Production of Indole Acetic Acid by selected *Streptomyces* spp.

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Abstract

Seaweeds were collected from Mandapam coastal area at Rameshwaram District. The genus of *Gelidiella acerosa* seaweeds was used in this study. 9 *Streptomyces* species were isolated by serial dilution method of 10^{-1} dilution. Various biochemical tests were carried out to characterize the species. SEM imaging proved that the morphological characteristics were well in the range of Actinomycetes. The spore morphology was found to be smooth *Streptomyces* species grown on yeast malt extract medium supplemented with 2mg/ml. L-tryptophan. Optical density was recorded. Tryptophan is a precursor for the production of IAA .Compounds from *Streptomyces* spp., were extracted with ethyl acetate. They were purified and identified by thin layer chromatography and the R_f value was recorded.

Key words: Seaweed, *Gelidiella acerosa*, *Streptomyces*, SEM, IAA, TLC.

INTRODUCTION

Streptomyces is the largest genus of Actinobacteria and the type genus of the family Streptomycetaceae. Over 500 species of *Streptomyces* bacteria have been described. As with the other Actinobacteria, *Streptomyces* are Gram-positive, and have genomes with high guanine and cytosine content. Found predominantly in soil and decaying vegetation, most *Streptomyces* produce spores, and are noted for their distinct "earthy" odour that results from production of a volatile metabolite, geosmin (Hwang *et al*, 1994).

A striking feature of the *Streptomyces* is the production of secondary metabolites coinciding and coordinately regulated with morphological differentiation. The result of extensive screening of *Streptomyces* has led to the discovery of more than 4000 antibiotic substances, many of which have been applied in human medicine, veterinary and agriculture. They produce over two-thirds of the clinically useful antibiotics of natural origin (e.g., neomycin and Chloramphenicol). The now uncommonly-used streptomycin takes its name directly from *Streptomyces*. Anand Prem.,*et al.,2006* .The secondary metabolites isolated from microbes especially from *Streptomyces*, exhibit either antimicrobial (antibacterial and antifungal) or antitumor and antiviral activities (Meera Kumari,et al .2013)

The genus *Streptomyces* includes aerobic, Gram-positive, filamentous bacteria which produce well developed vegetative hyphae (between 0.5-2.0 µm in diameter) with branches. They form a complex substrate mycelium that aids in scavenging organic compounds from their substrates. Although the mycelium and the aerial hyphae that arises from them are a motile, mobility is achieved by dispersion of spores. Spore surfaces may be hairy, rogues, smooth, spiny or warty. In some species, aerial hyphae consist of long, straight filaments, which bear 50 or more spores at more or less regular intervals, arranged in whorls (verticils).(James.,*et al*.,2010).

Marine seaweeds are rich source or structurally unique natural compounds Several of which shown wide Varity of biological activities (Selvakumar *et al.,* 2010)it is well known that even excellent drug candidates from seaweeds are often not developed because those seaweeds are rare difficult to collect or both. Seaweeds harbour a rich diversity of marine organisms in their tissues (Philippe Constant,*et al.,*1999). Seaweeds are host microorganisms for such as archea,bacteria,cyanobacteria and microalgae within their tissues where they reside in the extra and intra cellular space (C.J Kim *et al.,* 2010).

Seaweeds associated microorganisms are sources of wide variety of useful natural products like cytotoxins, antifouling agents, antibiotics, anti-inflammatory and antiviral compounds. *Streptomyces* sp. Annie Mathew 1995. has wide application for antiviral, antibacterial, antifungal, antitumor, insecticidal, immune-modulator and immune

suppressant etc. In this present investigation the IAA was produced by using *Streptomyces* sp which was isolated from *Gelidiella acerosa* seaweed.

Seaweeds used in this study

Kingdom: Planate
Phylum: Rhodophyta
Class: Florideophyceae
Order: Gelidiales
Family: Gelidiellaceae
Genus: Gelidiella
Species: *G. acerosa*

2. Materials and methods

2.1 Collection of sample:

The seaweed *Gelidiella acerosa* was collected from Mandapam coast. Then the sample wwas transported to the laboratory with minimum possible time to avoid the external microbial contamination and excessive proliferation. After bringing them to the laboratory all the epiphytic faunas were removed. Then it was washed with sterile sea water to remove the bacteria originating from environmental sea water.

