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Research Article

ANTAGONISTIC AND PLANT GROWTH PROMOTING POTENTIALS OF INDIGENOUS ENDOPHYTIC ACTINOMYCETES OF SOYBEAN (*GLYCINE MAX* (L) MERRIL)

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ABSTRACT
Use of indigenous endophytes is considered as an environmentally-friendly and ecologically efficient strategy. A total 15 endophytic actinomycetes belonging *Streptomyces sp., Micromonospora sp., Nocardia sp., Actinomadura sp., Microbispora sp.,* and *Actinoplanes sp.* were isolated from soybean (*Glycine max* (L) Merril) and were screened in vitro for the antagonistic activity against soil-borne fungal pathogens of soybean viz., *Rhizoctonia solani, Fusarium oxysporum, Sclerotium rolfsii, Collectotruchum truncatum, Macrophomina phseolina and Alternaria alternata*. The effective antagonists were further screened for their plant growth promoting (PGP) activity viz., production of plant growth regulators (auxins, gibberellins and cytokinins), siderophores and HCN. Nine endophytic isolates JDA 3, JDA 4, JDA 5, JDA 6, JDA 7, JDA 9, JDA 10, JDA 12 and JDA 15 were found to exhibit PGP trait were found to exhibit PGP traits. These endophytes thus; could be efficient biological control agent in sustainable crop production and offer unique opportunity for crop protection and biological control.

Keywords: *Endophytic Bacteria, Antagonistic Activity, PGP Activity, Biocontrol Agents*

INTRODUCTION
Global agriculture has to double food production by 2050 in order to feed the World's growing population and at the same time reduce its reliance on inorganic fertilizers and pesticides. To achieve this goal, there is an urgent need to harness the multiple beneficial interactions that occur between plants and microorganisms. It is essential to enhance the activities of microbes that benefit plant nutrition, control diseases and assist plants to cope with a variety of abiotic stresses to sustain and improve global food production in future climate scenarios while, maintaining environmental health. A diverse range of beneficial microorganisms have been found but their reliable use in field environments is yet to be fully realized (Ganesan *et al.,* 2007; Gupta, 2012). Keeping this in view, studies have been initiated to include crop protection and growth promotion using the native microorganisms, as a component of Integrated Pest Management (IPM).

Plants present an excellent ecosystem for microorganisms. Plant-microbial interactions have been large area of research interest since long and may range from beneficial to harmful. Microbial endophytes are typically defined as plant associated microbes that colonize living internal tissues of plants without causing any visible symptoms or immediate over-negative effects and can be isolated from surface disinfected plant tissue (Wilson, 1995, Hung and Annapurna, 2004). Virtually all plants are hosts to endophytic microorganisms and endophytes may usually be fungi, bacteria and actinomycetes (Pimentel *et al.,* 2006). These microorganisms include both commensal species, which have no direct effect on the host plant, and mutualistic symbionts (Procopio *et al.,* 2009). Intimate associations between endophytes and host plants can be formed without harming the plant and they have been demonstrated to improve and promote growth of host plants as well as to reduce disease symptoms caused by plant pathogens and/or various environmental stresses (Hasegawa *et al.,* 2006).

Endophyte-plant associations have been found to improve plant health and may help host plant to rescue from various biotic and abiotic stresses (Hasegawa *et al.,* 2006; Sapak *et al.,* 2008). They may also provide fitness benefits to host plants such as tolerance to herbivory, heat, salt, disease, and drought and increased below and aboveground biomass etc. (Faeth and Fagan, 2002; Backman and Sikora, 2008).

Thus, endophytic colonization improves the ecological adaptability of the host. Hence endophytes may be regarded as a true companion of host.

Endophytic actinomycetes have been defined as that can be isolated from the disinfected surfaces of plant tissues or that can be extracted from within the plant that do not cause visible harm to the host (Sharma *et al.,* 2011). A variety of actinomycetes inhabit a wide range of plants as symbionts, parasites or saprophytes (Matsukuma *et al*., 1994, Okazaki *et al*., 1995; Matsumoto *et al*., 1998). These enter the root system either through a root hair or directly through the epidermis. Once inside it invades the cortical tissues, passing from cell to cell, sometimes invading the plant cell walls.

