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Research Article

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Collection of Different Isolates of Actinomycetes from Major Groundnut Growing Regions of Andhra Pradesh

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ABSTRACT

Survey was conducted to collect the isolates of Actinomycetes in major groundnut growing areas of Andhra Pradesh (10 districts) viz., Anantapuramu, Kadapa, Kurnool, Chittoor, Nellore, Prakasam, Guntur, West Godavari, Vizianagaram and Srikakulam during Kharif, 2016. During the survey, 180 rhizospheric soil samples were collected and isolated a total number of fifty morphologically different Actinomycetes isolates. Basic identification of Actinomycetes was done by visual observation, general morphology, spore formation, colony morphology including Gram stain and biochemical tests and an earthy odour. The Actinomycetes isolated were maintained for in vitro antagonistic efficacy against stem rot pathogen Sclerotium rolfsii to identify potential isolates.

Keywords: *Ground nut, Biocontrol agents, Sclerotium rolfsii, Rhizosphere.*

INTRODUCTION

Ground nut stem rot caused by *Sclerotium rolfsii* Sacc. is a major constraint in the production and productivity of the crop. Management of stem rot disease become very difficult as soilborne nature of *S. rolfsii* with wide host range and lack of disease resistance in existing commercial cultivars. Though chemical pesticides have played an important role in increasing groundnut production and management of stem rot, their indiscriminate use for the control of disease has led to several environmental problems such as development of resistance in pathogens, pesticide residues and the destruction of beneficial organisms. Thus other alternative disease management options were considered among which biological control appears promising. Majority of the existing biocontrol agents for management of soil-borne diseases, were isolated from the rhizosphere. Biological control with potential Actinomycetes is receiving greater attention all over the world. Among Actinomycetes, *Streptomyces* being root-colonizing and rich producer of secondary metabolites become one of the important promising group of antagonists. Studies were conducted on the collection and isolation of Actinomycetes from different groundnut growing areas of Andhra Pradesh to identify potential isolates against stem rot pathogen *Sclerotium rolfsii*.

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Stem rot symptoms appear initially as yellowing of leaves and wilting of the branches near the plant base and continue to the top of the plant. Small brownish spots develop on the stem near the soil line and later spread to the top shoots and occasionally the spots are noticed on the roots near the soil line. Mass of white mycelia develops near the soil line around the affected areas of the stem. This is followed by production of sclerotia on the infected branches, which are initially white and later turn into brown colour (Aycock, 1966, Mathur & Sinha, 1970).

Sclerotium rolfsii Sacc., is one of the most common soilborne fungus causes stem rot disease of Groundnut (*Arachis hypogaea* L.) worldwide (Butler & Bisby, (1931), Singh & Mathur, (1953) and resulting major yield losses. In addition to groundnut, *S. rolfsii* attacks wide range of hosts and can cause disease at any stage of host plant during favourable conditions (Punja, 1985). The stem rot is most severe in certain states of USA with an average pod yield loss ranging from 10 – 25% with maximum loss upto 80% in having infected fields (Bowen et al., 1992). However, the intensity of losses differs with different geographical area, soil type and cultivation practices.

Nautiyal, (2002) reported that stem rot is the most economically important disease of groundnut, which accounts around 60 per cent loss in groundnut yields annually.

S. rolfsii perpetuates as heat, drought and fungicide-resistant recalcitrant sclerotia (Punja & Rahe, 1992) which are the primary inoculum for the disease, contributing greatly to the increase of inoculum potential in cropped soils (Coley &Smith, (1979), Punja, (1988).

MATERIALS AND METHODS

Collection of Soil sample Soil samples were collected at the rhizosphere of healthy groundnut plants adjacent to stem rot affected plants. After soil sample taken, it was packed in polyethylene bags to minimize moisture losses during transportation. Samples were air dried for one week then were crushed and sieved. The sieved soil samples were pretreated by mixing 1g of soil with 0.1g Calcium carbonate and incubated at 37°C for 2-5 days. This pretreatment enhances the population of *Streptomycetes spp*. in soil samples (Boroujeni et al., 2012).

