population, most widely distributed group of microorganisms in nature and may vary with the soil type. The name Actinomycetes is given to one of the eight families of the order Actinomycetales. The best known of these is the subclass *Actinobacteridae*, which includes the Order *Actinomycetales* whose members are commonly referred to as actinomycetes. Clusters of actinomycetes form long, thin filaments in the soil and have an important role in the environmental carbon cycle. A hardy group of bacteria, they are particularly adopt at surviving harsh conditions and breaking down tough substances in the soil, returning their components back down to the most basic structures. Soil microorganisms also influence above-ground ecosystems by contributing to plant nutrition, plant, soil structure and soil fertility.Among actinomycetes, the *streptomycetes* are the dominant. The non-streptomycetes are called rare actinomycetes, comprising approximately 100 genera.

The number and types of actinomycetes present in a particular soil would be greatly influenced by geographical location such as soil temperature, soil type, soil pH, organic matter content, cultivation, aeration and moisture content. Species diversity consists of species richness, the total number of species present, species evenness, and the distribution of species (Buckley & Schmidt, 2001). Methods to measure microbial diversity in soil can be categorized into two groups: biochemical-based techniques and molecular-based techniques. Typically, diversity studies include the relative diversities of communities across a gradient of stress, disturbance or other biotic or abiotic difference. Classical approaches for classification make use of morphological, physiological, and biochemical characters. The classical method described in the identification key by Nonomura, (1974) and Bergey's Manual of Determinative Bacteriology is very much useful in the identification of streptomycetes. Colour may also play an important role in the classification of the Streptomycetes. The diversity studies pave the way for exploring the microbial communities thus the way for accounting the biological properties of novel metabolites. Keeping in this mind, this piece of work has been carried out in exploring the actinomycetes community in the soil samples of industrial areas mainly plating industries.

Materials and Methods

Isolation of Microorganism

The actinomycetes strains used in this study were isolated from the soil of terrestrial environment from Kuruvampalaiyam, Electroplating industrial area (Coimbatore). Soil samples were brought to the laboratory in aseptic condition. Actinomycetes from the soil were isolated by spread plate technique on different media such as Starch-Casein Nitrate agar, Gauze Inorganic agar, Oat Meal agar, Water agar, Actinomycetes agar, Kusters agar after serial dilution with sterilized distilled water. Plates were kept for incubation at 37˚C for 7 days for the growth of microorganisms. Distinct colonies of actinomycetes were selected and streaked on a new plate. The isolated colonies were maintained in Starch-Casein agar slant and stored at 4˚C. The cultures to be used were revived by streaking on Starch-Casein agar and incubating at 28˚C for 7 days.

Cultural and Morphological Characterization of Actinomycetes

Actinomycetes colonies were identified on the basis of morphological characteristics following directions given by the International Streptomyces Project (Shirling & Gottlieb, 1966) and the Bergey's Manual of Systematic Bacteriology (William *et al.,* 1983). Morphology of spore bearing hyphae with entire spore chain was identified in SCN agar, different ISP media and was observed with a phase contrast microscope by using cover slip method. The cover slip with culture was observed under microscope for the morphology of substrate mycelium and aerial mycelium.

Colour Determination for Actinomycetes Identification

Nature of *Streptomyces* colony was observed for pigmentation in different ISP media. After 7days the isolates were divided by using a reference colour key (Tresner & Backus, 1963).

Biochemical Characterization

The biochemicals tests such as MR-VP, Indole, TSI, Oxidase, Catalase, Citrase, Estrase, Gelatinase, Caseinase, Urease, Decomposition of tyrosine, cellulose & pectin, Acid from carbohydrates and Nitrate

reduction were performed to characterize the biochemical property using the methods of Berd (1973) with minor modifications.