Almost all vascular plants examined to date were found to harbor several endophytic actinomycetes. Hasegawa *et al*., (2006) reviewed endophytic actinomycetes. A variety of actinomycetes inhabit a wide range of plants as symbionts, parasites or saprophytes, most of them belong to the genera, *Streptomyces* and *Microbispora*. Coombs and Franco, (2004) isolated 38 strains belonging to *Streptomyces*, *Microbispora, Micromonospora* and *Nocardia* from surface-sterilized root tissues of healthy wheat plants. Likewise Okazaki *et al.,* (1995) reported a total of 246 strains of actinomycetes of plant origin: belonged to *Streptomyces, Microbispora, Nocardia, Micromonospora, Actinomadura* and several others. Similarly, Takahashi and Omura (2003) successfully isolated 32 strains of *Streptomyces*, 33 *Microbispora* and 10 other rare actinomycetes from fallen leaves of 9 genera of higher plants. Rosenblueth and Martinez-Romero, (2006) listed 8 genera of plant-associated actinomycetes including *Arthrobacter, Curtobacterium, Kocuria, Nocardia, Streptomyces.*

The unique ecological niche has made endophytic bacteria as attractive and potentially promising tool for agricultural applications especially, for those bacteria having commercial features such as plant growth promotion and activation of plant defense mechanisms (Hallman *et al.,* 1997). Several bacterial endophytes have been reported as potential biocontrol agents that may improve and promote plant health (Reiter *et al.,* 2002).

Prieto *et al.,* 2011 also emphasized the use of indigenous bacterial endophytes with biocontrol activity against soil-borne phytopathogens is an environmentally friendly and ecologically-efficient way within the framework of integrated plant disease management. The biotechnological potential of endophytic isolates assessed by their antagonistic activity or by the *in vitro* production of enzymes, antibiotics, siderophores, and plant growth hormones is high. In spite of the great importance of microorganisms in agricultural ecosystems, only a very small part of the microbial diversity relevant to agriculture was carefully described. The great amount of information regarding the key role of endophytes in agriculture is yet to be explored. Hence, with the view of plant health and productivity the proposed studies with special reference to indigenous endophytic microbes for soybeans crop cultivar JS-335, as model phytosystem, have been initiated.

MATERIALS AND METHODS

Screening of Endophytic Microbes for Antagonistic Activity against Soil-borne Fungal Pathogens of Soybean Cultivar JS- 335

Endophytic actinomycetes: A total of 15 endophytic actinomycetes isolared from different parts of soybean viz., roots, stem, leaf at different growth stages of soybean (CV JS 335) were screened for their antagonistic activity against isolated soil-borne fungal pathogens. The endophytic isolates belonged to *Streptomyces sp., Micromonospora sp., Nocardia sp., Actinomadura sp., Microbispora sp.,* and *Actinoplanes sp.* (Table 1).

*Fungal pathogens:*Endophytic actinomycetes were screened for their *in vitro* antagonistic activity against the isolated fungal pathogens of soybean.The isolated plant pathogens viz., *Rhizoctonia solani, Fusarium oxysporum, Sclerotium rolfsii, Colletotrichum truncatum, Alternaria alternata* and *Macrophomina phaseolina* were used as test pathogens Stock cultures of the test pathogens were maintained on potato dextrose agar (PDA) at 4°C. Working cultures were established by transferring a stock agar plug containing the mycelium of each isolate onto PDA and incubated at 28 °C ± 2 °C for 7 days.

Table 1: Endophytic actinomycete isolates of Soybean

Isolate	Possible species
JDA 1	*Streptomyces sp.*
JDA 2	*Streptomyces sp.*
JDA 3	*Streptomyces sp.*
JDA 4	*Streptomyces sp.*
JDA 5	*Streptomyces sp.*
JDA 6	*Streptomyces sp.*
JDA 7	*Streptomyces sp.*
JDA 8	*Streptomyces sp.*
JDA 9	*Streptomyces sp.*
JDA 10	*Micromonospora sp.*
JDA 11	*Nocardia sp.*
JDA 12	*Nocardia sp.*
JDA 13	*Actinomadura sp.*
JDA 14	*Microbispora sp.*
JDA 15	*Actinoplanes sp.*

In vitro Assay for Antagonistic activity of Endophytic Actinomycete Isolates

Isolated endophytic actinomycetes were screened for their antagonistic activity against test pathogens. Stock culture of each isolate was maintained on glycerol yeast extract agar at 4°C. The assay for antagonism was performed on potato dextrose agar (PDA) by dual culture method as suggested by Yuan and Crawford (1995). Loopful of test antagonist endophytic actinomycetes was streaked onto one side of each PDA plate. The plates were incubated at 30°C for 72 hrs. A 5 mm diameter agar plug of fungal mycelium of test fungal pathogen was transferred onto the center of the other side of each plate. Fungal plugs were also placed on actinomycete uninoculated PDA plates separately for all the test pathogens as uninhibited control. The plates were incubated at 30°C and examined for inhibition of growth after 5th day. The experiment was performed in three replications.

