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BACILLUS AMYLOLIQUEFACIENS - A NOVEL PGPR STRAIN ISOLATED FROM JUTE BASED CROPPING SYSTEM

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ABSTRACT
The present investigation was made to assess the antagonism of new potential bacterial isolates, *Bacillus amyloliquefaciens* of plant growth promoting rhizobacteria to control *Macrophomina Phaseolina* disease complex of jute. *Bacillus* spp. are well known rhizosphere residents of many crops and usually show plant growth promoting activities that include bio control capacity against some phytopatogenic fungi. Amongst the isolated microorganisms, three in which one *Bacillus* and two others were initially selected based on dual culture technique, production of volatile substance (HCN) in NA, indication towards phytohormone *viz*. indole 3 acetic acid along with siderophore production. Among the isolated strains *Bacillus amyloliquefaciens* showed the best effect in phosphate solubilisation efficiency (73.33%), seed germination (96.66%) and seedling growth of jute, almost near the highest ability to pathogen inhibition (74.26%), reduction of stem rot disease severity (62.9%) in the green house test. The same strain was unique compared to all parameters and enhanced the activity of defence enzyme peroxidase (PO) even after challenge inoculation. The results of our present finding has the evidence of new and novel isolate of *Bacillus amyloliquefaciens* which has tremendous potentiality to control notorious pathogen, *M. phaseolina*, and also plays unique role in plant growth promoting activities in jute.

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) colonize plant roots, exert beneficial effects on plant development via direct or indirect mechanisms, they promote plant growth and/or reduce diseases. The rhizosphere supports large and active microbial populations capable of exerting beneficial, neutral or detrimental effects to the plant growth (Kloepper and Schroth, 1978).

The biological seed treatment differs from chemical means by its utilization of living microorganisms. There has been much research interest in PGPR and there is now an increasing number of PGPR being commercialized for crops (Bio-yield; Reddy *et al.*, 1999). However, it is also possible to inoculate seeds with bacteria that increase the availability of nutrients, including solubilizing phosphate, potassium, oxidizing sulphur, fixing nitrogen, chelating iron and copper. Phosphorus frequently limits crop growth in organic production (Lugtenberg, 2009). PGPR represent a wide variety of soil bacteria which when grown in association with a host plant, result in stimulation of growth of their host.

One of the most common ways that PGPR improve nutrient uptake for plants is by altering plant hormone levels, ability to alter available phosphate from insoluble to soluble form. This changes root growth and shape by increasing root branching, root mass, root and shoot length, and/or the amount of root hairs. This leads to greater root surface area, which in turn, helps it to absorb more nutrients (Dessaux *et al.*, 2009).

The soil borne root infecting fungi such as *Macrophomina phaseolina* is an important pathogen of jute (*C. olitorius* L. and *C. capsularis* L.) because they produce resting structure like sclerotia for their survival for a longer period of time under adverse environmental conditions. Some fungicides simultaneously offered protection against fungi through seed treatment, but it may not enhance the quality to a desired extent and chemical residues are major problems leading to ecological balance and human health hazards. Thus, alternative control measures for the control of *Macrophomina Phaseolina* should be developed.

Diversified populations of aerobic endospore forming bacteria such as species of *Bacillus* occur in agricultural fields and contribute to crop productivity directly or indirectly (Johri *et al.*, 2003). Mechanisms of growth promotion by PGPR-bio fertilizers include production of plant growth activators such as IAA, release of volatile growth-stimulating compounds (HCN) and inhibition of deleterious microorganisms via competition for iron by siderophore (Bandopadhyay *et al.*, 2006). Mechanisms of biological control by PGPR-bio fungicides include production of antifungal compounds including many types of antibiotics, and induction of host defences (induced systemic resistance). Various strains of species Bacillus amyloliquefaeciens are known as potential elicitors of ISR and exhibit significant reduction in the incidence or severity of various diseases in diverse hosts (Choudhary and Johri, 2008; Kloepper *et al.*, 2004).

