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STREPTOMYCES ANSP4 : AN ANTAGONIST OF *RHIZOCTONIA BATATICOLA* AND A PLANT GROWTH PROMOTER OF SOYBEAN

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Abstract

Root rot caused by *Rhizoctonia bataticola* is the most common soil borne disease of soybean. *Streptomyces* ANSP4, an indigenous isolate from the rhizosphere of pomegranate from Kanheri Sarap village of Akola district, demonstrated antagonistic potential against *R. bataticola* by dual culture technique. When evaluated under pot culture conditions *Streptomyces* ANSP4 treatment of *R. bataticola* inoculated seeds resulted in control of *R. bataticola* infection as evident from the remarkable improvement in all the tested parameters of plant growth. This biological treatment was more effective as compared to Carbendazim treatment. *Streptomyces* ANSP4 treatment of healthy seeds promoted plant growth as compared to control. ANOVA and T test confirmed the significance of the observations. *Streptomyces* ANSP4 was identified as *Streptomyces malachitospinus* by 16s rRNA gene sequencing and found to degrade cellulose, chitin, gelatin and starch. It also exhibited varying degree of growth in neutral to alkaline pH and tolerance to moderate concentrations of NaCl, $CaCO_3$, $CaSO_4$ and $CaCl_2$.

Key words: *Streptomyces*; *Rhizoctonia bataticola*; Biological control; Soybean

Introduction

Rhizoctonia bataticola is one of the prevalent soil borne disease of soybean in Akola district of India (Belkar *et al.,* 2016). It causes damping off, root rot, stem rot and charcoal rot resulting in reduction in crop yields (Yang and Navi, 2005, Mengistu *et al.,* 2011). Chemical fungicides commonly employed for controlling these diseases cause hazardous impacts on the ecosystem. (Yang *et al.,* 2011) Various microbial antifungal agents have been explored in past few decades in order to avoid the adverse effects of chemical fungitoxicants on rhizospheric ecosystem and soil health (Postma *et al.,* 2003). The biocontrol potential of *Streptomyces* against plant pathogens has been reported in many studies (Xiao *et al.,* 2000; Jeffrey *et al.,* 2015; Law *et al.,* 2017). *Streptomyces lydicus* 108 (Yuan and Crawford, 1995) has been patented from United states and *Streptomyces* Strain Di944 (Siva, 1999) has been patented from UK as biocontrol agents for agricultural applications. Intensive research has also been undertaken at International crop research institute for the semi-Arid tropics (ICRISAT) of A.P. India ICRISAT (Gopalkrishana *et al.,* 2013) regarding the agricultural applications of few *Streptomyces* Spp.

Since the indigenous strains have better application potential considering their acquaintance with the specific agro-climatic conditions of any region, *Streptomyces* spp. were isolated during our previous studies from rhizospheric soil of Kanheri Sarap village of Akola district of Maharashtra. The isolate *Streptomyces* ANSP4 had shown significant antagonistic potential against *R. bataticola* (Khendkar *et al.,* 2018). In the present investigation, *Streptomyces* ANSP4 was identified and evaluated for its biocontrol potential against *R. bataticola* and plant growth promotion of soybean seeds by pot study. *Streptomyces* ANSP4 was also studied for its enzyme profile and tolerance to salts and pH.

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Materials and Methods

Test of antagonism

The antagonistic potential of *Streptomyces* ANSP4 was evaluated by dual culture technique (Morton *et al.,* 1955). Fungal phytopathogen *R. bataticola* was inoculated as spot on one side of plate and strait lines of *Streptomyces* ANSP4 were inoculated on opposite side of *R. bataticola* on starch casein agar media. In control plate only *R. bataticola* was inoculated on one side of

plate. These plates were incubated for 7 to 10 days at 30°C and the per cent inhibition was calculated according to Skidmore *et al.,* (1976).