Analysis of Antagonistic Activity: The level of inhibition was defined as subtraction of radial mycelial growth γ_0 (in cm) of a control culture from the distance of the fungal growth in the direction of actinomycetes (γ in cm) in the test cultures. The level of inhibition was calculated adopting following formula (Yuan and Crawford, 1995).

$$\Delta \gamma = \gamma_0 - \gamma$$

Where,

$\Delta \gamma$ = The level of inhibition.

γ_0 = Mycelial growth fungal pathogen (in cm) in control culture.

γ = Mycelial growth fungal pathogen (in cm) in control culture.

Determination of Growth Inhibition Ratings: The antagonistic bioactivity of soybean endophytic actinomycete was evaluated in term of ratings as described by Yuan and Crawford, (1995) as shown below:

Level of Inhibition	Ratings
$\Delta \gamma > 2.0$ cm	+++
2.0 cm > $\Delta \gamma > 1$ cm	++
1 cm > $\Delta\gamma > 0.5$ cm	+
$\gamma < 0.5$ cm	-

Screening of PGP Traits among the Antagonistic Endophytes

The endophytic isolates exhibiting strong to moderate antagonistic activity against fungal pathogen were selected for determination PGP activity. The antagonistic endophytic isolates were screened for their ability to produce plant growth regulators (PGRs), siderophores and HCN.

Determination of Production of Plant Growth Regulators (PGRs): Culture extracts of antagonistic endophytes were analyzed for the presence of the PGRs viz., auxins (indole-3-acetic acid (IAA) and indole-3-pyruvic acid (IPyA)), gibberellins (GA3) and cytokinins isopentenyl adenine (iPa), isopentenyl adenosine (iPA) and zeatin (Z)).

Culturing of Endophytic Microorganisms: The endophytic actinomycetes were grown in Yeast extract malt extract medium (Shirling and Gottlieb, 1966). The cultures were incubated for 7 days at 26^0 C. For Auxins production the medium was amended with 5 ml of 5% L-tryptophan (El-Tarabily *et al.*, 2009).

Extraction Process: The extraction of the PGRs was carried out adopting the methodology suggested by Tien *et al.,* (1979) and Strzelczyk and Pokojska (1984). The cultures were centrifuged at 7,700 x g for 30 min. 100 mL of supernatant was collected in a separatory funnel.The pH of the supernatant was adjusted to 2.8 with 1 N HCl and extracted twice with 300 mL ethyl acetate. The ethyl acetate and the aqueous fraction were separated. The aqueous fraction was used for further extraction. The pH of aqueous fraction was adjusted 7.0 with 1 N NaOH and extracted with water saturated n-butanol. The n-butanol and aqueous fractions were separated. The above ethyl acetate and n-butanol fractions were evaporated to dryness under vacuum on rotary evaporator at $40\text{-}45^0$ C. The residue thus obtained was dissolved in absolute 2 mL of methanol and further filtered through Whatman filter paper and used for chromatography analysis.

Chromatographic Analysis: The culture extracts were further analyzed qualitatively for the presence of the PGRs viz., auxins [indole-3-acetic acid (IAA) and indole-3-pyruvic acid (IPYA)], gibberellins (GA3), and the cytokinins [isopentenyl adenine (iPa), isopentenyl adenosine (iPA) and zeatin (Z)] etc. by paper chromatography (Thimmaiah, 2004). Cochromatography with authentic compounds, specific color reactions with chromogenic reagents and visualization under UV light were used to establish identity.

Siderophore Production: Detection of siderophore production was carried out by $FeCl_3$ test (Cabaj and Kosakowska, 2007). 1-5 ml of ferric chloride solution (3 %) was added to 1 ml of culture filtrate. The formation of brown to purple color indicated the presence of siderophores (Neilands, 1981; Coleman, 1995; Wijesundera *et al.,* 1995; Logeshwaran *et al.,* 2009).