Isolation and identification of Actinomycetes by soil dilution plate method

Ken Knight's Agar medium was used for isolation of Actinomycetes (Allen, 1953). 10 g soil sample was suspended in 100 ml sterile water (10%) and agitated for 30 min at 420 rpm. These suspensions were considered as 10^{-1} dilution. From 10^{-1} suspension, took the 1 ml supernatant transferred to 9 ml of sterile distilled water and subsequently serially diluted to 10^{-3} , upto 10^{-6} etc. From the required dilution, 0.1 ml suspension was drawn and plated over the surface of Ken knight's medium by spread plate technique (Allen, 1953). All the plates were incubated at 28°C ± 2°C for 5 days. Colonies of Actinomycetes on agar plates were picked up on the basis of their morphological characteristics and isolated as pure culture by routine microbiological methods and maintained on Ken knight agar slants and as 20% (w/v) glycerol stock. The slant cultures were stored at room temperature and kept in dark while glycerol stocks were kept in freezer at -20ºC and -80ºC.

ISOLATION OF RHIZOSPHERE MYCOFLORA

Among the Actinomycetes, *Streptomyces* genus alone may represent 5-20 % of the total microbial isolates in non specific dilution plate counts. The colonies showing typical morphology were purified and stored at 4°C in agar slants and as glycerol stock at -20°C. Crawford et al., 1993 used the selective media for isolating diverse group of Actinomycetes which has low nutrient concentrations, were found to be very good by avoiding contamination and overgrowth of isolation media by other bacteria and fungi.

The name "Actinomycetes" was derived from Greek "atkis" (a ray) and "mykes" (fungus), and has features of both bacteria and fungi (Das et al., 2008).

Actinomycetes are soil organisms which have characteristics common to bacteria and fungi and yet possess sufficient distinctive features to delimit them into a distinct category. In the strict taxonomic sense, actinomycetes are clubbed with bacteria in the same class of Schizomycetes but confined to the order Actinomycetales which have gained prominence in recent years because of their potential for producing antibiotics (Kumar et al., 2005). Actinomycetes emits an earth odour of freshly ploughed soil due to the production of "GEOSMINS", the volatile compounds (Wilkin, 1996).

Actinomycetes population considered as one of the major group of soil population (Kuster, 1968). Actinomycetes are also present in marine sediments, but at lower numbers than in soil (Zheng et al., 2000). Actinomycetes are the most widely distributed group of micro organisms in nature which primarily inhabit the soil (Oskey et al., 2004). Most of the Actinomycetes are benign saprophytes, it is likely that their population depends on the available decomposable organic matter thus increase soil fertility. They compose 10-50% of the total microbial population in soil. They are second in abundance to bacteria with population range from 10^5 to 10^8 CFU per gram of soil and in composting manure pits it can reach upto 10^{10} CFU per gram of soil (Kanti, 2005). Streptomycin, gentamicin, rifamycin and erythromycin are some of the antibiotics which are in use presently are the products of Actinomycetes. The Actinomycetes are important in the field of agriculture and also pharmaceutical industries. Previous study showed that Actinomycetes isolated from soil have the potential to inhibit the growth of several plant pathogens (Jeffrey et al., 2007, 2008) isolated Actinomycetes from farming soil and reported the inhiition of *Erwinia amylovora* and A*grobacterium tumefaciens* growth by Actinomycetes.

Streptomyces are Gram positive bacteria with high G+C content that are mainly found in soil and decaying organic matter (Kampfer, 2006). Hayakawa, (2008) isolated *Streptomyces* by serial dilution method from soil. Similar method was used for isolation of *Streptomyces* by Collins et al. (1989) from vermicompost sample and Manasa et al. (2013) from rhizosphere soil. Dhanasekaran et al. (2009) collected, isolated and screened Actinomycetes for the production of novel bioactive compounds and reported that the Actinomycetes count was 12 x 10^4 cfu/g of soil. These organisms will grow in close association with the plant roots and are one of the important groups of root-colonizing micro-organisms (Franco-Correa et al., 2010; Nimnoi et al., 2010).