Results and Discussion

A total of 24 isolates were obtained from terrestrial soil samples from Kuruvampalaiyam, Eletroplating Industry, Coimbatore. Among which 6 were pigmented while other remaining colonies were non-pigmented. Six different types of media are used for the isolation of actinomycetes (Table 1). The maximum number of isolates with 6×10^4 colony forming unit per gram (cfu/g) was obtained from the starch casein agar. Next to SCA media the maximum number of isolates of 5×10^4 (cfu/g) was obtained from water. Isolates with 4×10^3 and 4×10^4(cfu/g) was obtained from the Kuster's and Oat meal agar. In Gauze Inorganic media the number of isolates obtained was 3×10^3(cfu/g). The number of isolates with 2×10^3 (cfu/g) was obtained from actinomycetes agar. Thus, the starch casein agar was found best for isolating *Streptomyces sp* from terrestrial soil samples from Industrial area, Coimbatore.

Morphology of the colonies was observed on starch casein agar (Fig 1). On third day the colonies appeared as chalky white spots, and the aerial mycelium arose from the surface of the agar plate in the form of single hyphae that subsequently branched heterogeneously.

After 5 days, they showed distinct differences in their aerial mycelia color, and some of the grey and white colonies showed fine droplets of extracellular exudates on their surface which are the characteristic features of *Streptomyces*. They were also found to be associated with scanty to profuse sporulation ability and had different colony morphological features. Based on the color coding proposed by Tresner and Backus (1963), the grouping was done for the spore color of the organisms and were arranged them into their respective color groups according to certain underlying color similarities.

Spore morphology of 24 isolates was identified in different media of ISP1-7 and Starch casein agar (Fig 2 and Fig 3). Different in spore chain of the isolates were observed under light microscope. Colony morphology was observed in starch casein agar for the growth of the isolates. The most number of isolates showed regular margin while few strains showed irregular margin. Among the 24 isolates 15 showed leathery colonies(KOA_1-1, KOA_1-2, KOA_1-3,KOA_1-4, KOA_1-6, KOA_1-7, KOA_1-8, KOA_1-9, KOA_1-11, KOA_1-12, KOA_1-13, KOA_1-16, KOA_1-17, KOA_1-18,KOA_1-20), 5 were butyrous (KOA_1-5, KOA_1-14, KOA_1-15, KOA_1-22, and KOA_1-24) and 4 puffy colonies (KOA_1-10, KOA_1-19, KOA_1-21, and KOA_1-23) were obtained. Different spore chains like spiral, short spiral, straight spiral, straight, long straight chains were observed in under microscope (Kozo opticals) (Table 2). In this study, a spectrum of colours like white, peach, pale yellow, green, ash, brown and gray colonies were observed. Based on the colour pattern *Streptomyces* isolates were thus grouped into five groups (Table 3). The percentage of white colour colony of *Streptomyces* sp. was found to be predominant compared to other coloured colonies. Similarly Vanajakumar *et al*., (1995) has reported white colour series of *Streptomyces sp.* as the dominant forms followed by the grey, yellow, and red colour series. The members of the genus Streptomyces are clearly distinguished from all other actinomycetes using a combination of biochemical properties. The 24 isolates selected in the study were found to be aerobic, Gram positive, non- motile, catalase positive, mostly produced musty odour.

Table.1 List of Isolates Obtained from Different Agar Media

STATE	DISTRICT	LOCATION	DIFFERENT MEDIA USED	TOTAL NUMBER OF ISOLATES	ISOLATE NAME
TAMIL NADU	COIMBATORE	KURUVAMPALAIYAM ELECTROPLATING INDUSTRIAL AREA	STARCH CASEIN AGAR	6×10^4cfu/g	KOA_1-1, KOA_1-2, KOA_1-3, KOA_1-4, KOA_1-5, KOA_1-6
			OAT MEAL AGAR	4×10^3cfu/g	KOA_1-7, KOA_1-8, KOA_1-9, KOA_1-10
			GAUZE INORGANIC AGAR	3×10^3cfu/g	KOA_1-15, KOA_1-16, KOA_1-17
			KLUSTER	4×10^4cfu/g	KOA_1-11, KOA_1-12, KOA_1-13, KOA_1-14
			WATER AGAR	5×10^4cfu/g	KOA_1-18, KOA_1-19, KOA_1-20, KOA_1-21, KOA_1-22
			ACTINOMYCETES AGAR	2×10^3cfu/g	KOA_1-23, KOA_1-24