HCN Production: All the isolates were screened for the production of hydrogen cyanide (HCN) by adopting the methods of Lorck, (2004) and Castric and Castric, (1983) with slight modifications. Endophytic bacterial cultures were grown in nutrient broth supplemented with 4.4 gm/l glycine (Samuel and Muthukkaruppan, 2011) and were streaked on nutrient agar supplemented with 4.4 gm/l glycine. A disc of Whatman filter paper No. 1 of diameter equal to the diameter of Petri plate (impregnated with alkaline picric acid solution (0.5 % w/v) was placed in upper lid of inoculated Petri plates under aseptic condition. Plates were sealed with parafilm and were incubated upside at 28 + 10C for 24-72 hr. The control plates without inoculation of endophytic bacteria were also maintained. Change in color from yellow to light brown, moderate brown or to strong reddish brown was taken as indication of HCN production.

RESULTS AND DISCUSSION

In vitro Assay of Endophytic Actinomycete Antagonistic Activity

A total of 15 endophytic actinomycetes were tested for their *in vitro* antagonistic activity against six different soil-borne fungal pathogens of soybean. The level of antagonistic effects was based on the values of $\Delta\gamma$ (cm) further grouped in terms of ratings. Results obtained showed that the isolates showed inhibition of fungal pathogens with varying effectiveness. The endophytic actinomycetes showed effective inhibition of R. *solani, F. oxysporum, S. rolfsii, C. truncatum, A. alternata* and *M. phaseolina.*

Of the 15 isolates two isolates JDA 5 (2.13 cm) and JDA 6 (2.03 cm) exhibited a strong inhibition of *R. solani* and belonged to +++ rating (Table 2). Four isolates JDA 2 (1.86 cm), JDA 9 (1.96 cm), JDA 11

(1.03 cm) and JDA 15 (1.7 cm) showed a moderate inhibition of *R. solani* belonged to ++ rating while, eight isolates JDA 1 (0.96 cm), JDA 4 (0.6 cm), JDA 7 (0.9 cm), JDA 8 (0.83 cm), JDA 10 (0.7 cm), JDA 12 (0.96 cm), JDA 13 (0.6 cm) and JDA 14 (0.83 cm) showed a good inhibition of mycelia growth with + rating. However, isolate JDA 3 (0.46 cm) did not show appreciable inhibition of mycelia growth of *R. solani*.
Isolate JDA 3 (2.23 cm) exhibited a strong inhibition of mycelia growth of *F. oxysporum* (Table 2) Nine isolates JDA1 (1.5 cm), JDA 2 (1.2 cm), JDA 7 (1.53 cm), JDA 8 (1.7 cm), JDA 11 (1.3 cm), JDA 12 (1.26 cm),

JDA 13 (1.1 cm) JDA 14 (1.8 cm) and JDA 15 (1.43 cm) exhibited a moderate inhibition of *F. oxysporum* and belonged to ++ ratings. However, isolate JDA 4 (0.63 cm), JDA 6 (0.86 cm), JDA 10 (0.66 cm) exhibited a good degree of growth inhibition of *F. oxysporum*

A relatively moderate to good antagonistic activity was observed against *S. rolfsii* (Table 2). Seven isolates JDA 5 (1.3 cm), JDA 8 (1.16 cm), JDA 10 (1.3 cm), JDA 11 (1.1 cm), JDA 12 (1.53 cm), JDA 13 (1.16 cm) and JDA 14 (1.6 cm) exhibited a moderate inhibition of mycelial growth and belonged to ++ rating. Only one isolate JDA 2 (0.53), JDA 9 (0.76) and JDA 15 (0.6) had + rating. However, other isolates did not any show appreciable growth inhibition potentials.

Isolates JDA 4 (1.63 cm) and JDA 7 (2.0 cm) showed growth moderate inhibition of *C. truncatum* (Table 2) and belonged to ++ rating Four isolates JDA 1 (0.86 cm), (0.63 cm), JDA 11 (0.96 cm), JDA 12 (0.5 cm) and JDA 14 (0.53 cm) exhibited a good degree of growth inhibition of *C. truncatum*. While, isolates JDA 2, JDA 5 and JDA 8 did not any show appreciable growth inhibition potentials.

However, 6 isolates viz., JDA 3, JDA 6, JDA 9, JDA 11, JDA 13 and JDA 15 didn't inhibit the growth of *A. alternate* (Table 2). Only two isolate JDA 7 (1.40 cm) and JDA 10 (1.16 cm) exhibited a moderate growth inhibition of *A. alternata* and had ++ rating.

