

## Screening of Biocontrol Potential of Indigenous *Bacillus* spp. Isolated from Rice Rhizosphere against *R. solani*, *S. oryzae*, *S. rolfsii* and Response towards Growth of Rice

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Different *Bacillus* strains were isolated and selected to assess their capability to inhibition of rice soil borne pathogens and evaluated in terms of plant growth promotion activities. Out of seventeen strains, four strains B-1, B-2, B-3 and B-4 showed strongly antagonistic activities against pathogens and showed significant plant growth promoting (PGP) activity. Based on the morphological and several biochemical approaches like P-solubilization, IAA production, HCN production, different specific C-utilization, catalase, oxidase activities and mechanism of antagonism was studied through protease and chitinase activities, all these six isolates were identified as *Bacillus* sp. All the strains showed strongly inhibited (upto 90%) the growth of phytopathogens like *Rhizoctonia solani*, *Sclerotium rolfsii* and *Sclerotium oryzae* invitro. For Plant growth promoting assay, strains B-1, B-2 and B-3 were capable of enhancing the vegetative growth and yield parameters (shoot/root length, biomass, chlorophyll content and yield) and showed significant increase than non-inoculated control plants. The present findings suggest that these three indigenous isolates may be exploited as a potential bioinoculant agent for biocontrol as well as plant growth promoter.

**Keywords:** *Bacillus*; phytopathogens; Rice; *R. Solani*; *S. Rolfsii*; *S. oryzae*.

Due to uncontrolled population growth the demand for rice in India is increasing (Joshi *et al.* 2009), so farmers heavily dependent on the use of high chemical fertilizers and pesticides to achieve the higher yields (Tilman *et al.* 2002). This dependence on chemical fertilizers and fungicides associated with problems such as environmental pollution, health hazards, interruption of natural ecological nutrient cycling and destruction ecological communities especially on soil microflora that directly supports the soil and crop health (Ramirez *et al.* 2010). Plant diseases which lead to the loss of crops are one of the major concerns in the agricultural scenario

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in India and there is need to control the diseases caused by the different pathogens. like soil borne and foliar, soil borne pathogens inflicts many diseases on rice like stem rot, seedling blight and sheath blight caused by *Sclerotium rolfsii*, *Sclerotium oryzae* and *Rhizoctonia solani*, respectively and causes loss of yield. A variety of alternative approaches have also been considered for control of pathogens (Ajilogba *et al.* 2013; Compant *et al.* 2005; Karlidag *et al.* 2007; Foldes *et al.* 2000; Leelasuphakul *et al.* 2008). Hence crop production and diseases management have to be achieved with fewer detrimental inputs or through biofertilizers and biocontrol agents (BCA) is important for improving and sustaining the crop productivity (Roberts *et al.* 1988; Leelasuphakul *et al.* 2008; Sethi and Adhikary, 2009a; Sethi and Adhikary, 2009b; Karlidag *et al.* 2007; Foldes *et*

al. 2000). Several microorganisms are being used in controls of pest and diseases of various crops included *Trichoderma*, *Pseudomonas* and *Bacillus* sp. Rhizospheric *Bacillus subtilis* based biocontrol agents are quite important in the management of pest and plant diseases (Jacobsen *et al.* 2004; Leelasuphakul *et al.* 2008; Ajilogba *et al.* 2013) is also known as effective biocontrol agent against several soil borne pathogens indicating their effectiveness in inducing disease resistance in the crop plants in addition; the increased growth response induced by these species has also been reported for many kinds of crops (Compant *et al.* 2005; Foldes *et al.* 2000; Karlidag *et al.* 2007). It was found that the efficient strains from outside a particular agro-climatic zone often fail to achieve the desired result as plant growth promoter (Sethi and Adhikary, 2014; Sethi and Adhikary, 2015; Azad, 1994). Therefore it becomes necessary to isolate and screen the native *Bacillus* strain and testing the efficacy of biocontrol and plant growth promoting activity.

Therefore in this present study the efficacies of indigenous *Bacillus* strains isolated from rice rhizospheric and non-rhizospheric soil of different regions of Odisha and compare their plant growth promoting and biocontrol activities against *R. Solani*, *S. rolfsii*, *S. oryzae* of rice plant.

