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Screening of Endophytic Actinomycetes for their Plant Growth Promoting Activity and for Biocontrol in Chilli (*Capsicum annum*) against *Sclerotium Rolfsii*

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Abstract: ***In the present study,sixty endophytic actinomycetes recovered from chilli plants were evaluated for their plant growth-promoting abilities. All endophytic actinomycetes isolates were used to evaluate phosphate solubilization, siderophores, ammonia, indole-3-acetic acid and gibberellic acid production, as well as antifungal activities. Out of sixty isolates the amount of indole-3-acetic acid (IAA) ranging between 2.33±0.14 to 121.92±0.44μg/ml was produced by 37 isolates and 32 isolates were positive for ammonia production ranging between 2.24±0.04 to 22.12±0.003 mg/ml. Among sixty isolates tested, the amount of hydroxamate-type siderophores were produced by18 isolates ranging between 4.1±0.2 to 129.4±0.1μg/ml, while catechols-type siderophores produced by 9 isolates ranging from 9.2±0.2 to 147.3±0.2μg/ml. Thirteen isolates showed the solubilisation of inorganic phosphorous ranging from 1.12 ± 0.04 to 20.07 ± 0.02 mg/100ml, gibberellic acid production was shown by 9 ranged from 26.50±0.006 to 56.33±0.002 μg/ml and 8 different isolates observed to produce HCN. The antagonistic activity was displayed by six isolates against Sclerotium rolfsii. On the basis of PGP traits and antifungal activity, isolate CR36 was selected for green house study which was identified as Streptomyces diastaticus subsp. ardesiacus. These results clearly suggest the possibility of using endophytic actinomycetes as bioinoculant for plant growth promotion, nutrient mobilization for sustainable agriculture.***

Keywords: Endophytic actinomycetes, chilli (*Capsicum annum)*, Plant growth promoting activity, Biocontrol

1. Introduction

Chilli (*Capsicum annum* L.) belong family *Solanaceae*is known as chief crop on the basis of its high consumption, nutritional and cash value to farmers and consumers. Its contain vitamin-C (Ascorbic Acid), vitamin-6 (Pyridoxine), vitamin-A and minerals like iron, copper, potassium. In India it is an important commercial crop. India is the largest consumer and exporter of chilies in the international market and exports dry chilli. Despite oflarger area, the productivity of chilli is low when compared to other countries. Among the bioagents, actinomycetes are a group of Gram-positive filamentous bacteria with high G+C ratio,represent a large portion of the rhizospheric microbial community and are prolific producers of diverse bioactive secondary metabolites with high commercial value, such as vitamins, alkaloids, plant growth factors, enzymes, and enzyme inhibitors (Gangwar*et al* 2014).

Sclerotium rolfsii is a soil borne phytopathogenic fungus affecting a large number of cultivated plants (Pattanapipitpaisal and Kamlandharn 2011). Control of *Sclerotium* rot, may be achieved by applying tremendous volume of fungicides every other day during the growing season. However, problems regardingthe efficacy of the chemicals and fungicide residues are ofincreasing concern and need to be solved because of thedirect effects on human health and the environment. Biologicalcontrol of phytopathogens by microorganisms has beenconsidered to be more natural and an environmentally acceptablealternative to the existing chemical treatment methods (Baker and Paulitz, 1996). Chitinolytic enzymes have been considered for biocontrolof pathogenic fungi due to their ability to degradechitin, which is a major structural component of most fungalcell wall (Chet, 1987). Several chitinolytic bacteria and fungi have been reported to be potentialbiological agents. For example, *Serratiamercescens* is used in control of *S. rolfsii* (Ordentlich*et al.*, 1988), *Paenibacillusillinoisensis* is for against *Rhizoctonia solani* Kuhn (Jung *et al* 2003).

Actinomycetes are also known to colonize the internal tissues of the plants and the association of actinomycetes with plants is found to confer various benefits such as the production of antimicrobials, extracellular enzymes, phytohormones and siderophores (Cao *et al* 2005). In this context, the utilization of plant growth promotion by endophytic bacteria and fungi has been widely reported; there is scant literature on the use of endophytic actinomycetes for plant growth promotion (Shutsrirung *et al* 2013).