Four isolates JDA 1 (0.96 cm), JDA 5 (0.76 cm), JDA 12 (0.9 cm) and JDA 15 (0.83 cm) exhibited a relatively good antagonistic activity against *A. alternata* with + rating. However, other isolates did not show any appreciable growth inhibition potentials.

Isolate JDA 9 (1.06 cm) and JDA 15 (1.83 cm) exhibited a moderate growth inhibition of *M. phaseolina* (Table 2) and had ++ rating. Two isolates viz., JDA 1 (0.56 cm) and JDA 8 (0.96 cm) had a good degree of growth inhibition with + rating. However, other isolates did not show any growth inhibitory activity.

All the 15 isolates showed antagonistic activity against one or other fungal pathogens. Out of 15 isolates 14 were found to inhibit the growth of more than one fungal pathogen. Among all the isolates JDA 1 and JDA 12 were found to be most effective antagonists inhibiting the growth of five fungal pathogens tested. Seven isolates JDA 5, JDA 8, JDA 9, JDA 10, JDA 11, JDA 14 and JDA 15 were found to inhibit the growth of four fungal pathogens whereas, four isolates JDA 2, JDA 4, JDA 7 and JDA 13 inhibited three fungal pathogens.

Only one isolate JDA 6 inhibited two fungal pathogens. Out of all the isolates tested *in vitro*, three isolates viz., JDA 3, JDA 5 and JDA 6 were found to be most potent growth inhibitors and possessed +++ level of inhibition.

Among all the endophytic isolates JDA 1-9 belonged to *Streptomyces* sp. and isolates were; JDA 10 (*Micromonospora* sp.), JDA 11 (*Nocardia* sp.), JDA 12 (*Nocardia* sp.), JDA 13 (*Actinomadura* sp.), JDA 14 (*Microbispora* sp.) and JDA 15 (*Actinoplanes* sp.).

Table 2: Anatagonistic activity of endophytic actinomycetes against soil-borne fungal pathogen of Soybean

Endophytic actinomycete	Level of inhibition ($\Delta\gamma = \gamma_0 - \gamma$)					
	R. solani	***F. oxysporum***	***S. rolfsii***	***C. truncatum***	***A. alternata***	***M. phaseolina***
JDA 1	0.96	1.5	0.46	0.8	0.96	0.56
JDA 2	1.86	1.2	0.53	0.46	0.06	0
JDA 3	0.46	2.23	0.1	0	0.46	0
JDA 4	0.6	0.63	0.23	1.63	0	0
JDA 5	2.13	0.2	1.3	0.4	0.76	0.36
JDA 6	2.03	0.86	0.1	0	0	0
JDA 7	0.9	1.53	0.16	20	1.4	0
JDA 8	0.83	1.7	1.16	0.36	0.36	0.96
JDA 9	1.96	0.33	0.76	0	0	1.06
JDA 10	0.7	0.66	1.3	0	1.16	0
JDA 11	1.03	1.3	1.1	0.96	0	0
JDA 12	0.96	0.26	1.53	0.5	0.9	0
JDA 13	0.6	1.1	1.16	0	0	0.26
JDA 14	0.83	1.8	1.6	0.53	0	0
JDA 15	1.7	1.43	0.6	0	1.83	1
Control (γ_0)	-	-	-	-	-	-

Screening of Plant Growth Promoting Activity among the Antagonistic Endophytic Actinomycetes

Out of 15 endophytic actinomycete isolates tested, 9 isolates were found to possess PGP trait (Table 3). However, isolates JDA 1 (*Streptomyces* sp.), JDA 2 (*Streptomyces* sp.), JDA 8 (*Streptomyces* sp.), JDA 11 (*Nocardia* sp.), JDA 13 (*Actinomadura* sp.) and JDA 14 (*Microbispora* sp.) did not exhibit any PGP activity. Eight isolates were positive for auxin production. Isolates JDA 3 (*Streptomyces* sp.), JDA 4 (*Streptomyces* sp.), JDA 5 (*Streptomyces* sp.), JDA 6 (*Streptomyces* sp.), JDA 9 (*Streptomyces* sp.), JDA 10 (*Micromonospora* sp.), JDA 12 (*Nocardia* sp.) and JDA 15 (*Actinoplanes* sp.) showed IAA production however, none of the isolate tested exhibited IPyA production. Only one isolates JDA 3 (*Streptomyces* sp.) exhibited gibberellins production. Only two isolates exhibited cytokinin production. Isolates JDA 3 (*Streptomyces* sp.) and JDA 5 (*Streptomyces* sp.) exhibited zeatin production while none of the isolate exhibited iPa and iPA production. Out of 10, three isolates viz JDA 3 (*Streptomyces* sp.), JDA 5 (*Streptomyces* sp.) and JDA 9 (*Streptomyces* sp.) were found have HCN production. Whereas, isolates JDA 5 (*Streptomyces* sp.), JDA 6 (*Streptomyces* sp.) and JDA 7 (*Streptomyces* sp.) exhibited siderophore production.