Table.2 Colony Morphology of *Streptomyces sp* on Starch Casein Agar

ISOLATES	GROWTH	VEGETATIVE	AERIAL	APPEARANCE OF COLONY	COLOUR OF SPORE MASS	CHAIN TYPE	SPORE SHAPE	NON DIFFUSIBLE	DIFFUSIBLE
KOA_1-1	M	VIOLET	VIOLET	LEATHERY	VIOLET	LONG STRAIGHT	SPHERICAL	-	VIOLET
KOA_1-2	G	YELLOW	WHITE	LEATHERY	WHITE	LONG STRAIGHT	SPHERICAL	-	-
KOA_1-3	M	WHITE	GREY	LEATHERY	GREY	LONG STRAIGHT	SPHERICAL	-	-
KOA_1-4	G	WHITE	GREY	LEATHERY	WHITISH GREY	LONG STRAIGHT	SPHERICAL	-	-
KOA_1-5	G	WHITE	WHITE	BUTYROUS	WHITE	LONG STRAIGHT	OVAL	-	-
KOA_1-6	G	WHITE	GREY	LEATHERY	WHITISH GREY	LONG STRAIGHT	SPHERICAL	-	-
KOA_1-7	G	WHITE	WHITE	LEATHERY	WHITE	SPIRAL	OVAL	-	-
KOA_1-8	M	ORANGE	ORANGE	LEATHERY	ORANGE	LONG STRAIGHT	SPHERICAL	-	-
KOA_1-9	G	WHITE	WHITE	LEATHERY	WHITE	SPIRAL	SPHERICAL	-	-
KOA_1-10	G	WHITE	PEACH	PUFFY	PEACH	LONG STRAIGHT	OVAL	-	-
KOA_1-11	G	WHITE	GREY	LEATHERY	WHITISH GREY	LONG STRAIGHT	SPHERICAL	-	-

Table.2 Colony Morphology of *Streptomyces* sp on Starch Casein Agar (Contd..)

ISOLATES	GROWTH	VEGETATIVE	AERIAL	APPEARANCE OF COLONY	COLOUR OF SPORE MASS	CHAIN TYPE	SPORE SHAPE	NON DIFFUSIBLE	DIFFUSIBLE
KOA_1-12	G	WHITE	ASH	LEATHERY	ASH	SHORT SPIRAL	SPHERICAL	-	-
KOA_1-13	G	BROWN	WHITE	LEATHERY	BROWN	LONG STRAIGHT	OVAL	-	BROWN
KOA_1-14	G	WHITE	WHITE	BUTYROUS	WHITE	SPIRAL	SPHERICAL	-	-
KOA_1-15	M	GREEN	GREEN	BUTYROUS	GREEN	SPIRAL	SPHERICAL	-	GREEN
KOA_1-16	G	WHITE	WHITE	LEATHERY	WHITE	LONG STRAIGHT	SPHERICAL	-	-
KOA_1-17	G	WHITE	WHITE	LEATHERY	WHITE	STRAIGHT CHAIN	SPHERICAL	-	-
KOA_1-18	G	WHITE	WHITE	LEATHERY	WHITE	SPIRAL	SPHERICAL	-	-
KOA_1-19	G	ORANGISH BROWN	WHITE	PUFFY	ORANGISH BROWN	SPIRAL	CYLINDRICAL	-	ORANGISH BROWN
KOA_1-20	G	BROWN	WHITE	BUTYROUS	BROWN	STRAIGHT CHAIN	SPHERICAL	-	BROWN
KOA_1-21	G	WHITE	WHITE	LEATHERY	WHITE	SPIRAL	SPHERICAL	-	-
KOA_1-22	M	WHITE	WHITE	BUTYROUS	WHITE	STRAIGHT SPIRAL	SPHERICAL	-	-
KOA_1-23	G	PALE YELLOW	WHITE	PUFFY	LITE YELLOW	LONG STRAIGHT	SPHERICAL		PALE YELLOW
KOA_1-24	M	WHITE	WHITE	BUTYROUS	ORANGISH BROWN	LONG STRAIGHT	SPHERICAL	-	-