Evaluation of antagonistic potential of *Streptomyces* against *R. bataticola* under pot culture

Fresh biomass of *Streptomyces* was collected and 20% biomass was mixed with talc powder. Fresh *R. bataticola* (R1) inoculum was prepared in potato dextrose broth. Five treatments were prepared for pot study. Surfaced sterilized seeds were soaked in fresh biomass of *R. bataticola* for 30 minutes (T3). Two different Treatments were given to these seeds *viz*. ANSP4 treatment (100mg/g of seeds) (T1) and Carbendazim treatment (0.2mg/g of seeds) (T2). Other treatments included only *Streptomyces* ANSP4 to surface sterilized seeds (T4), Seeds without any treatment were kept as control (C) (Xiao *et al.,* 2002; Konde *et al.,* 2008).

Statistical analysis

Statistical analysis was carried out using Graph pad prism software version 5 (Otto-Hanson *et al.,* 2012). ANOVA and T test were performed.

Characterization of *Streptomyces*

Stress tolerance

Streptomyces ANSP4 was grown on starch casein agar media with different concentrations of NaCl (2.5%, 5%, 7.5% and 10%), $CaCO_3$ (2.5% and 5%), $CaSO_4$ (2.5% and 5%) and $CaCl_2$ (2.5% and 5%) and on different pH (7, 8, 9, 10) (Carla *et al.,* 2008; Sakure *et al.,* 2015).

Enzymatic activity

Cellulose degradation:

Streptomyces ANSP4 isolate was inoculated on Czepak mineral salt medium and incubated for 2-3 days at 28°C. After incubation, the plate was flooded with 1% Congo red solution and excess dye was drained after 5 minutes. Solution of 1M NaCl was then added repeatedly until colour of Congo red solution disappeared. A clear zone around the colony indicated degradation of cellulose (Carder 1986; Sharma *et al.,* 2001).

Chitin degradation:

Streptomyces ANSP4 isolate was inoculated as spot on colloidal chitin agar plate (swollen chitin 2g/lit, ammonium sulphate 0.05g/lit., agar 1.8g/lit. and pH 7) and incubated up to 5 days. Chitinase activity was identified by clear zone around the colony (Palaniyandi *et al.,* 2013).

Hydrolysis of casein:

Streptomyces ANSP4 isolate was inoculated as spot on casein agar plate (10g/lit. casein, 10g/lit., glucose, 1.5g/lit., K_2HPO_4 and 15g/lit. agar) (Palaniyandi *et al.,* 2013) and incubated at 28°C for 5 days. A clear zone around the colony shows caseinase activity.

Hydrolysis of gelatin:

Inoculation of *Streptomyces* ANSP4 isolate was done on nutrient agar containing 15% gelatin and incubated for 3 to 4 days. After incubation plate was flooded with 1% $HgCl_2$. Clear zone around the colony indicated the hydrolysis of gelatin (Stefka *et al.,* 2004).

Hydrolysis of starch:

Streptomyces ANSP4 isolate was spot inoculated on starch agar plate and incubated at 28°C for 3-5 days. After incubation, the plate was flooded with 1% iodine, formation of a clear zone indicated the amylase activity (Shaw *et al.,* 1984; Santos *et al.,* 2003).

Identification of *Streptomyces* ANSP4

The molecular studies were followed to acquire accuracy in assigning at genus and species level of classification. Molecular phylogeny of Streptomycetes was determined by amplifying and sequencing genomic 16s rRNA regions of *Streptomyces* ANSP4 . Sequencing facility was availed from Yaazh Xenomics, Chennai.

Results and Discussion

Antagonist *Streptomyces* ANSP4 was isolated from rhizospheric soil of pomegranate (Khendkar *et al.,* 2018). Antifungal activity of this culture by dual culture technique exhibited 36.47% inhibition Fig. 1. Kunova *et al.,* (2016) have reported similar findings where *Streptomyces*

Fig. 1: Antifungal activity of *Streptomyces* ANSP4 against *R. bataticola*.

Table 1: Effect of different treatments on different parameters of soybean growth.