Our findings are in congruence with other reports. A number of endophytic actinomycetes exihibited suppression of different soil-borne plant pathogens including *Rhizoctonia solani, Phythium* spp. *Fusarium oxysporum,* and *Colletotrichum arbiculare* indicating their potential use as bio-control agents (Gangwar *et al.,* 2012). They demonstrated the antagonistic activity of endophytic actinomycetes *Streptomyces* sp., *Actinopolyspora* sp., *Nocardia* sp., *Saccharopolyspora* sp. *Pseudonocardia and Micromonospora sp.* against ten pathogenic fungi: *Aspergillus niger, A. versicolor, A. flavus, Alternaria brassicicola, Botrytis cinerea, Cheatomium globosum, Fusarium oxysporum, Penicillium penophilum, Phytophthora dresclea* and *Rhizoctonia solani.* El- Shatoury *et al.,* (2009) evaluated the anatagonistic potential of endophytic actinomycetes from *Achillea fragrantissima* (Forssk) Sch. Bip. (Compositae). They found that 16 isolates inhibited plant pathogenic fungi *Botrytis cinerea* and *Macrophomina phaseolina*.

Tangum and Niamsup (2012) that the endophytic actinomycete *Streptomyces* sp. P4 strain, isolated from sweet pea root was effective in restricting the radial growth of *Fusarium oxysporum* f.sp. *lycopersici*, an important phytopathogen of tomato. Scanning electronic microscopic analysis showed that the rupture of the *F. oxysporum* mycelial cell wall occurred at the area of interaction between *F. oxysporum* and

Streptomyces sp. P4. Taechowisan and Lumyong (2003) demonstrated the antagonistic activity of endophytic *Streptomyces aureofaciens* CMUAc 130 isolated from the roots of *Zingiber officinale* and *Alpinia galanga*, against *Colletotrichum musae*, *Fusarium oxysporum*, *Bipolaris* sp., *Drechslera* sp., *Rhizoctonia* sp., *Sclerotium* sp. and *Candida albicans* ATCC 90028. Taechowisan *et al.,* (2003) emphasized the use of antagonistic microorganisms, such as endophytic *Streptomyces* sp. is an ideal method of controlling plant diseases.

Table 3: Plant growth promoting potentials of antagonistic endophytic actinomycete isolates

Sr. no.	Endophytic actinomycetes	PGRs production							
		Auxins		Gibberellins	Cytokinins			HCN	Siderophore
		IAA	IPyA	GA3	iPa	iPA	Z		
1	JDA 1	-	-	-	-	-	-	-	-
2	JDA 2	-	-	-	-	-	-	-	-
3	JDA 3	+	-	+	-	-	+	+	-
4	JDA 4	+	-	-	-	-	-	-	-
5	JDA 5	+	-	-	-	-	+	+	+
6	JDA 6	+	-	-	-	-	-	-	+
7	JDA 7	-	-	-	-	-	-	-	+
8	JDA 8	-	-	-	-	-	-	-	-
9	JDA 9	+	-	-	-	-	-	+	-
10	JDA 10	+	-	-	-	-	-	-	-
11	JDA 11	-	-	-	-	-	-	-	-
12	JDA 12	+	-	-	-	-	-	-	-
13	JDA 13	-	-	-	-	-	-	-	-
14	JDA 14	-	-	-	-	-	-	-	-
15	JDA 15	+	-	-	-	-		-	-