The present study was carried out to evaluate and characterize of various PGPR related properties and induction of defence related enzyme by cellulolytic bacteria *Bacillus amyloliquefaciens* (Strain 7) and others isolated from soil-rhizosphere in singly and consortia assortment in response to

infection by *M. Phaseolina* (Tassi.) Goidanich.

MATERIALS AND METHODS

Culture media and organisms

Bacillus amyloliquefaciens (S_7) was isolated from the rhizosphere soils of jute growing areas of West Bengal using Nutrient Agar by serial dilution method (King *et al.*, 1954) and purified after single colony isolation from Tryptic soy Agar (TSA) with endospore stain kit. After performing 8 fold dilutions, 100 μL aliquots were placed on Nutrient Agar (NA) and incubated at 30 $\pm$ 2ºC for 48h before colonies were isolated. Single colony was taken as primary criterion for selection of the isolates.16S r DNA gene was PCR amplified with F (5'-AGAGTTTGATC CTGGCTC-3') and R (5'-GGTTACCTTGTT ACGACTT-3') primers in ABI 3730xl cycle sequencer. Amplicon was electrophoresed in a 1% Agarose gel and visualized under UV (Koumoutsi, *et al.,* 2004). Concentration of the amplicon was checked in a Nanodrop ND 2000. The amplicon was purified using Nucleospin purification column (Macherey-Nagel). Forward and reverse sequences were assembled and contig was generated after trimming the low quality bases. The sequence analysis was carried out using bioinformatic tool BLAST of NCBI. The PCR product was sequenced and submitted to genbank.

Table 1: Bio chemical parameters for Plant Growth Promoters

Sample No.	Siderophore Positive/ Negative	Radius of Halo zone (mm)	IAA Positive/ Negative	HCN positive/ Negative
S_2	+ +	5.0	+	+ +
S_3	+	4.5	-	+ +
S_4	+	2.1	-	+
S_5	+ +	5.0	+ +	-
S_7	+ + +	15.3	+ + +	+ +
S_{10}	+ + +	17.5	+	+
S_{12}	+ + +	18.0	+	+ + +

- = Negative, + = Scanty/Mild positive, + + = Moderate, + + + = Strong.

Table 2: Phosphate solubilisation in PKV media

Sl. No.	Sample/ Organism	Phosphate solubilisation (Pikovs'kays Agar)	Radius of clear zone (mm)	Solubilization Efficiency (SE %)
1.	S_2	+ +	16.0	45.45
2.	S_3	-	NA	-
3.	S_4	+	10	25
4.	S_5	+ +	14.0	27.27
5.	S_6	-	NA	-
6.	S_7	+ + +	22	73.33
7.	S_{10}	+ +	*	-
8.	S_{12}	-	NA	-

- = Nil, + = Scanty/Mild, + + = Moderate, + + + = Strong, * = No clear zone but utilize phosphate from available sources, NA = Not Applicable

Table 3: Dual culture studies amongst bio agents and Pathogens

Sample	Colony Diameter/ Radial growth (mm)		Inhibition Zone (mm)	% Inhibition
	Pathogen	Antagonist		
S_2	25.00	61.00	3.5	72.22
S_3	22.00	64.00	3.5	75.55
S_4	24.00	64.00	2.0	73.33
S_5	23.00	61.00	5.0	74.20
S_7	22.00	63.00	4.8	74.26
S_{10}	28.00	60.00	1.7	68.88
S_{12}	25.00	64.9	0.1*	72.33
S_{15}	90.0	-	-	-
C. D (p = 0.05)	0.687	0.868	0.581	1.652

* = Almost over growth, - = Nil.