Although the production of such plant hormones as indole-3-acetic acid (IAA) by endophytic actinomycetes has been suggested to be one of the mechanisms for plant growth stimulation Also their phosphate solubilization potentials help the plant to draw the immobilized phosphate out of the soil. Further, plant metabolism is enhanced by better iron sequestration through siderophores that are released by their inherent microbes which also help the host in developing resistance to infections. The objectives of this study were to isolate andscreen endophytic actinomycetes exhibiting a plant growth promoting activity, anddetermine their *in vitro* and *in vivo* antagonism towards afungal rot pathogen of chilli, *Sclerotium roslfsii*.

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2. Materials and Methods

Sample collection and isolation of endophytic actinomycetes

Roots,stem and leaves of thirty healthy (*Capsicum annuum* L) plants were collected from different located in Patiala and Ludhiana, India. Samples were sealed in plastic bags, brought to laboratory and stored at 4°C until further processing. The roots, stem and leaf segments were washed in running tap water to remove soil particles and sterilized by segmental immersion in 70% (v/v) ethanol for 5 minutes and sodium hypochlorite solution (0.9% available chlorine) for 20 minutes and then, surface-sterilized samples were washed in sterile water three times to remove surface sterilization agents. The samples were soaked in 10% (w/v) sodium bicarbonate solution for 10 minutes to retard the growth of endophytic fungi. These fragments were mashed using sterile pestle and mortar. An aliquot of 1 ml of root, stem and leaf suspension was poured on to Petri plates containing starch casein nitrate agar medium (SCA) and spread with the help of glass spreader. Petri plates were incubated at 28ºC for 7-10 days then examined for microbial growth. Isolated colonies were picked and streaked on SCA medium and then, sub-cultured on slants and stored at 4ºC.

Identification of actinomycetes

Endophytic actinomycetes isolates were picked from isolation plates and purified on SCAmedia. The isolates were identified according to morphological criteria, including characteristics of colonies on plate, morphology of substrate and aerial hyphae, morphology of spores and distinctive pigment production and reverse colony color. Biochemical characteristics were also studied as described by Bergey's Manual of Systematic Bacteriology and with published descriptions (Cao *et al* 2004, Tan *et al* 2006). Biochemical screening such as ability to hydrolyze starch, casein and as well as, decomposition of esculin, tween 80, tyrosine, xanthine and hypoxanthine by all the cultures were also done for the genus confirmation. Light microscopy and Gram-stain properties were used for the visual observation of the morphological and microscopic characteristics.

Screening of endophytic actinomycete isolates for Plant Growth Promoting traits

Production of Indole acetic acid (IAA)

Indole-3-acetic acid (IAA) was assayed based on the colorimetric method described by Gorden and Weber (1951) with some modifications. Endophytic actinomycetes were inoculated in 100 ml. Erlenmeyer flasks containing yeast malt extract broth for 10 days in a rotary shaker at 200 rpm,28ºC for 7 days. The 10 ml of grown culture was taken in eppendorff tubes and centrifuged at10,000 rpm for 20 minutes and 1 ml supernatant was mixed with 2 ml Salkowski's reagent and incubated for 30 min at room temperature. Development of a pink color indicated IAAproduction.

Phosphate Solubilization Potential

Screening of isolates for phosphate solubilization was done qualitatively by using NBRI-BPB agar medium. Actinomycetes cultures were inoculated on plates containing NBRI-BPB medium and incubated at 28^{0}C for 7 days and the colonies forming yellow halo zone were considered as phosphate solubilizers. The quantitative estimation was done by Olsen and Sommers 1987 method. Those isolates which showed positive qualitative results were selected and allowed to grow for 7 days in broth was carried out using Erlenmeyer flasks (250ml) containing 50 ml of Pikovskaya medium grown for 7 days.

Gibberellic acid and Ammonia production

Cultures inoculated in their YME broth were incubated at 37^{0}C for seven days and then centrifuged at 8000 rpm for 10 min. 15ml of the supernatant was pipetted out separately into the test tubes and 2 ml of zinc acetate solution was added. After 2 min, 2 ml of potassium ferrocyanide solution was added and centrifuged at 8000 rpm for 10 min. Five ml of supernatant was added to five ml of 30% hyperchloric acid. Absorbance was measured at 254nm in a UV-VIS spectrophotometer. From the standard graph prepared by using gibberellicacid solutions of known quantities, the amount of GA_3 produced by the culture was calculated and expressed as μg 25 ml^{-1} broth.