Endophytic actinomycetes isolated from surface sterilized wheat and barley roots showed antagonistic activity to wheat root pathoges *Gaeumannomyces graminis*, *Rhizoctonia solani* and *Pythium* sp. (Coombs *et al*., 2004). It was observed that 17 of 38 isolates displayed statistically significant activity *in planta* against *G. garminis*. Kafur and Khan (2011) isolated endophytic actinomycetes from surface sterilized leaves of *Catharanthes roseus* (L.) G. Don. 65 % of the isolates exhibited antifungal activity against fungi *Candida albicans, Botrytis cinerea, Curvularia lunata, Fusarium oxysporum, Fusarium solani* and *Rhizoctonia solani.*

Quecine *et al.,* (2008) reviewed that a number of endophytic actinomycetes suppressed wheat fungal pathogens including *R. solani*, *Pythium* sp. and *Gaeumannomyces graminis* var. tritici, both *in vitro* and *in planta*, indicating their potential use as biocontrol agents. Moussa *et al.,* (2011) demonstrated the antifungal spectrum of endophytic actinomycetes isolated from wild Egyptian plant. They found that 18.5 % were highly antagonistic to *Fusarium solani*, 12.3 % to *Phytophthora infestans*, 30.7 % to *Macrophomina* and 32.3 % to *Botrytis cinerea*. Two of the isolates showed the highest activities towards all the four tested fungi isolates were *Kitasatosporia* sp. and *Streptomyces* sp. They further demonstrated that in dual cultures, *Streptomyces* sp. inhibited 22.3 % of the *Fusarium oxysporum* growth, 19.6 % of the *Phytophthora infestans* growth, 30 % of the *Botrytis cinerea* growth and 16.2 % of the *Macrophomina phaseolina* culture growth in tested plates. Isolate *Kitasatosporia* sp. inhibited 21 % of the *Fusarium oxysporum* growth, 19.5 % of the *Phytophthora infestans* growth, 18.4 % of the *Botrytis cinerea* growth and 19.9% of the *Macrophomina phaseolina* culture growth.

Inderiati and Franco (2008) proposed that actinomycetes endophytes are promosing biological control agents for use in agriculture. Endophytic actinomycete *Streptomyces, Microbispora* and *Nonomurae* sp. were tested *in vitro* for antifungal activity against four fungal pathogens; *Alternaria solani, Phytophthora parasitica, Rhizoctonia solani* and *Pythium irregulare*. All the isolates tested inhibited the growth of at

least one of the phytopathogenic fungi and that 5 of the isolates inhibited the growth of all the fungal pathogens. Endophytic actinomycetes *Streptomyces sp.*, isolated from leaves and roots of healthy and wilting banana plants and were screened their antifungal activity against *Fusarium oxysporum* f. sp. cubense, a causative agent of banana Panama disease (Cao *et al.,* 2004). Confrontation test showed that 50 % of the *Streptomyces* strains from healthy plant had antagonistic activity however only 27 % of strains from wilting plant showed antagonisms. They also demonstrated that the endophytic isolates from roots had more significant fungal growth inhibition potentials as compared to leaf endophytes.

Actinomycetes are known to produce bioactive substances that are effective against phytopathogenic fungi (Ningthoujam *et al.,* 2009). Suwan *et al.,* (2012) also proposed that actinomycetes constitute a potential as biological control agents because of its intense antagonistic activity via the production of various antifungal substances such as chitinase and β-1, 3-glucanase. Various mechanisms have been implicated in the elucidation of antagonistic activity by endophytic actinomycetes towards soil-borne fungal plant pathogens that includes; production of inhibitory antifungal metabolites (for ex. antibiotics), cell wall degrading enzymes such as chitinases, glucanases, siderophores, induction of systemic resistance and competition for nutrients (El-Tarabily *et al.,* 2009; Moussa *et al.,* 2011).

El-Tarabily *et al.,* (2009) demonstrated the antagonistic activity of cucumber endophytic actinomycetes against *Pythium aphanidermatum.* They demonstrated the role of □-1,3, □ -1,4, and □ -1,6-glucanases, volatile compounds, inhibitory diffusible metabolites, siderophore and mycoparasitism in antagonistic activity. Moussa *et al.,* (2011) reviewed that volatile organic compound (VOC) produced by *Streptomyces* sp. and other species of actinomycetes were reported to cause growth abnormalities in different fungi, including *Fusarium oxysporum*. Shekhar *et al.,* (2006) purified a bioactive compound from endophytic *Streptomyces violaceusniger* that showed a strong antagonism towards various wood-rotting fungi and chitinase enzymes were associated with this inhibition.

