Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(6): 43-46

Received: 08-09-2018
Accepted: 10-10-2018

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Antagonistic activity of bacterial endophytes against major soilborne pathogens of soybean

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Abstract
The antagonistic effect of 30 bacterial endophytes of soybean collected from northern Karnataka and parts of Maharashtra against *Sclerotium rolfsii*, *Rhizoctonia bataticola* and *Fusarium oxysporum* were assayed *in vitro* through dual culture plate technique. The bacterial endophytes RB-KK-6 (40.78%), SB-BS-6 (50.08%) and LB-BU-1 (47.02%) were found effective against *S. rolfsii* and the isolates SB-DG-11 (47.41%), LB-BiN-8 (41.22%) were effective against *R. bataticola.* The effective bacterial endophytes against *F. oxysporum* were RB-HS-1 (41.99%), SB-BiJ-9 (40.07%), LB-BU-1 (54.20%) and LB-BV-2 (51.64%). Based on molecular characterization the effective bacterial endophytes were identified as *Acinetobacter* sp. (RB-HS-1), *Alcaligenes faecalis* (RB-KK-6), *Stenotrophomonas* sp. (SB-BiJ-9), *Bacillus pumilus* (SB- DG-11 & LB-BiN-8), *Paenalcaligenes* sp. (LB-BU-1), *Bacillus cereus* (SB-BS-6) and *Brevibacillus* sp. (LB-BV-2).

Keywords: Soybean, Bacterial endophyte, *Sclerotium rolfsii*, *Rhizoctonia bataticola* and *Fusarium oxysporum*

1. Introduction
Soybean (*Glycine max* (L.) Merril) also known as 'golden bean' or 'miracle bean', is one of the premier agricultural crops in India. Soybean, with over 40 per cent proteins and 20 per cent oil has now been recognized as a potential supplementary source of edible oil and nutritional food. It is gaining popularity on account of its unique characteristics and adaptability to varied agro-climatic conditions. The unique chemical composition of soybean seed, which includes a number of nutraceutical compounds such as isoflavones, tocopherol and lecithin has made it one of the most valuable agronomic crops in the world. Despite having made rapid stride for both coverage and total production, soybean still suffers on the productivity front. Soil borne plant pathogens can significantly reduce the yield and quality of soybean. Some of these pathogens are particularly challenging because they often survive in soil for many years and attack the crop irrespective of its crop growth stage. Biological control in agriculture is gaining much attention when environment and soil health are taken into account. Endophytes might interact more closely with the host plant and therefore, could be more effective biocontrol agents in sustainable crop production and offer unique opportunity for crop protection and biological control.

Endophyte refers to fungi or bacteria which, during entire or part of their life cycle, invades the tissues of living plants and cause unapparent and asymptotic infections entirely within plant tissues, but cause no symptoms of the disease [1]. Endophytes benefit the plant by promoting plant growth, improving resistance to multiple stress and offering protection from diseases and insects. Endophytic fungi are of biotechnological interest due to their ability to produce antibacterial, antiviral, anticancer, antidiabetic and immunosuppressive compounds that also act as biocontrol agents [2]. Inspite of the increased number of reports about the beneficial traits of endophytic microbes towards the crop plants protecting their host against pathogens and promotion of plant growth, there is a dearth of information regarding the use of different endophytic microorganisms for the management of soil borne fungal pathogens and growth promotion of soybean in India. India being a tropical country with great biodiversity offer more chances to chase the endophytes that are suitable to produce bioactive compounds. Hence, the present investigation was undertaken to tap the endophytic bacterial diversity of soybean and to screen them for the antagonistic activity against major soil borne pathogens.

2. Material and methods

Isolation of bacterial endophytes

Healthy soybean plants collected from major soybean growing areas of northern Karnataka *viz*., Belagavi, Bidar, Dharwad, Haveri and parts of Maharashtra *viz*., Kolhapur and Sangli were used for isolation as per the procedure suggested [3]. Roots, stems and leaves were washed to remove dirt and cut into sections. Surface sterilized sections were rinsed with 0.02 M potassium phosphate buffer three times (0.1 ml aliquot from the last wash was taken and transferred to Petri plate which served as sterility check).One gram of plant parts was macerated with nine ml of potassium phosphate buffer in pestle and mortar. Further serial dilution was made up to 10^{-6} dilution and were plated using the streak plate method. The plates were incubated for 48-72 h for the observation of the colonies and isolated colonies were picked up and streaked again on fresh nutrient agar plates for further purification.