The endophytic actinomycetes isolates were tested for the production of ammonia using the method described by Cappucino and Sherman (1992). In this method 20 μl of seed culture was propagated in 10 ml of peptone water and incubated at 28°C with shaking at 120 rpm for 10 d. Subsequently, 0.5 ml of Nesseler's reagent was added to the culture and the development of brown to yellow color indicated a positive test for ammonia production. The absorbance was measured at 530 nm using a Thermo scientific (Multiskan GO) spectrophotometer, compared with the standard curve of $(NH_4)_2SO_4$ and expressed in mg/ml.

Siderophore Production Ability

Screening for siderophore production by CAS agar assay was performed in order to detect the ability of endophytic actinomycetes to produce siderophores. Cultures were streaked onto CAS agar Petri dish and incubated for 7 days at 28°C. Bacteria produce an orange/purple halo in the blue medium when siderophores are secreted (Schwynand Neilands1987). For Quantitative estimation the actinomycetes isolates were grown in yeast malt extract broth and incubated at 28ºC for 10 days. The culturesupernatant was separated by centrifuging the cultures at 10,000 rpm for 15 minutes. Thenfollowed for Catechol-type siderophores produced by using the method of Arnow [9] and Hydroxamate type of siderophores production was assayed by the method of Csaky(1948).

Screening of actinomycete isolates for antagonistic activity

The actinomycetes isolates were evaluated for their antagonistic activity against*Sclerotium rolfsiiby* dual-culture *in vitro* assay. Five days old fungal discs (8 mm in diameter) at 28ºC were placed at the center of the plates containing PDA media. Five days old actinomycetes discs (8mm) grown on SCA at 28ºC were placed on opposite sides of the Petri plates. Plates without the actinomycetes disc serve as controls. All the plates were incubated at 28ºC for 14 days and colony growth inhibition (%) was calculated by using the formula: C – T/C x 100, where C is the colony growth of

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pathogen in control and T is the colony growth of pathogen in dual culture.

Chitinase Production

The test for chitinase production was performed by the procedure described by (Taechowisan and Lumyong 2003, Tang-um and Niamsup 2012).

Preparation of colloidal chitin

Colloidal chitin was prepared from the chitin (Hi Media) by the modified method of Hsu and Lockwood (1975).

Quantitative production of extracellular chitinase

For the quantitative estimation of chitinase activity 0.6% and 1% colloidal chitin concentration was used. Colloidal chitin broth was used as a production medium with pH 7 and incubated at 30ºC in the incubator shaker at 150-160 rev min^{-1} for 7 days. Spores were inoculated to a concentration of 105 ml^{-1}. Chitinase activity in the supernatant was determined by the procedure of Taechowisan*et al,* (2003), Tang-um and Niamsup (2012). The amount of N-acetyl glucosamine (GlcNAc) released in the supernatant was spectrophotometrically measured by the method of Somogyi-Nelson (Green *et al,* 1989) on the 520-nm absorbance. One unit (U) of chitinase activity was defined as the amount of enzyme required to produce 1 mol of reducing sugar per min. under the conditions of the experiment.

Evaluation of effectiveness of actinomycete isolate (CR36) as potential antagonist against *Sclerotium rolfsii in* green house

Inoculum preparation of potential antagonists

The potential isolate (CR36) was grown in nutrient broth medium for 5 days. The surface sterilized seeds of chilli were immersed overnight in the antagonist suspension containing 10^8cfu/ml.

Soil infestation

Soil was taken from field and sterilized by autoclaving at 1210C for 1 hr for 3 consecutive days. Seeds were grown in pots, using completely randomized block design (CRD) with 4 treatments and 3 replications each. 10 seeds were sown per pot containing sterile soil. The treatments were: (A) T0- Absolute Control (no pathogen and no actionmycete) (B) T1- Control (fungus only) (C) T2- Seeds treated with filtrate of potential actinomycete isolate + fungal spore suspension (D) T3- Seeds treated with filtrate of potential actinomycete isolate