Selection of seed sample and effect of *B. amyloliquefaciens* for Seedling growth and vigour

Highly popular and moderately susceptible variety JRO 524 was collected for further study from CSRSJAF (ICAR), Budbud ; Burdwan, WB, India. Two bacterial isolates (S_3, S_5) as *Alcaligenes faecalis* and *Bacillus amyloliquefaciens* (S_7) were cultured on Nutrient broth media and one fungus *Trichoderma aureoviride* (S_{12}) on PD broth (PDB) at (28-30) $\pm$ 2ºC. Selections are based on dual culture method (Bells *et al.*, 1982; Dhingra and Sinclair, 1985) for their antagonistic potential, compatibility and by production of phytohormone. Two-day-old cultures were harvested and centrifuged at 10,000 rpm for 10 min. Pellets obtained were suspended in distilled water and the inoculums load was adjusted to 1×10^8 CFU/mL based on absorbance in spectrophotometer (OD 0.48 at 610 nm). The suspension were mixed and air-dried under sterile conditions (Vidhyasekaran and Muthamilan, 1995; Nandakumar *et al.*, 2001). Control seeds were soaked in distilled water.

Compatibility among selected strains and others including Bacillus strains were tested using NA: PDA (1:1) medium following the method of Fukui *et al.* (1994). The most promising isolates based on different parameters screened early were selected for the seed treatment. Treated seeds were plated equidistantly on three layers of wet blotters on Perspex plates and incubated at 30 $\pm$ 2ºC under alternate cycles of 12/12h near UV light and darkness for 7 days.

On the 8th day of incubation the seeds were evaluated for the pycnidia of *M. phaseolina* following the procedures of international seed testing association, ISTA (ISTA, 1996). Treated seeds were also subjected to the paper towel method, in which the seeds were placed equidistantly between wet blotters and incubated at 30 $\pm$ 2ºC under 12/12 h alternate cycles of light and darkness for 14 days. After incubation, the seed germination percentage and root-shoot lengths of the seedlings were assessed and the vigour index was calculated based on the formula prescribed by Abdul-Baki and Anderson (1973). Application of bio formulations enhanced the biomass of jute under laboratory and glasshouse conditions compared with the untreated control and chemical treatments even with microbial mixture.

A pot culture was conducted to test the bio formulations under challenge inoculation with *M. phaseolina* using *Bacillus amyloliquefaciens* and others in single and in combinations against seed borne and early damping off disease. In all, charcoal-based seed treatment (10 g/kg of seed) with the spore suspension (2×10^8 spores/ml) of respective bio agent before sowing and 2% CMC (as sticker) based soil bio formulation mixture at 15 days after sowing was applied. The fungicide Carbendazim (Bavistin) 0.2% was used to compare and IAA soaked seed for compatibility. The plants inoculated by pathogen alone with a load 1×10^5 spores/ml served as

Table 4: Effects of Seed treatment in the green house test

Treatments	Germination (%)	Root Length (Cm)	Shoot Length (Cm)	Fresh Weight (Gm)	Dry Weight (Gm)	Vigour Index (VI)	Disease control (%)
T_1	93.33^{e}	3.75^{b}	11.4^{bc}	3.26^{de}	0.45^{cd}	1413.94^{d}	63.61^{c}
T_2	96.66^{f}	4.96^{j}	12.81^{e}	3.95^{fg}	0.55^{e}	1717.64^{g}	62.88^{c}
T_3	90^{d}	3.82^{e}	11.5^{bc}	3.87^{f}	0.47^{d}	1378.80^{cd}	62.43^{c}
T_4	93.33^{e}	4.63^{h}	12.58^{de}	3.48^{e}	0.41^{bcd}	1606.20^{e}	31.91^{abc}
T_5	86.66^{c}	4.1^{f}	11.61^{c}	2.85^{b}	0.36^{ab}	1361.42^{f}	31.73^{abc}
T_6	83.33^{b}	3.8^{d}	12.28^{d}	2.83^{b}	0.35^{ab}	1339.94^{c}	31.54^{abc}
T_7	86.66^{c}	3.75^{b}	11.20^{b}	3.12^{cd}	0.33^{ab}	1295.56^{bc}	20.23^{ab}
T_8	90^{d}	4.71^{i}	12.79^{e}	4.33^{h}	0.37^{ab}	1575.00^{e}	38.23^{abc}
T_9	90^{d}	4.27^{g}	14.2^{f}	4.16^{gh}	0.38^{abc}	1662.30^{fg}	48.34^{bc}
T_{10}	90^{d}	3.77^{c}	13.98^{f}	3.31^{de}	0.34^{ab}	1597.50^{ef}	25.62^{abc}
T_{11} (Diseased Control)	73.33^{a}	3.49^{a}	10.39^{a}	2.91^{bc}	0.30^{a}	1017.82^{a}	–
T_{12} (Healthy Control)	83.33^{b}	3.8^{d}	11.24^{b}	2.22^{a}	0.33^{a}	1253.28^{bc}	–