The utilization pattern of carbon sources by the strains can be used as an aid for species determination. Ken knight's Agar medium was used as basal medium for culturing Actinomycetes which produced highest bomass (15.6-24.4 mg/100 ml) among different media used.

Lakshmipathy and Kannabiran 2010 identified the potential strain VITDDK2 from the isolated Actinomycetes of the coastal region of Tamil Nadu by screening with antagonistic activity against *Klebsiella pneumoniae*, *Aspergillus flavus* and *Aspergillus niger*. They reported that strain VITDDK2 shared 93% similarity with *Streptomyces sp*. strain 346 by the Chitinolytic activity, Chemotaxonomic analysis, 16 S rRNA partial gene sequence and phylogenetic analysis.

RESULTS AND DISCUSSION

Isolation of different soil Actinomycetes

Roving survey was conducted to collect the isolates of Actinomycetes in major groundnut growing areas of Andhra Pradesh in 10 districts *viz.*, Anantapuramu, YSR Kadapa, Kurnool, Chittoor, PSR Nellore, Prakasam, Guntur, West Godavari, Vizianagaram and Srikakulam during the kharif, 2016. The data pertaining to survey is given in Table 1. During the survey 180 rhizospheric soil samples were collected from healthy roots of a plant present nearby disease affected plant and isolated a total number of fifty morphologically different Actinomycetes by

soil dilution plate technique using Ken Knight's Agar medium (Table 2, Plate 1). Basic identification of Actinomycetes was done by visual observation, general morphology, spore formation, colony morphology including Gram stain and biochemical tests and an earthy odour. Among other inhabitants of soil samples the isolates identified by zones of growth inhibition as the major evidence of antibiotics production. Among the fifty, twenty isolates were selected based on the growth rate of isolate on culture medium and zone of growth inhibition. All the twenty isolates further identified as Actinomycetes by general biochemical and morphological characters and proved that all were acid-fast negative, Gram stain positive and aerobic with aerial and substrate mycelia of different colors with spiral spore chains.

These twenty isolates were selected for *in vitro* antagonistic study and were maintained as pure culture by routine microbiological methods on Ken knight's agar slants.

Table 1: List of Surveyed villages in Andhra Pradesh

S.No	District	Mandals	Villages	No. of fields visited	No.of soil samples collected
1.	**Kurnool**	Gonegandla	Gonegandla	3	3
			Lingamdinne	3	3
		Pattikonda	Pattikonda	3	3
			Chinnahulthi	3	3
		Yammiganur	Banavasi	3	3
			Adoni	3	3
2.	**Kadapa**	Duvvur	Duvvur	3	3
			Gollapalle	3	3
		Vempalli	Vempally	3	3
			Tallapalle		3
		L.R.Pally	Lakkireddipalli	3	3
			Dinnepadu	3	3
3.	**Prakasam**	Kothapatnam	Alluru Kothapatnam	**3**	3
			Ethamukkala	**3**	3
		Chirala	Gavinivaripalem	**3**	3
			Ipurupalem	**3**	3
		Ulavapadu	Krishnapuram	**3**	3
			Chagallu	**3**	3
4.	**Anantapuramu**	Kalyandurgam	Kalyanadurgam	**3**	3
			Thimmasamudram	**3**	3
		Kadiri	Kadiri	**3**	3
			Yeguvapalle	**3**	
		Narpala	Narpala	**3**	3
			Bandlapalle	**3**	3
5.	**Guntur**	Cherukupalli	Gudavalli	**3**	3
			Kanagala	**3**	3
		Karlapalem	Karlapalem	**3**	3
			Ganapavaram	**3**	3
		P.V.Palem	Piittalavanipalem	**3**	3
			Khajipalem	**3**	3
6.	**Vizianagaram**	Merakamudidam	Merakamududam	**3**	3
			Ravivalasa	**3**	3
		Cheepurupalli	Sankupalem	**3**	3
			Mettapalle	**3**	3
		Dattirajeru	Lingarajapuram	**3**	3
			Dattirajeru	**3**	3
7	**West Godavari**	Chintalapudi	Chintalapudi	**3**	3
			Recharla	**3**	3
		Polavaram	Polavaram	**3**	3
			pattisam	**3**	3