2.1.1 Isolation of *Streptomyces:*

The *Streptomyces* were isolated by Biochemical test. The colonies were confirmed by SEM test.

2.1.2 Production of IAA:

The production of IAA by all *Streptomyces* spp. isolates was determined according to the method of Bane and Musarrat (2003). Discs (8 mm diameter) from colonies of the isolates, *Streptomyces* grown on yeast malt extract (YM) agar and incubated at 30°C for 5 d, were transferred to 5 mL YM broth containing 2 mg/mL L-tryptophan. These Khamna et al. cultures were incubated at 30°C with shaking at 125 rpm for 7 d and then harvested by centrifugation at 11,000xg for 15 min. One milliliter of the supernatant was mixed with 2 mL of Salkowski reagent; the appearance of a pink colour indicated IAA production. Optical density (O.D.) was read at 530 nm. The level of IAA produced was estimated against the IAA standard.

2.1.3 Extraction and Isolation of IAA:

All *Streptomyces* isolates were cultivated in 200 mL of YM broth containing 2 mg/mL L-tryptophan at a pH of 7.0. IAA was extracted from the supernatants with ethyl acetate according to the method described by Ahmad et al. (2005). Ethyl acetate fractions (10-20 mL) were applied to TLC plates (Silica gel G f254, thickness 0.25 mm, Merck, Germany) and developed in butanone-ethyl acetate ethanol- water (3:5:1:1). Spots with Rf values identical to authentic IAA were identified under UV light (254 nm) by spraying the plates with Ehmann's reagent (Ehmann1977).

Result and Discussion

The colour of the mycelium was found to be the following when the organism was grown using PDA medium.

The investigation accounted for the presence of *Streptomyces* sp in the Seaweeds of *Gellidiella acerosa* and *Gracilaria.* Nine *Streptomyces* were selected for this study. The results indicated the occurrence of the species only because of the usage of the selective media that is *Actinomycetes* isolation agar. The strains were found to grow well in potato dextrose agar and hence were used for further sub culturing. The change in colour increases gradually day by day. This colouration pattern may be due to the primary and secondary metabolites produced during the growth period. These primary and secondary metabolites are rich in certain

compounds such as amino acids, sugars, fatty acids, antibiotics etc. Aerial and substrate mycelia showing different colours were observed.

The characterisation of *Streptomyces* sp., were studied by the method recommended by International *Streptomyces* Project (Shirling and Gottlieb, 1966). The colouration patterns of the aerial and substrate mycelia were different in medium which provides certain nutrients for triggering of genes for the conversion as well as expression of other metabolic products, which in turn leads to different aerial and substrate mycelia colouration as observed by Dhevendaran and Annie(1999). This colouration difference may be due to the different nutrients providing by different media which in turn causes difference in the primary and secondary metabolites. Mycelia colour characteristics of selected species in potato dextrose agar was found to have white colour substrate mycelium and green colour aerial mycelium. This was found to grow the best in 5% sodium chloride concentration. The gram staining shows that the strain was gram positive. The sporophrore staining showed rectiflexibiles. With regard to the spore chains they may be grouped into 'sections of rectiflexibiles.

The scanning electron microscope images showed that the size of the spores were approximately 2.2µm, which confirms that this species belongs to the Actinomycetes group. The spore surface morphology was found to be smooth through examinations.

All *Streptomyces* isolates were screened for their ability to produce IAA; nine isolates had the ability and several reports have shown that *Streptomyces* sp. Previous studies demonstrated that *Streptomyces has* the ability to produce IAA. The purpose of this study was to investigate IAA production in *Streptomyces* and the role of IAA. L-tryptophan at 2 mg/ml concentration was the best for IAA production by this isolate, whereas at higher concentrations tryptophan exerts an adverse effect on production. L-tryptophan is generally considered as an IAA precursor, because its addition to IAA producing bacterial culture enhances IAA biosynthesis. The strain *Streptomyces* preferred tryptophan for production of IAA. Maximum IAA production was found in the medium amended with 0.5% tryptophan (Fig. 2).