3. Results

Isolation and screening of endophytic actinomycetes

A total of 60 endophytic actinomycetes strains were obtained from root,leaf and stem tissues of 30chilli plants (Table 1). Out of 60 isolates, 40 belonged *Streptomyces* sp. followed by *Nocardia*sp. (n=6) *Micromonospora*sp. (n=4),*Psuedonocardia*sp. (n=4) and *Saccharopolyspora*sp. (n=6). Maximum number of isoalteswere recovered from roots then stem and leaf tissues (Table 2).Results were found to be consistent with previous findings of endophytic actinomycetes isolation from, wheat roots, tomato roots, banana and mangrove plants (Coombs and Franco 2003, Gayathri *et al* 2013, Khushboo and Gangwar 2017).The predominance of *Streptomyces* sp. in rhizospheric microbial flora might be related to the infection of host plants (Sardi 1992).

Table 1: Occurence of endophytic actinomycetes isolates from chilli plants

Types	No. of isolates
Streptomyces sp.	40
Micromonosporasp.	4
Nocardia sp.	6
Psuedonocardia sp.	4
Sacchropolyspora sp.	6
Total	60

Table 2: Distribution of endophytic actinomycetes isolates from chilli plants

Type	No. of isolates		
	Root	Stem	Leaf
Streptomyces sp	19	16	5
*Micromonospora*sp.	-	2	2
Nocardia sp.	3	2	1
*Psuedonocardia*sp	2	2	-
Sacchropolyspora sp.	3	2	1
Total	27	24	9

Plant Growth Promoting (PGP) of the actinomycete isolates

Indole acetic acid (IAA) production

Thirty-seven out of 60 isolates were observed to produce the phytohormoneindole aceticacid in range from 2.33±0.14 to 121.92±0.44 µg/ml (Table 3). The maximum IAA production was reported byCR36 (*Streptomyces* sp.) (121.92±0.44 µg/ml) while isolate and *Micromonospora*sp was a weak producer. There are few reports in the literature on the IAA productionof endophytic actinomycetes. Goudjal*et al* (2016) reported that seven endosymbiotic actinobacteria from sand truffles (*Terfezialeonis* Tul.) produced IAA in the range of 35.9± 0.5–117.1± 0.6 µg/ml and *Streptomyces* sp. TL7 was the highest producer, while *Actinomaduraglauciflava* isolate produced the minimum yield of IAA. Nimnoi*et al*(2010)reported that only ten endophyticactinomycetes isolates obtained from *Aquilariacrassna* produced IAA in the range of 9.85± 0.31to 15.14±0.22µg/ml. The presence of actinomycetes mainly inside root tissues that produce IAA may play an important role in plant development and health (Oliveira *et al* 2010).

Phosphate solubilization potential

Out of 60 isolates, thirteen were observed to solubilize phosphate i.e formation of yellow color zone around the colony on NBRI-BPB medium. The quantitative estimation of phosphatesolubilization was also calculated as described in Table 2. All the isolates which were able tosolubilize phosphate on NBRI-BPB medium were further evaluated for amount of phosphatesolubilized. The amount of phosphate solubilized by actinomycete isolates obtained from chilli plants fell in the range from 1.12±0.04 to 20.07±0.02 mg/ml.The maximum amount of phosphate solubilization was shown by CR6 (*Streptomyces*). Passari*et al* (2015) reported only fourteen (63.6%) among the 22 endophytic actinomycetes isolates able to solubilize inorganic

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phosphate. The phosphate solubilization efficiency varied from 35 to 73% among the isolates, and the maximum phosphate solubilization were detected in *Streptomyces* sp. 34 (73%) followed by *Leifsoniaxyli* 24 (64%) and *Microbacterium* sp. 21 (59%). Quantitative estimation of phosphate solubilization by the endophytic actinomycetes ranged from 3.2 to 32.6 mg/100 ml, with the highest by *Streptomyces* sp. (32.6 mg/ 100 ml) followed by *Leifsoniaxyli* (31.5 mg/ 100 ml).

Production of gibberellic acid and ammonia

Nine out of 60 isolates were observed to produce the gibberellic acid. Gibberellic acid production ranged from 26.50±0.006 to 56.33±0.002µg/ml.From these isolates, maximum gibberellic acid production was reported in CS54 (56.33±0.002 µg/ml), followed byCR36 (48.91±0.01) evident from table 3. These results are supported by Solans (2011) who isolated 122 actinomycetes from *Ochetophila* plant. Among 122 isolates, maximum gibberellic acid production was reported in *Micromonospora sp.* (3.73µg/ml), followed by *Frankia sp.* (1.76µg/ml), *Actinoplanes sp.* (1.53µg/ml) and *Streptomyces sp.* (0.96µg/ml). Rashed *et al.,* (2015) tested 83 actinomycete isolates from marine sediment for gibberellic acid production which ranged from 11.2-121.5 µg/ml.