		Kothapeta	Kothapeta	**3**	3
			Vadapalem	**3**	3
8	**Chittoor**	Chandragiri	Sanambhatla	**3**	3
			Panapakam	**3**	3
		Narayanavanam	Kalyanapuram	**3**	3
			Samudayam	**3**	3
		Molakalacheruvu	Molakalacheruvu	**3**	3
			Devarapalle	**3**	3
9	**Nellore**	Kavali	Kavali	**3**	3
			Musunur	**3**	3
		Vidavalur	Vidavalur	**3**	3
			Dampuru	**3**	3
		Sullurpeta	Ramachandragunta	**3**	3
			Suggupalle	**3**	3
10	**Srikakulam**	Tekkali	Chakipalle	**3**	3
			Gudem	**3**	3
		Polaki	Polaki	**3**	3
			Nandigam	**3**	3
		Ranasthalam	Ranastalam	**3**	3
			Kotcherlass	**3**	3

Table 2: Rhizosphere soil samples collected from different districts of Andhra Pradesh

S. No.	District	Mandal/Place	Isolate
1	Anantapuramu	Kalyandurgam	KYD
2		Kadiri	KDR
3		Narpala	NPL
4	Kadapa	Lakkireddipalli	LRP
5		Vempalli	VPL
6	Kurnool	Pattikonda	PKD
7		Yammiganur	YMN
8		Gonegandla	GGD
9	Chittoor	Molakalacheruvu	MKC
10		Satyavedu	STD
11		Narayanavanam	NYV
12		Tirupati	TPT
13	Nellore	Kavali	KVL
14		Sullurpet	SLP
15	Prakasam	Kothapatnam	KPT
16	Guntur	Cherukupalli	CRP
17	West Godavari	Chintalapudi	CLP
18	Vizianagaram	Merakamudidam	MMD
19	Srikakulam	Tekkali	TKL
20		Ranastalam	RSL

Among the isolated twenty Actinomycetes five isolates *viz*., KYD, KDR, GGD, LRP and MKC were found to be effective in inhibiting the growth of *S.rolfsii*. Actinomycetes were present only in twenty soil samples collected and remaining soil samples are not having any Actinomycetes. The presence of Actinomycetes may depend on area of collection, soil type, soil P^H and soil temperature. In alkaline soils, 95% of the microbial isolates may be Actinomycetes as these are tolerant of alkaline conditions. Actinomycetes can be isolated more commonly from hotter soils than colder soils due to its usual spore recovery from dried hot soils. Actinomycetes may not tolerate

dessication but the spores they produce may tolerate dessication and can be recoverable after a drought. Optimum temperature for actinomycetes is 28-37°C but can grow at 55-65°C in compost heaps. They are aerobic hence do not grow well in wet soils.

Kamal and Sharma, (2014) isolated and purified Actinomycetes on Ken knights agar medium using streak and cross streak methods.

Several research workers isolated and characterized Actinomycetes from the rice rhizospheric soils (Tamreihao et al., 2016), Sugarbeet rhizosphere soil (Errakhi et al., 2009), rhizosphere of groundnut (Adilakshmi et al., 2013, Daniel jebaraj et al., 2017).

Similarly Taddei et al., 2006 isolated 71 *Streptomyces* spp. from soil samples collected at different places of Venezuela, among which 67 were of presumably new strains and four isolates shared 100% identity with reported *Streptomyces* spp.

Askar et al. (2014) also isolated 225 effective Actinomycetes from the soil samples collected at Saudi Arabia. *Streptomycetes* are Gram positive, filamentous bacteria in the Streptomycetaceae family (Phylum Actinobacteria, Order Actinomycetales (Anderson, 2001). They are widely distributed in soil (nearly 40% of soil bacteria) and rhizosphere, where they form very dynamic assemblies.

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