Streptomyces produced maximum IAA when it was grown using an YM broth at 30°C (Fig. 4), this temperature was found suitable for growth and IAA production of this isolate. Aldesuquy *et al.*, (1998) found that temperatures at 30°C were suitable for growth and IAA production of *Streptomyces* sp. Thin Layer Chromatography was used extensively for detection of IAA compare with standard,

From this study it is clear that *Streptomyces* can provide a rich source of IAA producing- *Streptomyces* spp. has the ability to produce a significant amount of IAA in a tryptophan-supplemented medium. Thus, our experiments have demonstrated that a considerable part of *Streptomyces* and bacteria associated with Seaweeds are able to synthesize IAA.

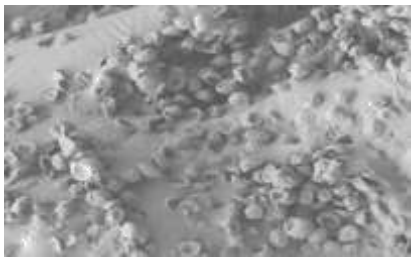
Table 1: Characterization of *Streptomyces* associated with seaweeds

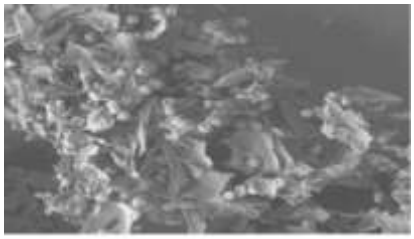
Strains	**Colour of Mycelium**		**Pigmentation**
	Aerial Mycelium	**Substrate Mycelium**	
AR1	Light Green	Yellow	-
AR2	Dark Green	Yellow	-
AR3	Blackish brown	Yellow	-
AR4	Green	Yellow	-
AR5	Grayish green	Cream white	-
AR6	Grey	Yellow	-

AR7	Grey	Brown	Pink
AR8	Dark green	Yellow	-
AR9	Yellowish green	Milky white	Light green

Figure 1: SEM Analysis of *Streptomyces sp.*

Image for AR3 isolates





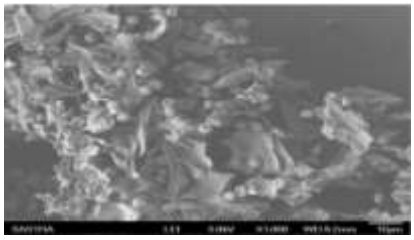


Image for AR8 Isolates

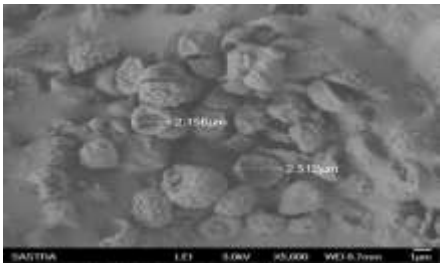


Image for AR9 Isolates.

Image for AR6 Isolate

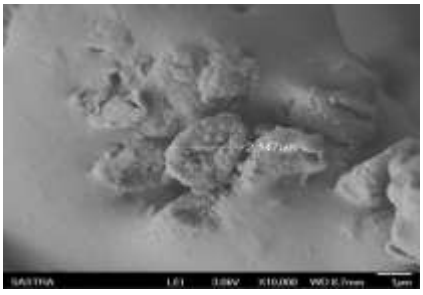


Image for AR7 Isolates

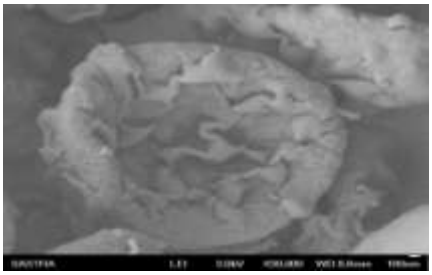


Image for AR4 Isolates

Table 2: Biochemical characteristics for nine isolates:

Name of the Test	AR1	AR2	AR3	AR4	AR5	AR6	AR7	AR8	AR9
Starch hydrolysis	+	+	+	+	+	+	+	+	+
Production of H2S	+	+	+	+	+	+	+	+	+
Citrate utilization	+	+	+	+	+	+	+	+	+
Catalase Test	+	+	+	+	+	+	+	+	+
Indole production	–	–	–	–	–	–	–	–	–
TSI	Alk/Alk	Alk/Alk	Acid/Alk	Acid/Alk	Alk/Alk	Alk/Alk	Alk/Alk	Alk/Alk	Alk/Alk
Methyl red Test	–	–	–	–	–	–	–	–	–
Voges-proskauer	–	–	–	–	–	–	–	–	–

Table3: Standard OD of IAA production.