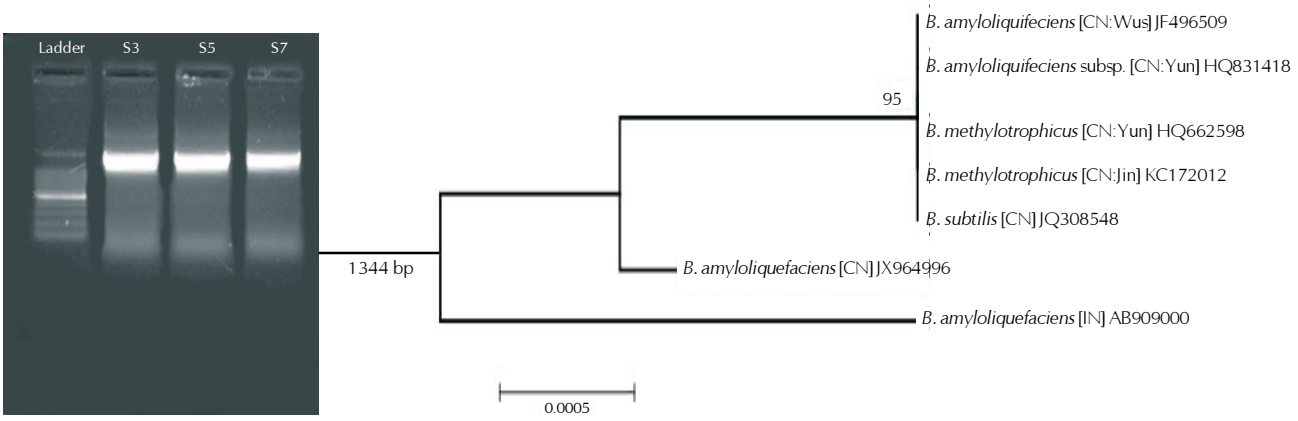

Figure 1:a. Amplicon size (1344 bp) of *B. amyloliquefaciens* (S_7). b. Constructed phylogenetic tree of *B. amyloliquefaciens* (AB909000)

inoculated control. Twenty one days after inoculation observations on development of early wilting or drooping of leaf symptoms were made. Post harvest data of the jute yield was recorded for individual treatment. Induction of defence-related protein and experimental findings in single and in combinations were used in the induction of defence reactions in jute. The experiment was conducted using completely a randomized block design with three replications on a poly greenhouse bench. The humidity in the poly greenhouse was maintained at around RH 80%. The temperature was adjusted to 28ºC (day)/20ºC (night).

The details of treatments in the green house test are as follows:

T_1: ST with S_3 + SA with S_3 15 DAS

T_2: ST with S_7 + SA with S_7 15 DAS

T_3: ST with S_{12} + SA with S_{12} 15 DAS.

T_4: ST with Carbendazim 50WP @ 2 gm/kg of seed.

