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Original Article

EFFECT OF ACTINOMYCETES ON GROWTH OF *OCIMUM SANCTUM*

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ABSTRACT

Objective: The study was aimed to isolate and identify Actinomycetes from rhizosphere soil of Malik Deenar College of pharmacy, Kasaragod, Kerala, and to screen the plant growth promoting the activity of isolated actinomycetes on *Ocimum sanctum*.

Methods: The Actinomycetes were isolated from rhizosphere region soil by serial dilution and pour plating method. Then they were identified to a generic level based on morphological characters, and biochemical characters. Plant growth promoting activity was screened by placing 3 sets of Ocimum sanctum seedlings in different pots and the shoot length was measured after 10 d and compared with control.

Results: 19 different types of isolates were chosen based on their morphological characteristics and used for assessments of plant growth promoting activity. Among the 19 isolates, 17 isolates were identified as *Streptomyces* spp. And 2 were belongs to *Nocardia* spp. The actinomycetes isolates which belong to *Streptomyces* spp were shown plant growth promoting activity.

Conclusion: The result of this study revealed that the rhizosphere soil has different types of actinomycetes, among these Streptomyces spp. are more abundant and common. These Streptomyces spp. have predominant activity on plant growth promotion with respect to Ocimum sanctum.

Keywords: Actinomycetes, Rhizosphere, Ocimum *sanctum,* Plant growth promoter, Secondary metabolite

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INTRODUCTION

Plant rhizosphere soil represents a biological niche with a diverse microflora comprised of bacteria, fungi, protozoa, and algae. This community is supported nutritionally by a high input of organic material derived from the plant roots and root exudates that are necessary for microbial growth [1]. The Actinomycetes are widely distributed in nature, predominantly found in soil, especially in the rhizosphere. These are a diverse group of gram-positive filamentous bacteria belongs to the order Actinomycetales. These organisms are profoundly known for their secondary metabolism and a wide array of metabolites viz, antibiotics enzymes, immunosuppressant's, antitumor, cytotoxic and pharmacological agents, etc [2]. Actinomycetes secondary metabolites can influence plant development by producing plant hormones, increasing the availability of mineral nutrients and also excreting antibiotics or toxins that act in the biological control of pathogens in the rhizosphere. They are well known for their extensive production of compounds with phosphate-solubilizing ability and compounds with high biotechnological potentials such as chitinase and antibiotics [3].

Actinomycetes are gram-positive bacteria with high guanine+cytosine content of over 55%in their DNA, which have been recognized as sources of several secondary metabolites, antibiotics, and bioactive compounds that affect microbial growth. Actinomycetes have filamentous nature, branching pattern, and conidia formation, which are similar to those of fungi. For this reason, they are also known as ray fungi [4]. A large number of actinomycetes have been isolated and screened from soil in the past several decades, accounting for 70%-80% of relevant secondary metabolites available commercially. Actinomycetes are a potential source of many bioactive compounds, which have diverse clinical effects and important applications in human medicine. It has been estimated that approximately one-third of the thousands of naturally occurring antibiotics have been obtained from actinomycetes [5].

Ocimum sanctum commonly known as thulasi or holy basil is an aromatic perennial plant and used as traditional medicine. It is commonly used in Ayurveda. Ocimum sanctum has been recommended for the treatment of bronchitis, malaria, diarrhea, dysentery, skin diseases, insect bite and so on [6].

MATERIALS AND METHODS

The identification and characterization of actinomycetes from rhizosphere soil require culture media, different kinds of reagents and instruments.

Collection of sample

The soil sample was collected from the rhizosphere region of Malik Deenar College of pharmacy, Kasaragod, Kerala from a depth of 12-15 cm from the soil surface in sterilized polythene covers

Isolation of actinomycetes

The soil sample was serially diluted and pour plated on starch casein nitrate medium and plates were incubated at 28 ° c for 7-8 d. Antifungal antibiotics griseofulvin 150 mg/l and fluconazole 150 mg/l was added to media to prevent fungal contamination.