## MATERIALS AND METHODS

### Isolation and maintenance of *Bacillus* sp.

Soil samples were collected from rhizosphere and non-rhizospheric soils in and around Odisha. *Bacillus* sp. was isolated by soil dilution method. Soil samples (10g) from rice rhizosphere transferred to 90 ml sterile distilled water and mixed thoroughly by shaking the flask on a rotatory shaker for 5 min. After serial dilution 0.1 ml suspension was spread over presterilized and cooled down nutrient agar plates in triplicates. The inoculated plates were incubated at  $30 \pm 2$  °C for 2 days. Rough and abundant colonies with waxy growth and irregular spreading edge were obtained and maintained on the Nutrient Agar Medium and kept at 4°C for further use.

Physiological and biochemical characterization of isolates were performed by using HiBacillus Identification Kit KB013 and Hi Carbohydrate TM Kit Hi25TM (Himedia) followed

by as per the manufacture's instruction. The culture strains were identified following the Bergey's Manual of Determinative Bacteriology<sup>15</sup>.

### *Bacillus* sp. inoculum preparation

The inoculum preparation was carried out according to the method described (Sethi and Adhikary, 2009a; Sethi and Adhikary, 2009b). Loopfuls of each isolated *Bacillus* sp. from 2-3 days old cultures were transferred separately to 50 ml nutrient broth (NB) medium and incubated overnight at  $28 \pm 2$  °C for 2-3 days. Viability was confirmed by taking loopful of cultures and restreaked on nutrient agar medium. This inoculum was prepared to be used in vitro for antifungal activities of the *Bacillus* isolates and also their biocontrol activities in vivo. The inoculums also used to net house experiments were prepared from a 24 h old shaken culture of each of the *Bacillus* isolate incubated at  $28 \pm 2$  °C. *Bacillus* culture suspension containing  $10^7$  cfu/ml was used as inoculant.

### Isolation and preparation of phytopathogenic fungal sp.

Samples of diseased leaf stem, root parts and seeds were randomly collected and these fungal pathogens were isolated from infected plants and diseased seeds following water agar and blotter techniques. Besides some other standard fungal pathogens were also used in this study such as *Rhizoctonia solani*, *Sclerotium rolfsii* and *Sclerotium oryzae* obtained from different agricultural research institutes, India. Fungal strains and inoculum was prepared by culturing it on Potato dextrose agar (PDA) for 10 days in Petridishes. The microconidial suspension of these three pathogens was prepared by pouring 1 ml of sterile water in each petridish. The inoculum was then scrapped with the help of a sterile spatula from the surface of the Petridishes. The spores from the mycelia of the fungi to get a concentration of  $10^6$  spore/ml concentration and applied in sterile soils as method described (Ajilogba *et al.* 2013; Adebay and Ekpo 2005).

### Phosphate Solubilisation

Phosphate solubilization is an important and complex phenomenon for selectively screening the bacteria having P-solubilizing activity which have the ability to release inorganic phosphate from tricalcium phosphate. Pikovskaya's medium, is a selective medium for phosphate solubilizing

microorganisms (PSM) was used to which tricalcium phosphate (TCP) has been added as it will enhance formation of halo zones. The medium was sterilized and poured into sterile petridishes (Himedia). Isolates were streaked on the Petridishes and incubated for 3 days at  $28\pm 2^\circ\text{C}$ . *Bacillus* isolates that were able to solubilize developed clear zones around colonies (Pikovskaya 1948).

#### **Determination of Indole Acetic Acid Production (IAA)**

Luria Bertani Broth (Himedia) was prepared and freshly grown cultures were inoculated into 10 ml LB broth in each test tube and incubated at  $28\pm 2^\circ\text{C}$  for 24 h at 120rpm. Grown cultures removed from each test tube and centrifuged at 10,000 rpm for 15 min. An aliquot of 1 ml of supernatant was transferred into a fresh tube to which two drops of orthophosphoric acid were added. The mixture was incubated at room temperature for 25 minutes. The development of a pink colour indicated the presence of indole acetic acid (Gordon and Weber 1951.).

#### **Detection of Hydrogen Cyanide (HCN) Production**

Production of HCN was detected according to the method described (Lorck 1948). 24h freshly grown cells were spread on agar medium which contained 4.5 g/l glycine had been added and a sterilized filter paper saturated with 1% solution of picric acid and 2% sodium carbonate was placed in the upper lid of the Petridish, which was then sealed with parafilm and incubated at  $28\pm 2^\circ\text{C}$  for 4 days, a change in colour of the filter paper from yellow to reddish brown was an index of cyanogenic activity while no colour change represent no cyanogenic activity.