S.N.	Treatments	Average shoot height(cm)	Average pod number.	Average seed weight (g)	Average plant dry weight (g)
1	Control (C1)	36.74	8.800	0.7316	12.70
2	*R.bataticola*+ *S.*ANSP4 (T1)	37.58	12.20	0.9790	17.20
3	*R. bataticola*+Carbendizim (T2)	15.54	4.400	0.5208	6.640
4	*R. bataticola* (T3)	1.480	0.2000	0.0320	1.460

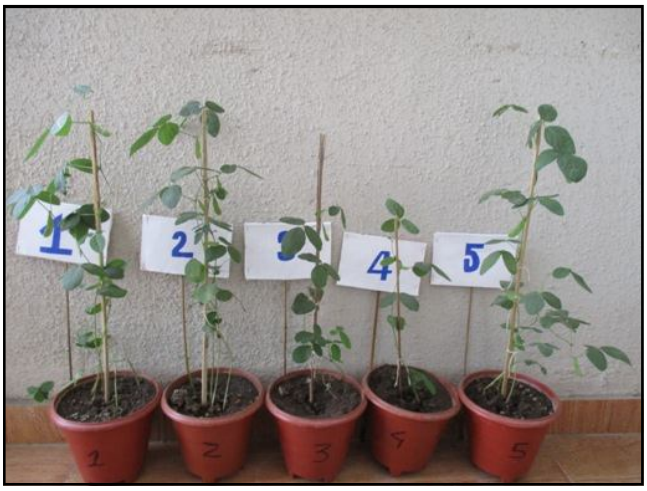

Fig. 2: Pot study of *Streptomyces* ANSP4 for soybean.
Pot 1- Control, Pot 2- *R. bataticola* inoculated seeds treated with *Streptomyces* ANSP4, Pot 3- *R. bataticola* inoculated seeds, Pot 4- *R. bataticola* inoculated seeds treated with Carbendizim and Pot 5- *Streptomyces* ANSP4 inoculated seeds.

Table 2: Significant difference between different groups.

S. No.	Parameters Checked	T test (P value)		ANOVA (P value) (Between 4 groups)
		T1 vs T3	T1vs C1	
1	Plant height	0.0079	0.5476*	0.0016
2	No. of Pods	0.0097	0.0119	0.0010
3	Seeds weight	0.0119	0.0079	0.0006
4	Plant dry weight	0.0079	0.0079	0.0010

*- Not significant, T1- *R. bataticola* inoculated seeds treated with *Streptomyces* ANSP4 , T2- *R. bataticola* inoculated seeds treated with Carbendizim, T3 - *R. bataticola* inoculated seeds and C1- Control.

Table 3: Efficacy of seed treatment with *Streptomyces* ANSP4 for growth of soybean plant.

	Growth parameters	Control (C)	*Streptomyces* ANSP4 (T4)	P values between C Vs T4 (by T test)
1	Average of shoot height(cm)	36.74	38.32	0.2222
2	Average of pod no.	8.800	14.80	0.0119
3	Average of seed weight (g)	0.7316	0.994	0.0079
4	Average of plant dry weight (g)	12.70	18.44	0.0079

T4- *Streptomyces* ANSP4 inoculated seeds.

anulatus CMJ58I showed 41.75% inhibition against *Rhizoctonia solani* FW408 in dual culture technique.

Antagonistic potential of *Streptomyces* ANSP4 was studied by evaluating effects of its application on average shoot height, number of pods, seed weight and plant dry weight Fig. 2, table 1.

Streptomyces ANSP4 treatment to *R.bataticola* inoculated seeds resulted in increase in most of the parameters of plant growth as compared to *R.bataticola* inoculated seeds without any treatment.. Moreover this positive effect was more prominent as compared to the effect of treatment of chemical fungicide Carbendizim. Significance of these observations was analyzed by T test and ANOVA. P values were obtained in the range of 0.0079 to 0.0119 in t Test and 0.0006 to 0.0016 in ANOVA. These findings supported the significance of antagonistic bioactivity of *Streptomyces* ANSP4 culture Fig. 3, table 2.