M – Moderate, G – Good, + Present, - Absent

Table.3 Grouping of *Streptomyces sp*. Based on Colour Hormony Code

COLOUR HORMONY CODE	SPORE MASS COLOUR	ISOLATES	SERIES DESIGNATION
WHITE	WHITE	KOA_1-5, KOA_1-7, KOA_1-9, KOA_1-14,KOA_1-16, KOA_1-17, KOA_1-18, KOA_1-19, KOA_1-20, KOA_1-21, KOA_1-22, KOA_1-23, KOA_1-24	Albus, Albidoflavus, Bosroemi, Albosporeus, Niveus, Virgatus
GREY	GREY BROWN ASH	KOA_1-3, KOA_1-4, KOA_1-6, KOA_1-11 KOA_1-13 KOA_1-12	Scabies, Violaceus, Cinereus, Hygroscopicus, Reticuli, Diastaticus, Flavoviridis, Griseus, Aureus, Chromogenes Rimosus, Flavus Cinereus, Cinereo-ruber
YELLOW	PALE YELLOW	KOA_1-2, KOA_1-23	Virgatus, Helvolus, Erythrochromogenes, Flavus, Sulphureus
RED	PEACH ORANGE	KOA_1-10 KOA_1-8	Fradiae, Roseoflavus, Roseus, Roseochromogenes, Fuscus, Ruber, Roseoviolaceus, Cinnamomeus
VIOLET	LILAC	KOA_1-1	Lavndulae, Roseus
GREEN	GREEN	KOA_1-15	Griseus, Viridis

Table.4 Distribution of *Streptomyces sp.* with Respect to Different Media

DISTRICT	LOCATION	DIFFERENT MEDIA USED	ISOLATE DESIGNATION	SPECIES IDENTIFIED
	KURUVAMPALAIYAM ELECTROPLATING INDUSTRIAL AREA	STARCH-CASEIN AGAR	KOA_1-1 KOA_1-2 KOA_1-3 KOA_1-4 KOA_1-5 KOA_1-6	*S. lavendulae* *S. erythrochromogenes* *S. cinereus* *S. reticuli* *S.albidoflavus* *S.scabies*
		OAT MEAL AGAR	KOA_1-7 KOA_1-8 KOA_1-9 KOA_1-10	*S.albus* *S.roseus* *S.albus* *S.fradiae*
		GAUZE et al	KOA_1-15 KOA_1-16 KOA_1-17	*S. griseus* *S. albus* *S. albus*
		KUSTER	KOA_1-11 KOA_1-12 KOA_1-13 KOA_1-14	*S.griseus* *S. cineruber* *S. flavus* *S. albidoflavus*
		WATER AGAR	KOA_1-18 KOA_1-19 KOA_1-20 KOA_1-21 KOA_1-22	*S. albidoflavus* *S. albus* *S. albidoflavus* *S. albus* *S. albus*
		ACTINOMYCETES AGAR	KOA_1-23 KOA_1-24	*S. albus* *S. albidoflavus*

Fig.1 Spore Morphology on Starch Casein Agar (400x)