Out of 60 only 32 isolates were positive for ammonia production at levels ranging from 2.24±0.04 - 22.12±0.003 mg/ml. Isolate CR4 produced the maximum amount of ammonia (22.12±0.003 mg/ml) followed by CR60 (21.67±0.003 mg/ml) then CR36 (18.27±0.003 mg/ml).

Siderophore production ability

Twenty-one out of 60 isolates from chilli leaves and root tissues produced siderophores which was noticeable by orange zones around the colonies on the CAS agar plates.The diameter of halo formed varied from 2 to 3.2 cm on the CAS agar plates and the most of theisolates belonged to *Streptomyces* sp., 9 isolates produced catechol type of siderophores (9.2±0.2 to 147±0.2 µg/ml) and maximum production was observed by isolate*Streptomyces* sp.CR29 (55.6±0.4 µg/ml) and minimum, isolate CR11 (9.2±0.2 µg/ml). While hydroxamate type were produced by 18 isolates (4.1±0.2 to 129.40±0.1 µg/ml), maximum being produced by isolate *Streptomyces* sp.CR36 (129.40±0.1 µg/ml) and minimum by isolate CR20 (4.1±0.2 µg/ml) (Table 2). Khamna *et al* (2009) also reported a *Streptomyces* isolate CMU-SK126 showing high siderophore of 16 µg/ml followed by *Streptomyces* CNU-GIN004 which produced 3.94 µg/ml of siderophore. Our results are also in agreement with Gangwar *et al*(2014) reported that nine isolates produced the amount of hydroxamate-type of siderophore ranging between 5.9-64.9 µg/ml and only four isolates were able to produce catechol-type of siderophore in the range of 11.2-23.1 µg/ml. Production of siderophores has also been known important for antagonism to phytopathogensand to improve growth of plants (Tan *et a*l 2006).

Antagonistic activity of actinomycete isolates against *Sclerotium rolfsii*

Out of 60 isolates, six against (10%) displayed antagonistic activity against *Sclerotium rolfsii*. Isolate CR36 exhibited maximum percent inhibition of 38.65±0.02% against *Sclerotium rolfsii*, followed by CL25(20.98±0.04%) CR43 showed minimum inhibitory activity of 9.3±0.027% (Table 4). Himaman *et al* (2016), out of the 477 actinomycete isolates (95 endophytes and 382 rhizosphere) only 48 isolates (10.1%) displayed activity against all three fungal pathogens. All actinomycete strains were screened against*C. eucalypti* and *Cylindrocladium*sp and *T. destructans* revealed that the272 isolates (57.0%) inhibited*C. eucalypti*, 118 isolates (24.7%) inhibited *Cylindrocladium* sp. and 241 isolates (50.5%) could inhibit*T. destructans.*

Quantitative production of extracellular chitinase

On the basis of maximum antifungal activity as well as hydrolytic enzymes production, the endophytic actinomycete isolate CR36 was selected for qualitative production of chitinase enzyme by plate agar assay. A clear zone surrounding the actinomycete colony was observed, indicating that *Streptomyces* CR36 produced chitinase. Maximum chitinase activity was observed on 4th day 0.09 U/ml at 0.6% colloidal chitin concentration. With 1% colloidal chitin substrate concentration, the maximum activity of CR36 was observed 0.55 U/ml on 5th day (Fig 1) as compared to standard (Fig2). Similar observations were reported by Young and Bell (1985) and Neugebour*et al,* (1991) during production of chitinase from *S. marcescens* and *S. lividans*, where by enzyme production was observed at exponential stage i.e. 84 h.