In vitro evaluation of bacterial endophytes

Soil borne fungal pathogens *viz.*, *Sclerotium rolfsii*, *Rhizoctonia bataticola* and *Fusarium oxysporum* isolated from infected soybean plant samples were used for screening of the endophytes. Dual culture plate technique was adopted for testing the antagonistic activity of the endophytes against the soil borne pathogens on PDA plates. The fungal pathogen was inoculated at one side of Petri plate and bacterial endophyte was streaked on the opposite side of the plate. For this, actively growing cultures of both endophyte and pathogens were used. Petriplate inoculated only with the pathogen served as control. Per cent inhibition over control was worked out according to the formula [4]

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition
C = mycelial growth in control (mm)
T = mycelial growth in treatment (mm)

Molecular characterization of the effective endophytes: The total genomic DNA from pure culture of the different isolates of bacteria were extracted by the CTAB (Cetyl Trimethyl Ammonium Bromide) method [5] with some modifications. PCR amplification of rDNA sequences were conducted by using the universal bacterial primers. Finally, the amplified products of the representative samples were sent for sequencing at Chromos Biotech Ltd., Bengaluru. The obtained sequence results were analyzed using Basic Local Alignment Search Tool (BLAST) algorithm available at http://www.ncbi.nlm.nih.gov.

3. Results and Discussion

A total of 30 bacterial endophytes (6 from root, 13 from stem and 11 from leaf) were obtained from 25 different locations of northern Karnataka and parts of Maharashtra. They were subjected for *in vitro* screening against major soil borne pathogens of soybean *viz*., *Sclerotium rolfsii*, *Rhizoctonia bataticola* and *Fusarium oxysporum* for their antagonistic activity.

A total of six bacterial root endophytes were evaluated against the three pathogens by dual culture technique and results are presented in Table 1.Efficacy of root endophytes against three pathogens ranged from 0.14 to 41.99 per cent mycelial inhibition. Among the six endophytes evaluated against *S. rolfsii*, the isolate RB-KK-6 showed the maximum mycelial inhibition of 40.78 per cent. The root isolate RB-HS-1 showed the maximum mycelial inhibition of 41.99 per cent against *F. oxysporum*. None of the isolates showed any inhibition above 40 per cent against *R. bataticola*.

Table 1: *In vitro* evaluation of soybean bacterial root endophytes against *Sclerotium rolfsii*, *Rhizoctonia bataticola* and *Fusarium oxysporum*

Bacterial endophyte	Per cent inhibition		
	S. rolfsii	***R. bataticola***	***F. oxysporum***
RB-HS-1	16.15(23.68)*	27.94(31.90)*	41.99(40.37)*
RB-HB-2	10.10(18.51)	26.72(31.11)	25.02(30.00)
RB-HY-3	8.74(17.18)	0.42(2.86)	23.46(28.96)
RB-DM-4	0.14(1.64)	17.83(24.97)	33.52(35.36)
RB-DK-5	5.15(13.09)	0.47(3.03)	14.19(22.12)
RB-KK-6	40.78(39.67)	20.72(27.07)	33.66(35.43)
S.Em. ±	0.37	0.71	0.31
C. D. @ 1%	1.46	2.81	1.24

* Arc sine values

Results clearly indicated that all 13 bacterial stem endophytes significantly inhibited the mycelial growth of all the three pathogens. Efficacy of stem endophytes against three pathogens ranged from 5.21 to 50.08 per cent mycelial inhibition. Among the 13 bacterial stem endophytes evaluated against *S. rolfsii*, the isolate SB-BS-6 showed the maximum mycelial inhibition of 50.08 per cent. The isolate SB-DG-11 showed the maximum inhibition of 47.41 per cent against *R. bataticola.* The stem isolate SB-BiJ-9 showed the maximum mycelial inhibition of 40.07 per cent against *F. oxysporum* Table 2.