Identification of actinomycetes

Identification of the strains was carried out based on their morphological, physiological and biochemical characters to the genus level following the direction mentioned in the Bergey's manual of systematic bacteriology and international Streptomyces. The morphological methods consist of macroscopic and microscopic characterization. Macroscopically the actinomycetes isolates were differentiated by their colony character, e. g. size, shape, colour, presence of mycelium and texture etc. Spore chain arrangement was studied by the coverslip method. The isolates were studied under oil immersion objective. Based on spore chain arrangement the isolates were classified into different genera of Actinomycetes. Staining and biochemical methods includes gram staining, acid-fast staining, catalase test, gelatine hydrolysis, hydrogen sulphide production test, carbohydrate fermentation test, starch hydrolysis test [7-12].

Screening of plant growth promoting activity

The inoculum is prepared by inoculating 3-5 colonies of each actinomycete strain into starch casein nitrate broth and then inoculums were incubated at 28 ° c for 6-8 d.

Soil was collected and sterilized by autoclaving for 3-4 d, then the soil was checked for sterility by placing a small amount of soil in a

sterile petri plate and incubated and observed for the growth of microorganisms. Add the sterilized soil to separate pots and then add the seedlings of *Ocimum sanctum*. For the preparation of seedling first, collect the seeds of *Ocimum sanctum* from the same plant and allow to sprout on a single area and wash the seedling properly by using water. Then place that seedling on the pots which contain sterile soil. Prepare 3 sets of such seedling with soil. Then to the first set, add starch casein nitrate broth along with actinomycetes and water daily. To the second set (control 1) of seedlings, add starch casein nitrate broth and water daily. To the final set (control 2) of seedling add water daily. Then check the growth of plant up to 10 d.

Table 1: Isolation and enumeration of actinomycetes

Soil sample	Number of colonies		
	10^{-1}	10^{-3}	10^{-5}
Papaya	57	22	4
Acacia	66	31	9
Coconut	60	20	2

Table 2: Morphological character of actinomycetes

Isolate no.	Microscopic observation			Macroscopic observation		Identified as
Ac 1	Gram reaction	Acid fast staining	Spore arrangement	Colony morphology	Pigmentation (back view)	
Ac 2	+	-	Spira	Dry, powdery, grey white	Grey	*Streptomyces* spp.
Ac 3	+	+	Short chains	White, medium, cottony	Absent	*Nocardia spp.*
Ac 4	+	-	RA	White, cottony, irregular	Green	*Streptomyces spp.*
Ac 5	+	-	Flexibilis	Dry, powdery, whitish brown	Brown	*Streptomyces spp.*
Ac 6	+	-	Flexibilis	White, round, cottony	Brown	*Streptomyces spp.*
Ac 7	+	-	Rectus	Greenish white, cottony, round	Green	*Streptomyces spp.*
Ac 8	+	-	RA	Greyish white, cottony, round	Grey	*Streptomyces spp.*
Ac 9	+	-	Straight	White, very small, cottony	Grey	*Streptomyces spp.*
Ac 10	+	-	Rectus	Yellowish white, large, dry	Absent	*Streptomyces spp.*
Ac 11	+	-	Rectus	Small, white, dry, powdery	Absent	*Streptomyces spp.*
Ac 12	+	-	Straight	Small, dry,brown	Brown	*Streptomyces spp.*
Ac 13	+	-	Spira	White, dry, powdery	Light pink	*Streptomyces spp.*
Ac 14	+	-	Spira	White, cottony, medium	Brown	*Streptomyces spp.*
Ac 15	+	-	Rectus	Yellowish white, large, dry	Absent	*Streptomyces spp.*
Ac 16	+	-	Spira	Greyish white, cottony	White	*Streptomyces spp.*
Ac 17	+	-	Rectus	Light greyish white, large	Creamish white	*Streptomyces spp.*
Ac 18	+	-	Rectus	Dark, greyish white, powdery	White	*Streptomyces spp.*
Ac 19	+	-	Straight	Light, greyish white, medium round	Creamish yellow	*Streptomyces spp.*
	+	+	Short chains	Brownish white, irregular, raised.	Brown	*Nocardia spp.*