T_5: ST with Carbendazim 50WP 0.2% + S_3 + SA with S_3 and S_7 (1:1) 15 DAS

T_6: ST with Carbendazim 50WP 0.2% + S_7 + SA with S_3 and S_{12} (1:1) 15 DAS

T_7: ST with Carbendazim 50WP 0.2% + S_{12} + SA with S_7 and S_{12} (1:1) 15 DAS

T_8: SS with IAA@ 25 ppm + SA with S_3 + S_7 + S_{12} (1:1:1) 21 DAS

T_9: T_4 + T_8 (1:1) + Quizalofop ethyl (Targa super) sprays @1.5 ml/lit. 30 DAS.

T_{10}: ST with S_3 + S_7 + S_{12} (1:1:1) + SA with S_3 + S_7 + S_{12} (1:1:1) 21 DAS.

T_{11}: Control (Inoculated/Diseased)

T_{12}: Control (Un-inoculated/Healthy).

ST: Seed Treatment, SS = Seed Soaking, SA = Soil Application, DAS = Day's after Sowing.

Assay of peroxidase

Leaf samples were collected at different time intervals and homogenized with liquid nitrogen in a pre-chilled mortar and pestle. 1g of leaf sample was homogenized with 2-3 mL of 0.1 M sodium phosphate buffer (pH 7.0) at 4ºC. The homogenate was centrifuged for 20 min. at 18,000 rpm. Supernatant was used as a crude enzyme extract and stored on ice till the assay is carried out. O-dianisidine (1mg/mL methanol) was used as substrate for assaying isoperoxidase (Putter, 1974). Sample extract electrophoreses in 8% PAGE buffered gel at low temp. (4-8ºC) and each lane was loaded with 20μL of proteins for regulation of gene expression. After electrophoresis the gel finally incubated and stained for 30 min. in a solution at dark place containing all the necessary compounds particularly 0.05% benzidine and freshly prepared 0.042% = 12.3 mM hydrogen peroxide in acetate buffer (20 mM, pH 4.2). Washed