Evaluation of effectiveness of actinomycete isolates as potential plant growth promoters and antagonists against *Sclerotium rolfsii* in chilli under green house

The most potent isolate *Streptomyces* sp. CR36 identified by 16S rRNA gene sequencing as strains of *Streptomyces diastaticus subsp.ardesiacus* (Table 5). Maximum increase in seed germination, root length, shoot length, root fresh weight, root dry weight, shoot fresh weight, shoot dry weight was observed in treatment with isolates CR36. Similar parameters were observed more for CR36+*Sclerotium rolfsii*as compared to *Sclerotium rolfsii*alone (Table 3). These phenomena may be related to the production of growth regulators by actinomycetes. Results are in accordance with Kaur*et al*(2015) who observed that soil inoculation by Cs1and Cs44 isolates enhanced root depth, shoot length and dry weights of root and shoot which may due to nitrogen fixation, auxins, production or unidentified compounds. There was no wilting and rot symptoms in the treatments with inoculation of *S. aureus*, NPK and NPK + *S. aureus*. Incidence of disease was reported in *S. aureus* with *Fusarium oxysporum* (26%). Costa *et al.,* (2013) reported two*Streptomyces* isolates for the control of *P. aphanidermatum* in cucumber (*Cucumis sativa* L.) under greenhouse conditions. Isolate 16R3B was able to reduce 71% damping-off incidence whereas isolate 14F1D/2 reduced the disease incidence by 36%. Damping off control in cucumber, mainly for the isolate 16R3B suggested for its use in greenhouse cucumber. The results found under greenhouse conditions with the isolate CR36 proved their potential as a biocontrol agent to reduce the rot, caused by *Sclerotium rolfsii* in this planting system.

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Table 3: Abilities of some endophytic actinomycetes from chilli plant on production of IAA, siderophore and solubilizing of insoluble-phosphate and HCN

Genus	Isolates	IAA production (µg/ml)	Phosphate solubilization (mg/100ml)	Gibberellic acid (µg/ml)	Ammonia production (mg/ml)	Siderophore Production	
						Hydroxamate (µg/ml)	Catechol (µg/ml)
Streptomyces	CR2	5.64±0.30	-	-	3.04±0.002	-	-
Streptomyces	CR3	22.81±0.09	2.38 ± 0.04	-	-	-	-
Streptomyces	CR4	31.17±0.49	8.07± 0.06	-	22.12±0.003	-	-
Streptomyces	CL5	8.08±0.08	-	-	16.29±0.003	-	-
Streptomyces	CR6	46.67±0.43	20.07 ± 0.02	-	18.83±0.01	-	-
Streptomyces	CR7	4.51±0.06	4.22 ± 0.04	-	7.175±0.01	-	-
Streptomyces	CS8	13.8±0.10	7.19 ± 0.05	-	12.48±0.003	-	-
Streptomyces	CS9	25.76±0.20	1.12 ± 0.04	-	5.15±0.01	-	-
Streptomyces	CR11	-	-	-	-	6.3±0.1	-
Streptomyces	CS13	22.17±0.17	4.058 ± 0.03	-	9.41±0.005	36±0.2	9.2±0.2
Micromonospora	CL14	44.03±1.67	10.14 ± 0.05	26.50± 0.006	9.49±0.003	71.5±0.2	20.2±0.3
Streptomyces	CL15	5.42±0.14	-	-	11.51±0.003	-	-
Micromonospora	CR17	2.33±0.14	2.37 ± 0.03	-	-	14.5±0.1	-
Nocardia	CR19	12.43±0.24	-	-	3.88±0.003	-	
Nocardia	CR20	-	-	-	-	4.1±0.2	-
Streptomyces	CR21	30.42±0.28	-	-	3.21±0.002	4.5±0.2	-
Streptomyces	CL22	5.44±0.05	-	-	8.37±0.004	-	-
Streptomyces	CR24	15.68±0.16	-	39.77 ±0.05	2.31±0.001	-	-
Sacchropolyspora	CL25	32.83±0.30	-	-	-	-	-
Sacchropolyspora	CR27	-	-	-	-	78.8±0.3	-
Streptomyces	CR29	26.76±0.26	-	-	6.27±0.01	-	147.3±0.2
Streptomyces	CS30	10.87±0.56	-	41.68 ±0.04	6.35±0.006	-	-
Streptomyces	CS33	8.38±0.04	-	-	6.20±0.003	-	-
Nocardia	CL35	11.83±0.61	-	-	-	102±0.2	-
Streptomyces	CR36	121.92±0.44	12.14 ± 0.02	48.91 ±0.01	18. 46±0.003	129.4±0.1	51.4±0.3
Streptomyces	CR39	2.43±0.15	-	36.08 ±0.02	18.27 ±0.003	105±0.1	-
Streptomyces	CL40	6.57±0.18	7.62±0.005	44.60 ±0.005	11.65±0.005	57.1±0.2	-
Streptomyces	CS41	31.75±0.82	-	-	11.36±0.005	72.4±0.1	-
Streptomyces	CR42	75.94±0.78	11.38 ± 0.05	45.30 ±0.02	7.02±0.003	-	-
Psuedonocardia	CS43	20.89±0.43	-	-	4.51±0.029	-	27.3±0.2
Micromonospora	CL45	3.36±0.18	-	-	-	-	
Streptomyces	CL47	-	-	-	-	-	22.4±0.3
Psuedonocardia	CS48	3.72±0.17	-	-			
Psuedonocardia	CS48	3.72±0.17	-	-	3.13±0.01	25.4±0.1	134.3±0.1
Streptomyces	CR49	16.23±0.18	-	-	9.56±0.006	93.1±0.1	-
Streptomyces	CR50	20.70±0.14	-	37.49 ±0.01	10.01±0.006	94.6±0.3	64.3±0.2
Streptomyces	CL52	34.08±0.36	-	-	12.70±0.003	-	-
Streptomyces	CL53	10.83±0.14	-	-	16.14±0.005	115.8±0.2	-
Streptomyces	CS54	3.07±0.15	-	56.33 ±0.002	14.64±0.003	-	-
Streptomyces	CR55	4.50±0.24	-	-	12.25±0.003	49.5±0.1	20.3±0.2
Streptomyces	CL58	37.4±1.25	-	-	2.24±0.04	25.4±0.2	-
Streptomyces	CR60	4.22±0.08	2.02 ± 0.04	-	21.67±0.003	-	-