Table 2: *In vitro* evaluation of soybean bacterial stem endophytes against *Sclerotium rolfsii*, *Rhizoctonia bataticola* and *Fusarium oxysporum*

Bacterial endophyte	Per cent inhibition		
	S. rolfsii	***R. bataticola***	***F. oxysporum***
SB-BJ-1	28.10(31.99)*	8.33(16.77)*	30.06(33.23)*
SB-BJ-2	32.55 (34.77)	12.41 (20.62)	28.32 (32.14)
SB-BU-3	5.21 (13.17)	11.71 (20.00)	26.98 (31.28)
SB-BU-4	40.07 (39.26)	12.11 (20.35)	33.84 (35.56)
SB-BK-5	34.08 (35.70)	8.30 (16.73)	17.47 (24.69)
SB-BS-6	50.08 (45.03)	21.99 (27.94)	32.42 (34.69)
SB-BKh-7	40.04 (39.24)	16.96 (24.30)	28.26 (32.10)
SB-BV-8	35.13 (36.34)	15.63 (23.28)	33.88 (35.58)
SB-BiJ-9	18.40 (25.39)	30.10 (33.26)	40.07 (39.26)
SB-DM-10	13.32 (21.39)	32.26 (34.60)	18.25 (25.28)
SB-DG-11	15.78 (23.39)	47.41 (43.50)	37.49 (37.74)
SB-DK-12	18.19 (25.23)	35.35 (36.47)	22.92 (28.59)
SB-DD-13	17.24 (24.52)	7.85 (16.25)	26.11 (30.71)
S.Em. ±	0.26	0.31	0.21
C. D. @ 1%	1.04	1.23	0.82

* Arc sine values

A total of 11 bacterial leaf endophytes were evaluated against the three pathogens. Efficacy of leaf endophytes against three pathogens ranged from 0.22 to 54.20 per cent mycelial inhibition**.** Among the 11 bacterial leaf endophytes evaluated against *S. rolfsii*, the isolate LB-BU-1 showed the maximum mycelial inhibition of 47.02 per cent The isolate LB-BiN-8 showed the maximum inhibition of 41.22 per against *R. bataticola.* The leaf isolates LB-BU-1 showed the maximum mycelial inhibition of 54.20 per cent followed by the isolate LB-BV-2 with an inhibition of 51.64 per cent against *F. oxysporum* Table 3.

Table 3: *In vitro* evaluation of soybean bacterial leaf endophytes against *Sclerotium rolfsii*, *Rhizoctonia bataticola* and *Fusarium oxysporum*

Bacterial endophyte	Per cent inhibition		
	S. rolfsii	***R. bataticola***	***F. oxysporum***
LB-BU-1	47.02 (43.27) *	17.91 (25.03) *	54.20 (47.39) *
LB-BV-2	30.95 (33.79)	18.79 (25.68)	51.64 (45.92)
LB-BV-3	37.98 (38.03)	35.77 (36.72)	29.80 (33.07)
LB-HA-4	3.69 (11.02)	12.03 (20.29)	23.48 (28.97)
LB-BiB-5	16.78 (24.17)	0.22 (1.56)	19.52 (26.20)
LB-BiI-6	18.17 (25.22)	10.66 (19.05)	17.57 (24.76)
LB- BiI -7	15.28 (23.00)	0.31 (2.52)	12.44 (20.64)
LB- BiN -8	18.35 (25.35)	41.22 (39.92)	30.58 (33.55)
LB-DN-9	17.29 (24.56)	30.81 (33.70)	32.87 (34.96)
LB-KK-10	27.69 (31.73)	0.96 (4.41)	17.64 (24.82)
LB-SK-11	21.95 (27.92)	35.23 (36.39)	31.60 (34.19)
S.Em. ±	0.33	0.98	0.37
C. D. @ 1%	1.30	3.93	1.49

* Arc sine values

Based on the dual culture method of screening, eight effective bacterial isolates were selected. The bacterial endophytes RB-KK-6 (40.78%), SB-BS-6 (50.08%) and LB-BU-1 (47.02%) were effective against *S. rolfsii* and the isolates SB-DG-11 (47.41%), LB-BiN-8 (41.22%) were effective against *R. bataticola.* The effective bacterial endophytes against *F. oxysporum* were RB-HS-1 (41.99%), SB-BiJ-9 (40.07%), LB-BU-1 (54.20%) and LB-BV-2 (51.64%).

Endophytic bacteria use different mechanisms like production of HCN, hydrolytic enzymes, siderophore and antibiotics to out compete the fungal phytopathogens. Further, some effective endophytes excrete extra cellular lytic enzymes that are responsible for their antagonistic abilities. The results are in agreement with the earlier works [6-8].