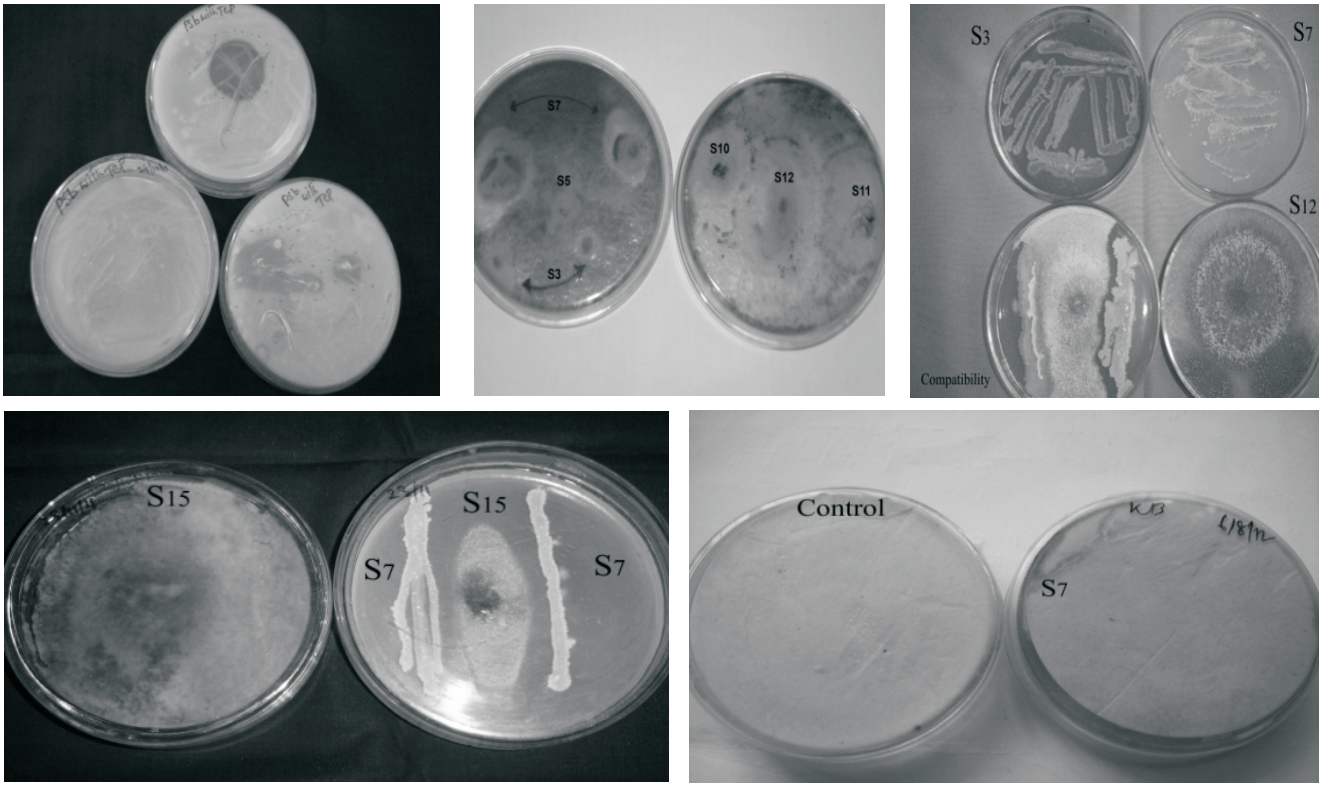

Figure 2.c. Phosphate solubilisation, d. Siderophore detection in CAS, e. Compatibility of bioagents, f. Dual culture and g. HCN test.

gel repeatedly for visualize the bands clearly.

RESULTS AND DISCUSSION

The isolate was identified based on maximum identity score as *Bacillus amyloliquefaciens* (AB909000) of which few sequences are selected, aligned using software Clustal W2 which closely related 99.18% with two China isolates JX964996 and JQ308548 and distantly 98.51% with China Inner Mongolia, Wushen isolate JF496509 (Fig. 1.a and b). Further *Alcaligenes faecalis* (S_3) and *Trichoderma aureoviride* (S_{12}) were also sequenced and got the genbank accessions AB901364 and AB916337 which shows compatible relations with each other (Fig. 2.e).

Efficient isolates were screened and finally selected before seed priming on the basis of their response (Fig. 2. c, d and g) in siderophore production in CAS, ability to convert insoluble Tri calcium phosphate to soluble form in Pikovs'kays agar (Pikovskava,. 1948), capacity of L-Tryptophan mediated IAA (Brick *et al.*, 1991) similar to FZB 42 (Idris *et al.*, 2007), HCN positive (Bakker and Schipper, 1987). Amongst all the isolates S_7 having with highest intensity 73.33% (Nguyen, *et al.*, 1992; Anandaraj *et al.*, 2003) to response against phosphate solubilization (22 mm clear zone), IAA (71 μg/mL); equally better intensity to siderophore by 15.3 mm Orange-yellow halo zone (Schwyn and Neiland, 1987) and HCN tests. Whereas S_3, S_4 are IAA negative but HCN positive and S_5 have no impacts in HCN test (Table 1 and 2). Besides two fungi S_{10} and S_{12} also shows better response against biochemical tests.

The antagonistic study by dual culture technique revealed that all the antagonists i.e. five bacterial and two fungal isolates had the ability to inhibit the pathogen growth significantly. The inhibition ranged from 68.88% - 75.55% (Table 3). To evaluate the efficacy of bio formulations under challenge inoculation using *B. amyloliquefaciens* and others in single and in combinations against seed borne and early damping off disease revealed that the jute seeds which were treated with bio formulation using the strain *B. amyloliquefaciens* as T_2 showed the highest peroxides activity in terms of number and intensity of banding pattern singly and even when in mixture as T_9 compared with others. These results showed that certain individual and compatible mixtures of plant growth promoters could provide a broad spectrum of ISR activity against pathogens.