These studies revealed an important observation that treatment of *Streptomyces* ANSP4 to normal seeds resulted in improvement in all growth parameters as compared to control Fig. 4, table 3. The increase was remarkable for all the plant growth parameters except plant height. It clearly indicated significant plant growth promoting potential in *Streptomyces* ANSP4 for soybean.

Presence of antagonistic and plant growth promoting potential together in the same strain of *Streptomyces* was reported earlier by Houssam (2009); Jeffrey *et al.*, (2015); Maria *et al.*, (2015). In our findings also *Streptomyces* ANSP4 appeared to possess both the bioactivities.

Tolerance of *Streptomyces* ANSP4 on concentrations of different salts was studied table 4 considering the saline soil of some regions in Akola District.. No growth of *Streptomyces* ANSP4 was observed at 7.5% and 10% NaCl concentrations and 5% $CaCl_2$ concentration. However *Streptomyces* ANSP4 exhibited varying degree of growth at 2.5% and 5% concentrations of NaCl, $CaCO_3$ and $CaSO_4$ and at P_H 7 to 10

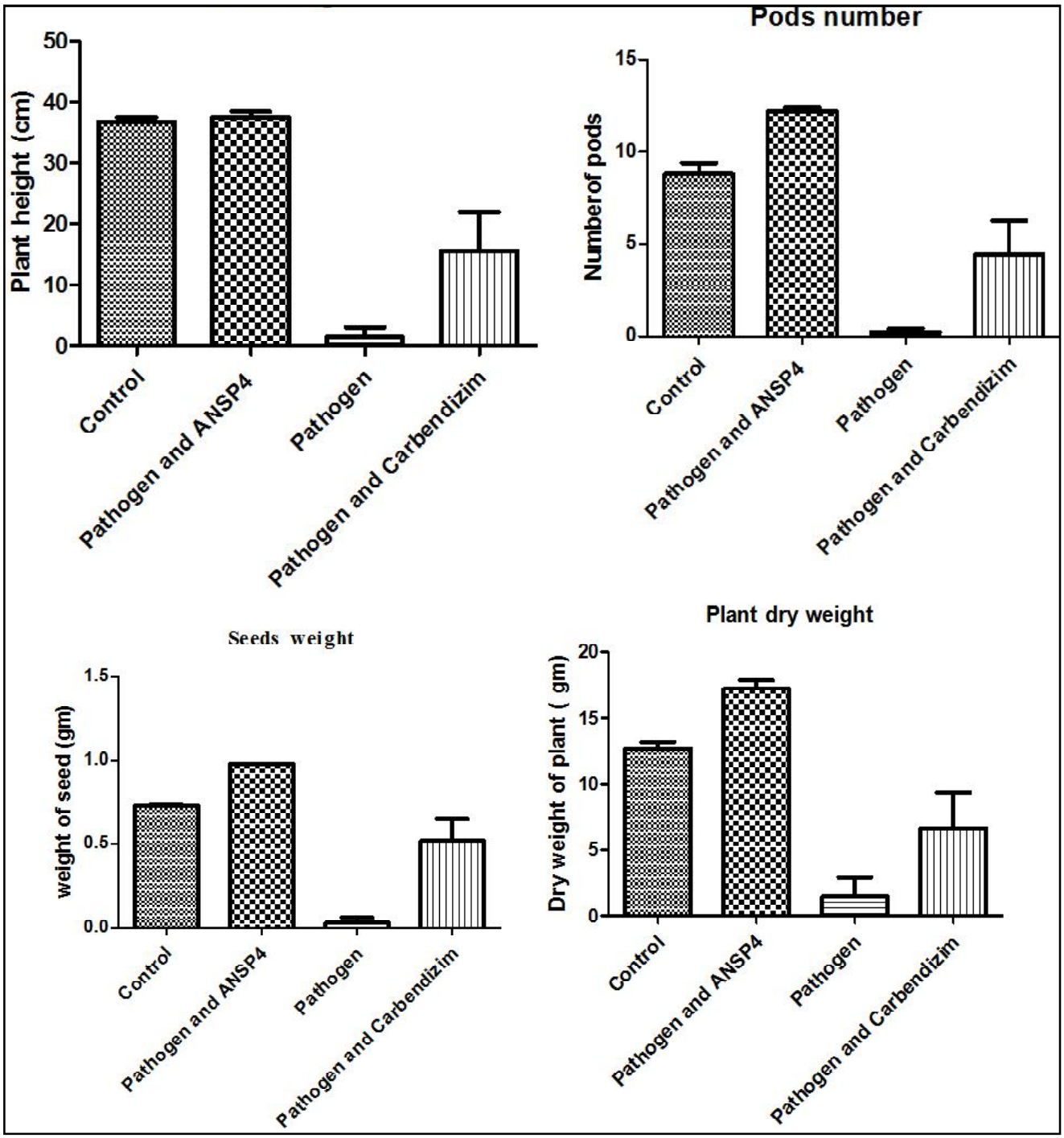

Fig. 3: Effect of different treatments on Soybean growth.
Note- Pathogen – *R. bataticola*

Table 4: Effect of different concentration of $CaSO_4$, $CaCO_3$ and $CaCl_2$ on the growth of *Streptomyces* $ANSP_4$.

S.No.	Isolates	$CaCo_3$		$CaSO_4$		$CaCl_2$	
		2.5%	5 %	2.5%	5 %	2.5%	5 %
1	$ANSP_4$	++++	++	+++	++	+	-

Poor growth +, moderate growth ++, good growth +++, luxuriant growth ++++

(luxuriant growth to poor growth). Thus *Streptomyces* ANSP4 seems to have application potential for neutral to alkaline and non saline to slightly saline soils.

Streptomyces ANSP4 could degrade cellulose Fig. 5A and chitin Fig. 5E and could hydrolyze gelatin Fig. 5B, starch Fig. 5C and casein Fig. 5D.