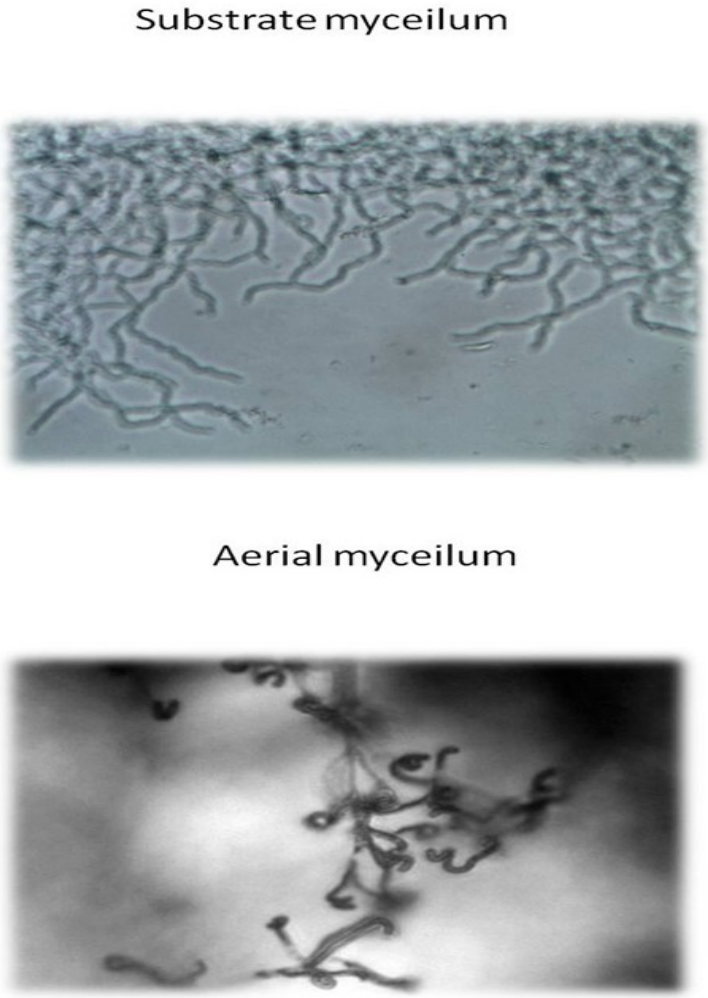


Fig.2 Growth of Isolates on Starch Casein Agar