The results on bio priming of seeds by S_7 known as *B. amyloliquefaciens* singly shows the better effects in all the parameters studied under poly greenhouse (Table 4).

Results based on Duncan's Multiple Range of Test (DMRT). Means with the same common letters in each column are not significantly different according to Duncan's multiple range test at P = 0.05.

It was the most efficient isolate regardless of crop seeds and form of inoculants used at green house studies even when it was applied with other bio agents (S_3 and S_{12}), agrochemicals (Carbendazim), growth regulator (IAA) and herbicides (Quizalofop ethyl) for post emergence effect in an integrated manner. The growth regulator chemicals Indole, 3 acetic acid have immediate effect on fresh weight basis where as bacterial antagonist S_3 and fungal bio agent S_{12} found to have intermediate effect with respect to their potential in enhancing

Figure 3: h. Antagonism by S_7 & S_{12} against Pathogen (S_{15}) in a consortial manner, i. Effect of bio agents on plants after sowing under green house, j. Analysis of Pox in native PAGE 30 DAS.

the percent seed germination, seedling biomass on dry weight basis and seedling vigour on possible extent of the crop seeds used. Among two forms of formulation the charcoal based seed treatment with respective bio agent singly or in compatible combination along with seed treating chemicals gave better responses with respect to enhanced germination of seed.

Whereas, CMC based Soil formulation gave better response in towards seed vigour index either in terms of estimated biomass on dry weight basis. The growth regulator IAA used for seed soaking and gave better performance in stimulating germination, root elongation and shoot length over of some treatments using bio agents or chemicals.

The results proved that the newly isolated bio agents have the synergistic effects with each other and potentially compatible when applied as mixture with different agrochemicals. However, *Bacillus amyloliquefaciens* (AB909000) has been proved as excellent bio-control agent of M. Phasiolina which could be applied with *Trichoderma aureoviride* and *Alcaligenes faecalis* in integrated way without any adverse effect to the plant. It is presumed that Bacillus *amyloliquefaciens* might have the role in production of bio active molecule of cyclic lipopeptide (CLP's) like compound which may induce in plant growth that could be explored in future.

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REFERENCES

Abdul-Baki, A. A. and Anderson, J. D. 1973. Vigour determination in soybean seed by multiple criteria. *Crop Sci.* **13(6):** 630-633.

Anandaraj, M., Ramachandran, V., Srinivasan, K. and S. Hamza. 2003. Phosphate solubilizing bacteria isolated from the rhizosphere soil and its growth promotion on black pepper (Piper nigrum L.) cuttings. First International meeting on microbial phosphate solubilisation. *Springer* **(2007):** 325-331.

Bakker, A. W. and Schipper, B. 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* sp. mediated plant growth stimulation. *Soil Biol. Biochem.* **19:** 451-457.

Bandopadhyay, A. K., Bandopadhyay, A., Majumdar, A., Samajpati, N. and Bhattacharyya, S. K. 2006. Investigations on some fungal antagonist plant growth promoting rhizobacteria for siderophore production and bio control of diseases in Jute. *J. Mycopathol. Res.* **44(1):** 165-166.

Bell, D. K., Wells, H. D. and Markham, C. R. 1982. *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. *Phytopathol.* **72:** 379-382.

Brick, J. M., Bostock, R. M. and Silversone, S. E. 1991. Rapid in situ assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. *Appl. Environ. Microbiol.* **57:** 535-538.

Choudhary, D. K. and Johri, B. N. 2008. Interactions of Bacillus spp. and plants – with special reference to induced systemic resistance (ISR). *Microbiol. Res.* **164:** 493-513.

Dessaux, Y., Hinsinger, P. and Lemanceau, P. 2009. Rhizosphere: So many achievements and even more challenges. *Plant Soil.* **321:** 1-3.