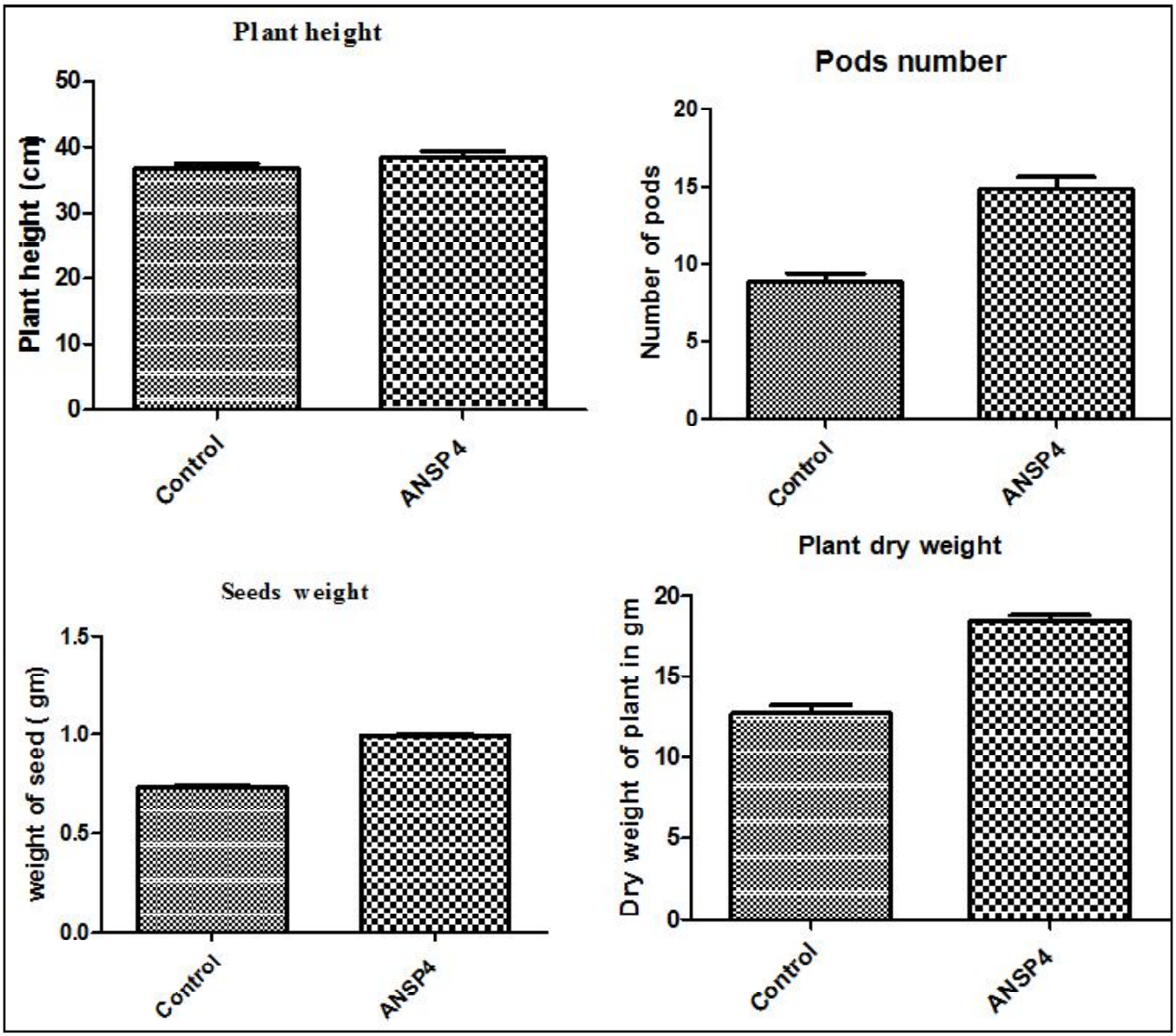

Fig. 4: Effect of *Streptomyces* ANSP4 on plant growth parameters of Soybean.

Identification of *Streptomyces* ANSP4

Streptomyces ANSP4 was sequenced by 16s rRNA gene sequencing. Polymerase chain reaction was carried out using 8F and 1541R primer. The 16s rRNA gene sequence was blast using NCBI blast similarity search tool.

ANSP4 was identified as *Streptomyces malachitospinus*. Nucleotide sequence of this organism is

>STREPTGGCATTCATTTTCGGTGTTCGACTT
CGTCCAATCGCCAGTCCCACCTTCGACAGCTCC
CTCCCACAAGGGGTTGGGCCACCGGCTTCGGG
TGTTACCGACTTTCGTGACGTGACGGGCGGTGT
GTACAAGGCCCGGGAACGTATTCACCGCAGCA