Molecular characterization of the effective bacterial endophytes: Eight effective bacterial endophytes of soybean were characterized based on cultural, morphological and molecular methods and results revealed that the isolatesRB-HS-1 was similar to *Acinetobacter* sp., the root isolate RB-KK-6 has shown similarity to *Alcaligenes faecalis*, the isolate SB-BiJ-9 was identified as *Stenotrophomonas* sp., the isolates SB- DG-11 and LB-BiN-8 had shown similarity to *Bacillus pumilus*, the isolate LB-BU-1 was similar to *Paenalcaligenes* sp., the isolate SB-BS-6 was similar to *Bacillus cereus* and the isolate LB-BV-2 has shown similarity to *Brevibacillus* sp. The bacterial endophytes were identified by 16S rDNA sequence analysis. Similar endophyte identification procedure was followed [9] where tomato bacterial endophytes were identified as *Luteimonas aestuarii*, *Pseudomonas lini*, *Bacillus pumilus* and *Bacillus* sp. by using16S rDNA sequence analysis. Kuklinsky-Sobral [10] isolated *Stenotrophomonas maltophila* from root samples of soybean. Yuliar *et al.* [11] isolated the endophytic bacteria and identified them by 16S rDNA sequence analysis as *Acinetobacter*, *Bacillus* sp., *B. pumilus*, *B. cereus*, *Alcaligenes* sp. and *Stenotrophomonas* sp. and evaluated them for plant growth promotion and antagonistic activity against *R. solani*. Nhu and Diep [12] isolated and screened the endophytic bacteria for plant growth promotion and the effective endophytes were identified as *Bacillus* sp., *Bacillus subtilis* and *Acinetobacter* sp.

Table 4: Comparative analysis of sequence similarity of association of native bacterial endophytes

Source	Endophyte	Comparison with already reported endophytes/ bioagents				
Stem	SB-BS-6	*Bacillus cereus* strain AIMST1.Cit.6**(***Citrus* sp., leaf, Malaysia)	*Bacillus cereus* strain SS. SNC06 **(**Mangrove plants, root, India**)**	*Bacillus cereus* strain LB8 **(***Capillaris* sp.**,** China**)**	*Bacillus cereus* strain KK2 **(**River water**,** India**)**	*Bacillus* sp.strain KCN26 **(**Banana rhizosphere, India**)**
		(100% similarity)			(99% similarity)	
Leaf	LB-BU-1	*Paenalcaligenes* sp. UN24 **(**soil and cow dung**,** China**)**	*Paenalcaligenes suwonensis* strain ABC02-12 (Spent mushroom compost**,** Korea)	*Paenalcaligenes* sp. strain JLT40(Shallow sea sediment, China)	*Paenalcaligenes suwonensis* strain TAP1 ((Soil, India)	*Paenalcaligenes suwonensis* strain NK7 (Soil, India)
		(100% similarity)	(99% similarity)			
	LB-BV-2	*Brevibacillus* sp. ZQ2(Soil, China**)**	*Brevibacillus laterosporus* strain DY23 **(***Nicotiana tabacum*, stem**,** China**)**	*Brevibacillus halotolerans* strain LAM0313 **(**Rice rhizosphere**,** China**)**	*Brevibacillus laterosporus* strain ZQ2 (Apple rhizosphere, China)	*Brevibacillus laterosporus* strain IHB B 2667 (Bamboo rhizosphere, India)
		(100% similarity)	(99% similarity)			
Root	RB-HS-1	*Acinetobacter* sp. LCE1 **(**Water, Argentina**)**	*Acinetobacter* sp. KT46 **(**Mine soil**,** China**)**	*Acinetobacter johnsonii* strain 0185 **(***Thalassia hemprichii*, China**)**	*Acinetobacter johnsonii* strain CIP 64.6 **(***Toona sinensis*, China**)**	*Acinetobacter oryzae* strain B23 **(***Oryza sativa***,** China**)**
		(99% similarity)				

Contd……

Source	Endophyte	Comparison with already reported endophytes/ bioagents				
Stem	SB-BiJ-9	*Stenotrophomonas* sp. EnB-bsy2 **(**Black soybean seeds**,** China)	*Stenotrophomonas maltophila* strain FLA3**(**Rice rhizosphere**,** India**)**	*Stenotrophomonas* sp. strain HPC107 **(**Soil**,** India**)**	*Stenotrophomonas* sp. strain SCT-8 **(***Bursaphelenchus xylophyilus*,China**)**	*Stenotrophomonas* sp. strain A18 **(***Microcystis aeruginosa***,** China**)**
		(99% similarity)				