Dhingra, O. D. and Sinclair, J. B. 1985. Basic Plant Pathology Methods, CRC Press, Inc., Boca Raton, Florida, USA. p. 335.

Fukui, R., Schroth, M. N., Hendson, M. and Hancock, J. G. 1994. Interaction between strains of Pseudomonadsin sugar beet sphermospheres and the relationship to pericarp colonization by *Pythium ultimum*in soil. *Phytopathol.* **84:** 1322-1330.

Idris EES, Iglesias, D. J., Talon, M. and Borriss, R. 2007. Tryptophan-dependent production of Indole-3-Acetic Acid (IAA) affects level of plant growth promotion by Bacillus amyloliquefaciens FZB42. *Mol Plant Microbe Interact.* **20:** 619-626.

ISTA 1996. International rules for seed testing. *Seed Sci. Technol.* **21:** 25-30.

Johri, B. N., Sharma, A. and Viridi, J. S. 2003. Rhizobacterial diversity in India and its influence on soil and plant health. *Adv. Biochem. Eng. Biotechnol.* **84:** 49-89.

King, E. O., Ward, M. K. and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanine and fluorescein. *J. Lab. Clin. Med.* **44:** 301-307.

Kloepper, J. W. and Schroth, M. N. 1978. Plant Growth Promoting Rhizobacteria in radishes. *In: Proceedings of IVth International Conference on Plant Pathogenic bacteria.* pp. 879-882.

Kloepper, J. W., Ryu, C. M. and Zhang, S. 2004. Induced systemic resistance and promotion of plant growth by Bacillus spp. *Phytopathology*. **94:** 1259-64.

Koumoutsi, A., Chen, X. H., Henne, A., Liesegang, H., Hitzeroth, G., Franke, P., Vater, J. and Borriss, R. 2004. Structural and functional characterization of gene clusters directing nonribosomal synthesis of bioactive cyclic lipopeptides in Bacillus amyloliquefaeciens strain FZB42. *J. Bacteriol*. **186:** 1084-1096.

Kushwaha, A., Baily, S. B., Maxton, A. and Ram, G. D. 2013. Isolation and characterization of PGPR associated with Cauliflower roots and its effect on plant growth. *The Bioscan*. **8(1):** 95-99.

Lugtenberg, B. and Kamilova, F. 2009. Plant Growth Promoting Rhizobacteria. *Ann. Rev. Microbiol*. **63:** 541-556.

Nandakumar, R., Babu, S., Viswanathan, S., Raguchander, T. and Samiyappan, R. 2001. Induction of systemic resitance in rice against sheath blight disease by *Pseudomonas fluorescens*. *Soil Biol. Biochem*. **33:** 603-612.

Nguyen, C., Van, W., Le Tacon, F. and Lapayrc, F. 1992. Genetic variability of phosphate solubilizing activrty by monocaryotic and dicaryotic mycelia of the ectomycorrhizal fungus Larcaria bicolor (Maire) PD. Orton. *Plant and Soil*. **143:** 193-199.

Pikovskava, R. 1948. Mobilization of P in sail in connection with vital activity by some microbial species. *Microbiologica*. **17:** 362-370.

Putter, J. 1974. *Peroxidases. In: H.U. Bergmeyer (eds). Methods of enzymatic analysis, New York: Verlag Chemie Academic Press*. **2:** 685-690.

Reddy, M. S., Rodriguez-Kabana, R., Kenney, D. S., Ryu, C. M., Zhang, S., Yan, Z., Martinez-Ochoa, N. and Kloepper, J. W. 1999. Growth promotion and induced systemic resistance mediated by a biological preparation. *Phytopathology*. **89:** S39.

Schwyn, B. and Neiland, J. B. 1987. Universal chemical assay for the detection and determination of siderophores.

Vidhyasekaran, P. and Muthamilan, M. 1995. Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt. *Plant Dis*. **79:** 782-786.