El-tarabily *et al.,* (2009) reported the plant growth promoting traits of endophytic actinomycetes isolated from cucumber. They reported that endophytic *Actinoplanes campanulatus, Micromonospora chalcea* and *Streptomyces spiralis* showed auxin (IAA and IPyA), gibberellins (GA3) production and *Streptomyces spiralis* had iPa production ability. However, none of the isolate had zeatin production ability. Moreover, *Micromonospora chalcea* and *Streptomyces spiralis, Streptomyces sp.* ans *Microbispora sp.* showed HCN production.

Ningthoujam *et al.,* (2009) reported that actinomycetes are prolific producers of various bioactive compounds such as antibiotics, siderophores, chitinases, and phytohormones and have phosphate solubilizing abilities. Several endophytic actinomycetes act as plant growth promoter by producing of phytohormone, indole-3- acetic acid (IAA) or iron chelating molecules, siderophores *in vitro* (Shenpagam *et al.,* 2012).

Several species of *Streptomyces* including *S. violaceus, S. scabies, S. griseus, S. exfoliates, S. coelicolor* and *S. lividans* were reported to secrete indole-3-acetic acid (IAA) when fed with L-tryptophan (Maulis *et al.,* 1994). Igrarashi *et al*., (2002) isolated *Streptomyces hygroscopicus* from *Pteridium aquilinum* and found that *S. hygroscopicus* produced novel pteridic acids A and B as plant growth promoters with auxin-like activity. Gangwar *et al.,* (2012) reported that seventeen endophytic actinomycetes isolates produced IAA in the range of 18 - 42 □g/ml. The maximum IAA (42 □g/ml) was produced by *Saccharopolyspora* W3 while *Strptomyces albosporus* W2, *Streptomyces roseosporus* W9 and *Actinopolyspora* W20 produced the minimum yield of IAA (18 □g/ml).

Verma *et al.,* (2011) reported the plant growth promoting potentials of three endophytic *Streptomyces,* strains recovered from surface sterilized root tissues of *Azadirachta indica* A. Juss. (Meliaceae). It was observed that the three selected strains prolifically produce IAA and siderophores that play vital role in promotion of plant growth and in suppression of *Alternaria alternata.* Interestingly, *Streptomyces* strain AzR-051 produced the highest amount of IAA at 13.73 µmol/ ml, compared to strains AzR-049 and AzR-010 9.22 µmol ml(-1) and 10.43 µmol/ml respectively. It also produces siderophores higher than the other two strains. Cao *et al.,* (2005) demonstrated potential of siderophore-producing *Streptomyces* endophytes for the biological control of fusarium wilt disease of banana. Rungin *et al.,* (2012) demonstrated that an

endophytic *Streptomyces* sp. GMKU 3100 isolated from roots of a Thai jasmine rice plant (*Oryza sativa* L. cv. KDML105) showed the highest siderophore production. Gangwar *et al.*, (2012) reported that out of 35 endophytic actinomycete isolates 19 were observed to produce catachol type siderophore in range of 1.3 - 20.32 □g/ml while hydroxamate- type siderophore was produced by only nine isolates in the range of 13.33 - 50.66 µg /ml. The isolate *Strptomyces roseosporus* W9 produced maximum Catechol type (20.32 µg/ ml) and isolate *Streptomyces globisporus* W26 produced maximum hydroxamate type (50.66 µg/ ml) siderophore. Nimnoi *et al*., (2010) who reported that *Pseudonocardia halophobica* isolated from roots of *Aquilaria crassna* exhibited high ability for siderophore production and produced 39.30 µg/ ml. Siderophores are produced by various soil microbes to bind Fe_3^+ from the environment, transport it back to the microbial cell and make it available for growth. Microbial siderophores are also utilized by plants as an iron source. Even though siderophores do not promote plant growth directly; they deliver iron to plants and provide nutrition to stimulate plant growth (Gangwar *et al*., 2012).

Conclusion

Nine endophytic actinomycetes JDA 3, JDA 4, JDA 5, JDA 6, JDA 7, JDA 9, JDA 10, JDA 12 and JDA 15 were found to exhibit PGP trait. Hence, considered as efficient endophytic strains as they possible dual ability of antagonizing funal pathogen and plant growth promotion; with the view of plant health and productivity. Thus, these promising endophytes may be commercially formulated effective bio-control agents for the management of soil-borne fungal pathogens of Soybean. These isolates were further selected for further studies on their effect on plant growth parametes, diseases incidences and induction of systemic resistance in Soybean.

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