Table 4: Potential antagonistic activity of *Capsicumannum* (chilli) endophytic actinomycetes against *Sclerotium rolfsii*

Isolate	Inhibition (%)
	Sclerotium rolfsii
CL25	20.98±0.04
CR27	10.22±0.08
CR36	38.65±0.02
CR43	9.3±0.027
CL52	11.3±0.027
CR58	13.2±0.027

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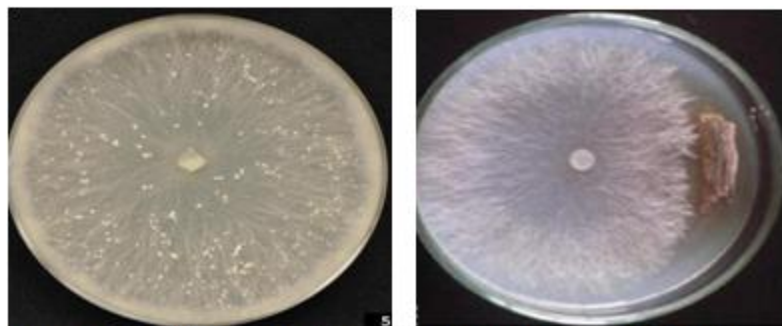

Figure 1: (A) *Sclerotium rolfsii* (B) *Sclerotium rolfsii* +CR36

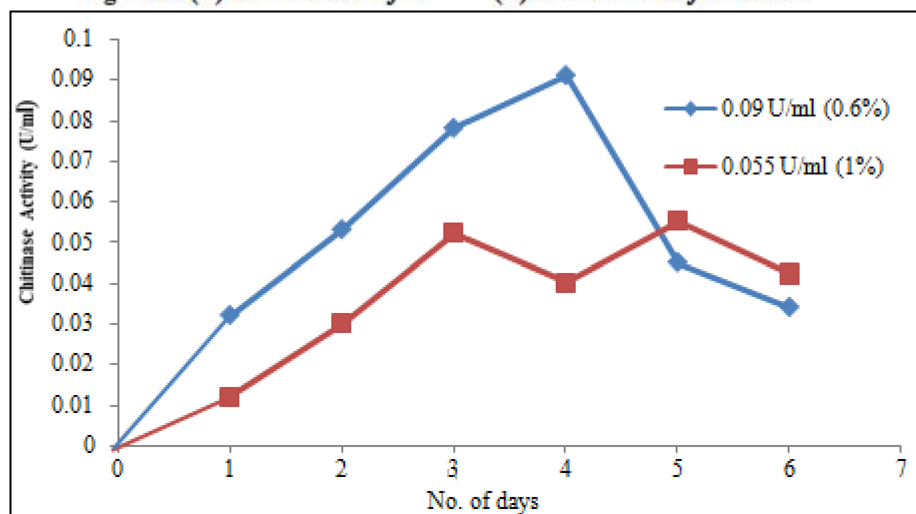

Figure 2: Quantitative production of chitinase by CR36 isolate

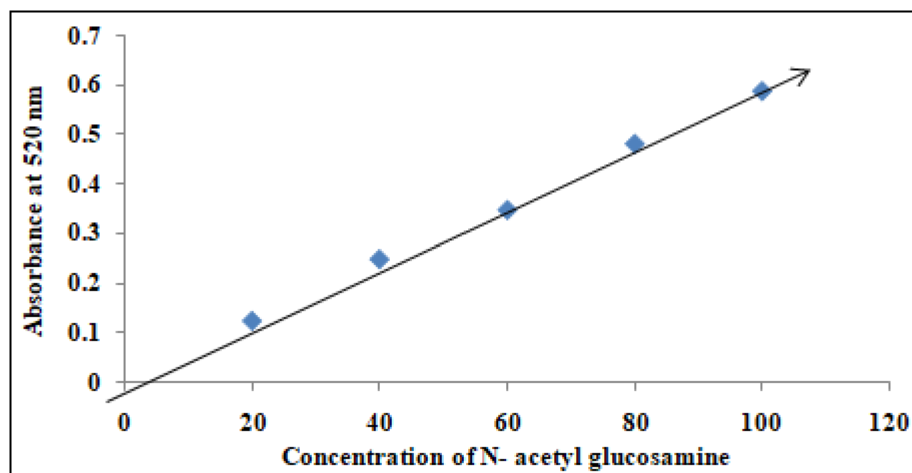

Figure 3: Standard curve of N-acetyl glucosamine

Table 5: Sequence producing significant alignments *Streptomyces diastaticus subsp. ardesiacus* (CR 36)

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	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Streptomyces diastaticus subsp. ardesiacus strain JB3 16S ribosomal RNA gene, partial sequence	1194	1194	99%	0.0	99%	KY451773.1
☐	Streptomyces sp. TN640 partial 16S rRNA gene, strain TN640	1186	1186	100%	0.0	99%	HE860515.1
☐	Streptomyces sp. strain HN33 16S ribosomal RNA gene, partial sequence	1181	1181	99%	0.0	99%	MG687441.1