ATGCTGATCTGCGATTACTAGCGACTCCGACTT
CATGGGGTCGAGTTGCAGACCCCAATCCGAAC
TGAGACCGGCTTTTTGAGATTCGCTCCACCTTG
CGGTATCGCAGCTCATTGTACCGGCCATTGTAGC
ACGTGTGCAGCCCAAGACATAAGGGGCATGATG
ACTTGACGTCGTCCCCACCTTCCTCCGAGTTGA
CCCCGGCGGTCTCCCGTGAGTCCCCAACACCC
CCGAAGGGGCTTGCTGGCAACACGGGACAAGG
GTTGCGCTCGTTGCGGGACTTAACCCAACATCT
CACGACACGAGCTGACGACAGCCATGCACCAC
CTGTACACCGACCACAAGGGGGCGACCATCTCT
GGCCGTTTCCGGTGTATGTCAAGCCTTGGTAAGG
TTCTTCGCGTTGCGTCGAATTAAGCCACATGCTC
CGCCGCTTGTGCGGGCCCCCGTCAATTCCTTTGA
GTTTTAGCCTTGCGGCCGTACTCCCCAGGCGGG
GCACTTAATGCGTTAGCTGCGGCACGGACAACG
TGGAATGTTGCCCACACCTAGTGCCCACCGTTT
ACGGCGTGTACTACCAGGGTATCTAATCCTGTTC