Concentration	OD at 530nm
0.1	0.36
0.2	0.45
0.3	0.54
0.4	0.63
0.5	0.71

Table4: IAA Production by selected *Streptomyces* species.

Isolates	IAA Concentration (OD at 530nm)	Cell dry weight
AR1	1.6451	0.25
AR2	1.2044	0.42
AR3	1.8736	0.49
AR4	1.1241	0.22
AR5	1.4663	0.46
AR6	1.2263	0.35
AR7	0.8759	0.58
AR8	2.4089	0.38
AR9	1.7294	0.27
Standard	1.4351	0.32

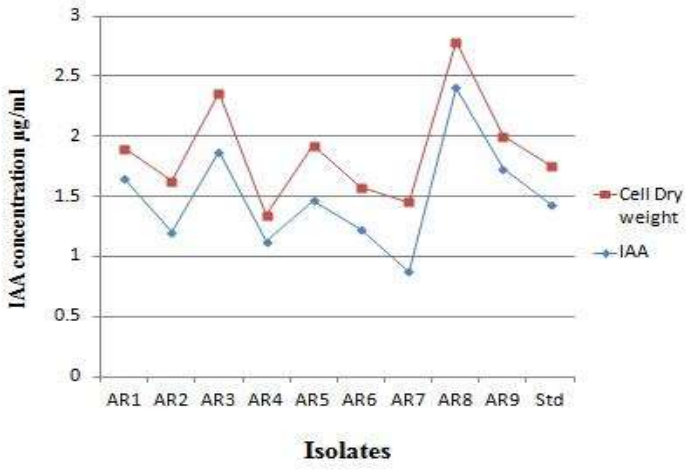
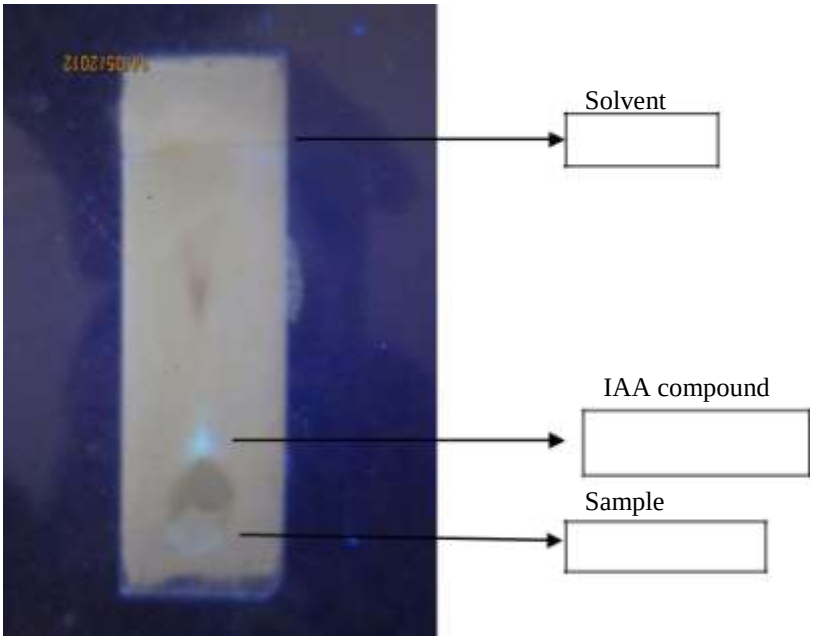


Table 5: Effect of Incubation period on IAA Production.

Isolates	24hrs(OD at 530nm)	48hrs(OD at530nm)	72hrs(OD at530nm)	96hrs(OD at 530nm)
AR1	0.54	1.34	1.47	1.55
AR2	0.91	1.00	1.16	1.23
AR3	0.19	0.22	0.34	0.63
AR4	0.40	0.43	0.75	0.89
AR5	0.55	0.40	0.68	0.78

AR6	0.94	1.06	1.21	1.29
AR7	0.98	0.84	1.18	1.26
AR8	0.67	0.73	0.85	1.34
AR9	0.65	0.84	0.89	0.99
Standard	0.59	0.68	0.79	0.86

Fig:2 TLC for AR1 Isolate



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