Leaf	LB- BiN -8	*Bacillus pumilus* strain ATCC 7061 **(***Halocnemum strobilaceum***,** Saudi Arabia**)**	*Bacillus pumilus* strain SBMP2 **(***Malus domestica*, fruit, India**)**	*Bacillus* sp. KCN26 **(**Banana rhizosphere**,** India**)**	*Bacillus pumilus* strain CTSP14 **(**Soy paste and soy sauce**,** China**)**	*Bacillus pumilus* strain CCGE2028 **(***Phaseolus vulgaris***,** Mexico**)**
		(100% similarity)	(99% similarity)			
Stem	SB-DG-11	*Bacillus pumilus* strain HM-7 **(***Cucumis melo*, root, China**)**	*Bacillus pumilus* strain MGB4043 **(**Mangrove soil**,** China**)**	*Bacillus pumilus* strain BN1 (Hot spring water, Pakistan)	*Bacillus pumilus* AGERI-PB1 **(***Saccharum* sp., root, Egypt**)**	*Bacillus pumilus* strain BJQ-Z1 (Soil, China)
		(99% similarity)				
Root	RB-KK-6	*Alcaligenes* sp. strain G51 **(**Acidic soil**,** India**)**	*Alcaligenes faecalis* strain L48 **(***Pheretima carnosa***,** China**)**	*Alcaligenes faecalis* subsp. *faecalis* strain UN27 (Soil and cow dung, China)	Bacterium endosymbiont LvG2H-17-10411 **(***Onthophagus taurus***,** USA**)**	*Alcaligenes faecalis* subsp. *faecalis* strain PK13 **(***Wasabia japonica*, petiole, China**)**
		(100% similarity)	(99% similarity)			

4. Conclusions

Diversified culturable endophytic bacteria with an addition advantage of antagonism against soilborne pathogens were identified and characterized. Further investigation on their bioefficacy, plant growth promotion activity under field condition and mechanism of action against the pathogens can be carried out which helps in complete understanding and usage of endophytes as effective bioagents in plant diseases management.

5. References

1. Wilson D. Endophyte- the evolution of the term, a clarification of its use and definition. Oikos. 1995; 73:274-276.
2. Pawle G, Singh SK. Antimicrobial, antioxidant activity and phytochemical analysis of an endophytic species of *Nigrospora* isolated from living fossil *Ginkgo biloba*. Curr Res Environ Appl Mycol. 2014; 4(1):1-9.
3. McInroy JA, Kloepper JW. Survey of indigenous bacterial endophytes from cotton and sweet corn. Plant Soil. 1995; 173:337-342.
4. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 1974; 150:850.
5. Murray MG, Thompson WF. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 1980; 8:4321-4325
6. Senthilkumar M, Swarnalakshmi K, Govindasamy V, Lee YK, Annapurna K. Biocontrol potential of soybean bacterial endophytes against charcoal rot fungus *Rhizoctonia bataticola*. Curr Microbiol. 2009; 58:288.
7. Dalal J, Kulkarni N. Antagonistic and plant growth promoting potentials of indigenous endophytic bacteria of soybean (*Glycine max* (L.) Merrill). Curr Res Microbiol Biotechnol. 2013; 1(2):62-69.
8. Yang T, Du W, Zhou J, Wang XX, Dai CC. Effects of the symbiosis between fungal endophytes and *Atractylodes lancea* on rhizosphere and phyllosphere microbial communities. Symbiosis. 2013; 61:23-36.
9. Akshatha S. Role of endophytes in suppression of plant nematode infecting vegetables. *M. Sc. (Agri.) Thesis*, Univ. Agric. Sci., Dharwad, Karnataka (India), 2016.
10. Kuklinsky-Sobral J, Araujo WL, Mendes R, Pizzirani-Kleiner AA, Azevedo JL. Isolation and characterization of endophytic bacteria from soybean (*Glycine max*) grown in soil treated with glyphosate herbicide. Plant Soil. 2005; 273(1):91-99.
11. Yuliar, Suciatmih, Supriyati D, Rahmansyah M. Biodiversity of endophytic bacteria and their antagonistic activity to *Rhizoctonia solani* and *Fusarium oxysporum*. Gl J Biol Agric Health Sci. 2013; 2(4):111-118.
12. Nhu VTP, Diep CN. Isolation and characterization of endophytic bacteria in soybean (*Glycine max* L. (Merrill)) cultivated on alluvial soil of Can Tho city, Vietnam. Int J Innov Eng Technol. 2017; 8:202-221.