RA–Retinaculum ageratum-Negative+Positive

Table 3: Biochemical character for identification of actinomycetes

Isolate no.	H_2S	Gelatine	Starch	Carbohydrate fermentation						Catalase
				Glucose	Lactose	Sucrose	Starch	Starch	Starch	
Ac 1	-	+	+	-	-	-	Alk	Alk	Alk	+
Ac 2	-	-	+	A	-	A	Alk	Alk	Alk	+
Ac 3	-	-	+	-	-	A	-	-	-	+
Ac 4	-	-	+	-	-	A	-	-	-	+
Ac 5	-	-	+	-	-	-	-	-	-	+
Ac 6	-	-	+	A	-	-	Alk	Alk	Alk	+
Ac 7	-	+	+	A	-	-	Alk	Alk	Alk	+
Ac 8	-	+	+	A,G	G	A	-	-	-	+
Ac 9	-	+	+	A,G	A,G	G	-	-	-	+
Ac 10	-	+	+	A,G	-	-	-	-	-	+
Ac 11	-	+	+	G	A	G	Alk	Alk	Alk	+
Ac 12	+	-	+	A	-	-	A	A	A	+
Ac 13	+		+	A	-	-	-	-	-	+
Ac 14	-	+	+	G	A	A,G	-	-	-	+
Ac 15	-	-	+	G	Alk	A,G	Alk,G	Alk,G	Alk,G	+
Ac 16	-	-	+	-	Alk,G	A	-	-	-	+
Ac 17	-	-	+	-	Alk	A	-	-	-	+
Ac 18	-	-	+	-	-	-	A	A	A	+
Ac 19	-	-	+	A	-	-	Alk	Alk	Alk	+

H_2S: H_2S productionA: Acid production, Gelatine: Gelatine hydrolysis Alk: Alkaline production, Starch: Starch hydrolysis G: Gas production, -: No change+: Positive

RESULTS AND DISCUSSION

Large number of actinomycetes were found in rhizosphere region of Malik deenar college campus, Kasaragod, Kerala. The soil sample was collected and serially diluted and plated on starch casein nitrate agar. From these actinomycetes isolates 19 different types of isolates were chosen based on their morphological characteristics and assessments of plant growth promoting activity. The results were shown in table 1.

Identification of actinomycetes

The chosen 19 isolates were identified Based on colony character, staining character and biochemical character. The morphological and biochemical character are tabulated (table 2.). Among the 19 isolates 17 isolates were identified as *Streptomyces* spp. And 2 were belongs to *Nocardia* spp.

Screening for plant growth promoting activity

The plant growth promoting activity was studied by growing the 3 sets of *Ocimum sanctum* seedling for 10 d. Then the shoot length was measured and compared with the control. In this, the shoot length of control 1(water+starch casein nitrate broth) was 6.30 cm. and shoot length of control 2(water) was 6.27 cm. The isolate Ac 8 showed highest shoot length about 11.04 cm followed by the isolate Ac 3 (9.70 cm), Ac 7 (9.29 cm) and these organisms belongs to Streptomyces spp. The value of shoot length was tabulated in the table 4.

Table 4: Value of shoot length

Isolate no.	Shoot length of *Ocimum sanctum* (cm)
Control 1	6.30
Control 2	6.27
Ac 1	8.89
Ac 2	9.06
Ac 3	9.70
Ac 4	8.13
Ac 5	8.29
Ac 6	9.41
Ac 7	9.29
Ac 8	11.04
Ac 9	6.66
Ac 10	6.79
Ac 11	6.82
Ac 12	6.71
Ac 13	5.54
Ac 14	6.54
Ac 15	8.13
Ac 16	7.88
Ac 17	7.05
Ac 18	7.58
Ac 19	7.71

CONCLUSION

The results of this study revealed that the rhizosphere soil have different types of actinomycetes, among these *Streptomyces* spp. are more abundant and common. These Streptomyces spp. have predominant activity on plant growth promotion with respect to *Ocimum sanctum*

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AUTHORS CONTRIBUTIONS

All the author has contributed equally

CONFLICT OF INTERESTS

Declared none

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