☐	Streptomyces sp. strain HN22 16S ribosomal RNA gene, partial sequence	1181	1181	99%	0.0	99%	MG687440.1
☐	Streptomyces sp. strain HN3 16S ribosomal RNA gene, partial sequence	1181	1181	99%	0.0	99%	MG687437.1
☐	Streptomyces thermospinosisporus partial 16S rRNA gene, strain A-203	1181	1181	99%	0.0	99%	LT899927.1
☐	Streptomyces sp. strain KMSt12 16S ribosomal RNA gene, partial sequence	1181	1181	99%	0.0	99%	KX950884.1
☐	Streptomyces sp. strain CXSt1 16S ribosomal RNA gene, partial sequence	1181	1181	99%	0.0	99%	KX950855.1
☐	Streptomyces sp. strain ACBT12 16S ribosomal RNA gene, partial sequence	1181	1181	99%	0.0	99%	KX815284.1
☐	Streptomyces sp. strain HN25 16S ribosomal RNA gene and 16S-23S ribosomal RNA intergenic sp	1181	1181	99%	0.0	99%	MF397913.1
☐	Streptomyces diastaticus strain WZ902 16S ribosomal RNA gene, partial sequence	1181	1181	99%	0.0	99%	MF193899.1

4. Conclusion

This study implies that the presence of antifungal*Streptomyces diastaticus subsp. ardesiacus*(CR 36) may play a major role in the controlof rot caused by *S. rolfsii*. Results of this experimentcould be considered in improving the strategy to use *Streptomyces diastaticus subsp. ardesiacus*as a biocontrol agent.

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