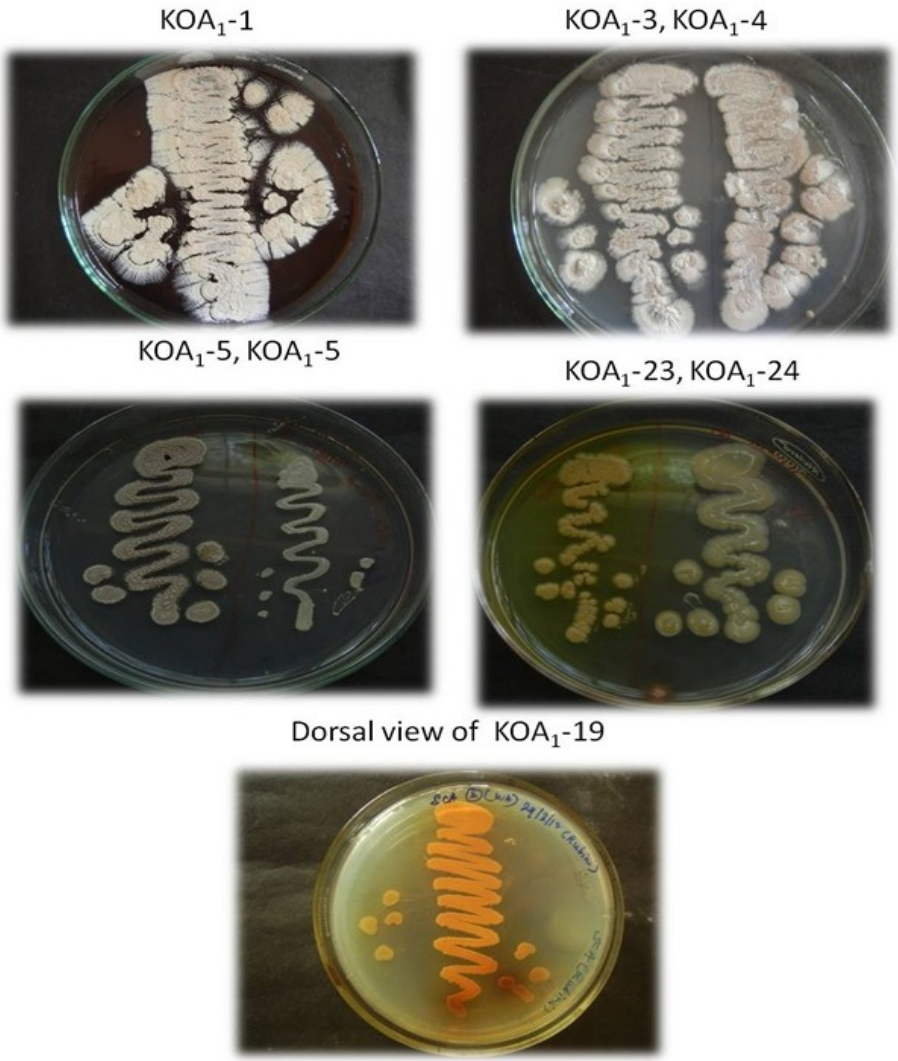
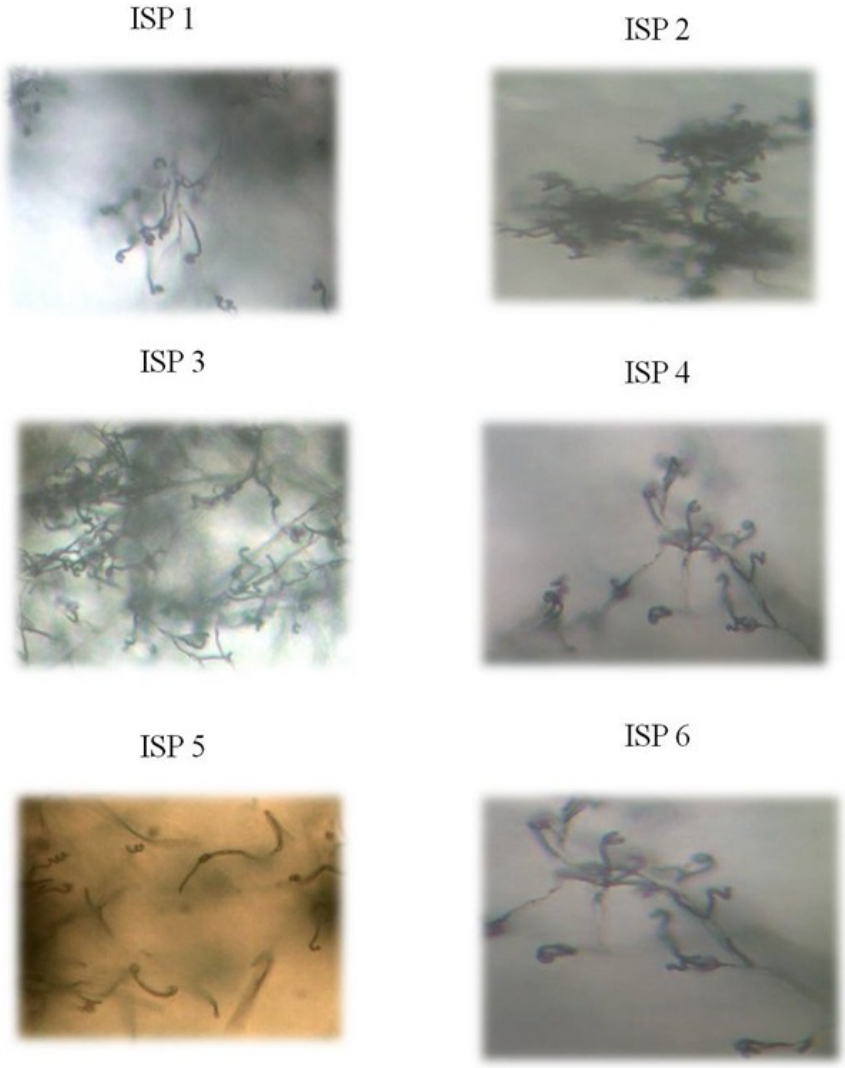