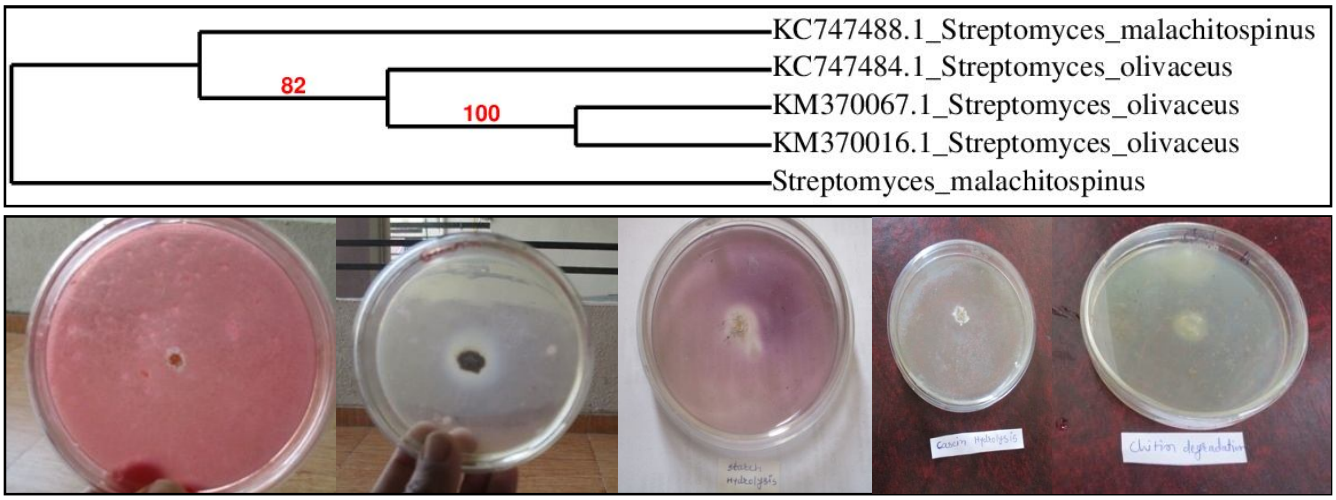

Fig. 5: Enzymatic activity.

GCTCCCCACGCTTTCGCTCCTCAGCGTCAGTAT
CGGCCCAGAGATCCCGCCCTTCGCCACCGGTGT
TTCTCCTGATATCTGCGCAATTTACCGCTACACC
AGGAATTCCAATCTCCCCTACCGAACTCTAGCC
TGCCGTATCGACTGCAGAACCCGGGGTTTAATC
CCCTGGGCTTTCCCAATCGACGTGACTAGCCCG
CCTTACCAAGCTCTTTTACGCTCCAAATATTTCT
GGACAACGCTTGCGCCCTACTTATTACCGCGTC
TGCCTGGCTACGTATATAGCCGGGCGCTTTCTTT
CTGTAAGTTACCGGTTACTTTTCTCTTTCTTTCC
CTGCCTGAAAGTGGGTTTTCAACCCGAAAGGC
CGTCATTCCTTCAGCCTGCTGTCGCCTGCCTTCA
GTGCTTTTCTGCCCATTGGGTCAATTAATCTCTA
CCCCTTGGCTTACCTTTCCGTTTAACGATATCCT
TGGACCCTGTGTTCTCAAAATCCATATAGTTC

Sequence of *Streptomyces malachitospinus* ANSP4 was deposited under the GenBank accession number MG787342.

Phylogenic tree

S. malachitospinus ANSP4 was found to control *R. bataticola* infection and increase plant growth for soybean. This biocontrol treatment was more effective as compared to Carbendiazim treatment for all the growth parameters in pot trails. Thus *S. malachitospinus* ANSP4 could be explored in future studies for biocontrol of fungal phytopathogens and growth promotion of soyabean .Its application potential could be investigated for other crops as well.

Acknowledgement

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