Fig.3 Spore Morphology on Different ISP Media



The utilization of starch and casein showed that these isolates produced the extracellular enzyme amylase and caseinase respectively to metabolize the polymeric components of the nutrient mixture to monomeric forms for their growth. They mostly degraded tyrosine and cellulose. They do not degrade pectin except for few species variables. Apart from these enzymes they also produce esterase, urease, and gelatinase and variably produce oxidase and citrase. Positive reaction for the catalase enzyme revealed that the isolates could withstand the stress conditions generated by reactive oxygen species. The test on triple sugar iron agar revealed that these organisms would not produce gas and acid when incubated in carbon sources such as glucose, sucrose and lactose except for few species variables. The MR test has the ability to oxidize glucose with the production and stabilization of high concentration of acid end product. They mostly showed MR negative except for few species variables. The isolates were reduced from nitrate to nitrite (Pridham & Lyons, 1960). Thus analysis of the morphological and cultural characteristics of the isolated organisms allowed us to determinate its probable taxonomic classification which clearly indicates that they belong to the genus *Streptomyces*.

At the species level of genus *Streptomyces*, the 24 isolates were identified by combining the morphological and biochemical characters with reference to Bergey's Manual of determinative bacteriology and Waksman (1943). The strains KOA_1-7, KOA_1-9, KOA_1-16, KOA_1-17, KOA_1-19, KOA_1-21, and KOA_1-22 were identified as *S. albus*. Isolates KOA_1-5, KOA_1-14, KOA_1-18, KOA_1-20, and KOA_1-24 were identified as *S. albidoflavus*. Other isolates obtained belonged to *S. griseus* (KOA_1-11), *S. cineruber* (KOA_1-15), S. cineruber (KOA_1-12), *S. roseus* (KOA_1-8), *S. fradiae* (KOA_1-10), *S. cinereus* (KOA_1-3), S. flavus (KOA_1-13), *S. reticuli* (KOA_1-4), *S. scabies* (KOA_1-6) and *S. lavendulae* (KOA_1-1). Species of Streptomyces identified in our study were found to possess similar characters as referred by William *et al*., (1983).

Results clearly showed that the diversity was observed in different types of medium used for processing the sample taken from Kuruvampalaiyam, Electroplating Industry, Coimbatore. The maximum number of species was observed in Starch casein agar, comprising of 6 species (*S. lavendulae, S. erythrochromogenes, S. cinereus, S. reticuli, S.albidoflavus, and S.scabies*). Four numbers of species was observed in Oat Meal Agar (*S. albus, S. roseus* and *S. fradiae*). Two numbers of species was observed in Gauze Inorgnic agar (*S. griesus* and *S. albus*). Four numbers of species was observed in Kuster's agar (*S. griesus, S.cineruber, S.flavus* and *S. albidoflavus*). Two numbers of species was observed in Water agar (*S. albus* and *S. albidoflavus*). Two numbers of species was observed in Actinomycetes agar (*S.albus* and *S. albidoflavus). S. albus* and *S. albidoflavus* were found repeatedly to be present in different media's used except in Starch casein agar (Table 4). Further the studies will focus on extraction of biometabolites from predominant species to explore its bioactivity.

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