#### **Determination for the production of different cell wall degrading enzymes of *Bacillus* isolates**

##### **Chitinase activity**

The different isolates obtained were screened for chitinase production based on the halo produced on plates with minimal salts medium amended with chitin. The qualitative assay for chitinase production was performed following the method (Dunne *et al.* 1997) and modified method given by (Roberts and Selitrennikoff, 1988). Isolates were inoculated by spotting on the plates having chitin minimal medium (CMM) as sole source of carbon and incubated at  $30\pm 2^\circ\text{C}$  for 10

days. These plates were examined for development of clear zones around colonies.

#### **Preparation of colloidal chitin**

5.0 g of chitin was added to 60 ml of concentrated HCl (acid hydrolysis) by constant stirring using a magnetic stirrer at  $4^\circ\text{C}$  and kept in refrigerator overnight. The resulting slurry was then added to 2000 ml. of ice-cold 95% ethanol and kept at  $26^\circ\text{C}$  for overnight (ethanol neutralization). Then it was centrifuged at 3000 rpm for 20 min at  $4^\circ\text{C}$ . The pellet was washed with sterile distilled  $\text{H}_2\text{O}$ . The final colloidal chitin was stored at  $4^\circ\text{C}$  until further use.

#### **Final chitinase detection medium**

The final chitinase detection medium per litre comprises of 4.5 g colloidal chitin, 0.3 g magnesium sulphate, 3.0 g ammonium sulphate, 2.0 g potassium dihydrogen phosphate, 1.0 g citric acid monohydrate, 15 g agar, 0.15 g bromocresol purple and 200  $\mu\text{l}$  of tween-80. The pH of the media was maintained at 4.7 and autoclaved at  $121^\circ\text{C}$  for 15 min. The fresh culture of *Bacillus* isolates to be tested for chitinase activity were inoculated into the sterile plates containing chitinase detection medium and incubated at  $28\pm 2^\circ\text{C}$  for seven days and observed for the colored zone formation. Chitinase activity was identified due to the formation of purple colored zone. Color intensity and diameter of the purple coloured zone were taken as the criteria to determine the chitinase activity after three days of incubation.

#### **Protease activity**

The production of protease, a fungal cell wall degrading enzymes which is an important mechanism of fungal growth inhibition was detected for all selected isolates. Protease activity was determined according to the modified method described (Berg *et al.* 2002). Skim milk agar medium (5.15/100ml) was used for detection of protease activity, 48h old freshly grown cultures were inoculated on skim milk agar medium and incubated at  $28\pm 2^\circ\text{C}$  for two to three days. *Bacillus* sp. which shows the protease activity gave a clearance zone indicating the production of protease enzyme. The degree of protease activities were measured based on the diameter of clearance zone.

#### **Carbon Utilization study**

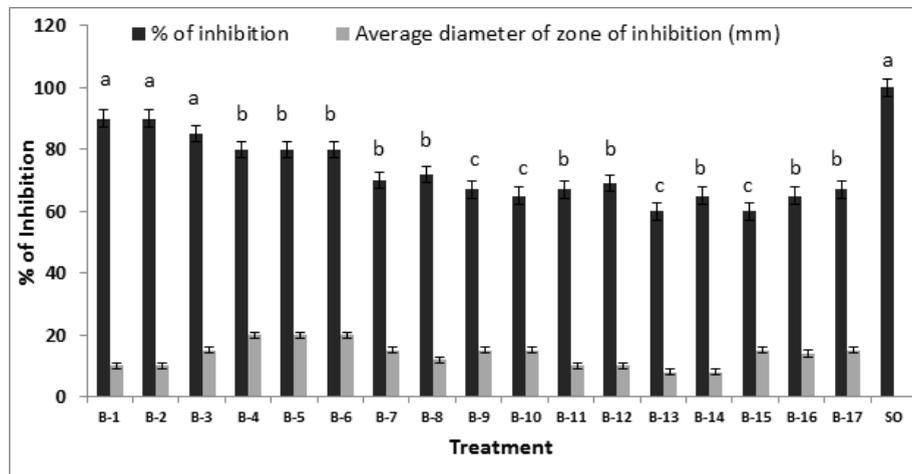
Different carbon utilization of *Bacillus* sp. was studied by the HiCarbo Utilization kit

(Himedia). Hi carbokit was used to study the biochemical profile of organisms is a standardized colorimetric identification system utilizing 35 carbohydrate utilization test. The test was based on the principle of change of pH and substrate utilization by selected bacteria. On incubation or organism undergo metabolic changes which are indicated by a spontaneous colour change in the media. A combination of 35 tests for utilization of carbohydrate tests. Kit contains Part A, Part B each having 12 carbohydrates utilization tests and Part C containing 11 sugars and 1 control. The organism was pure cultured and culture inoculums

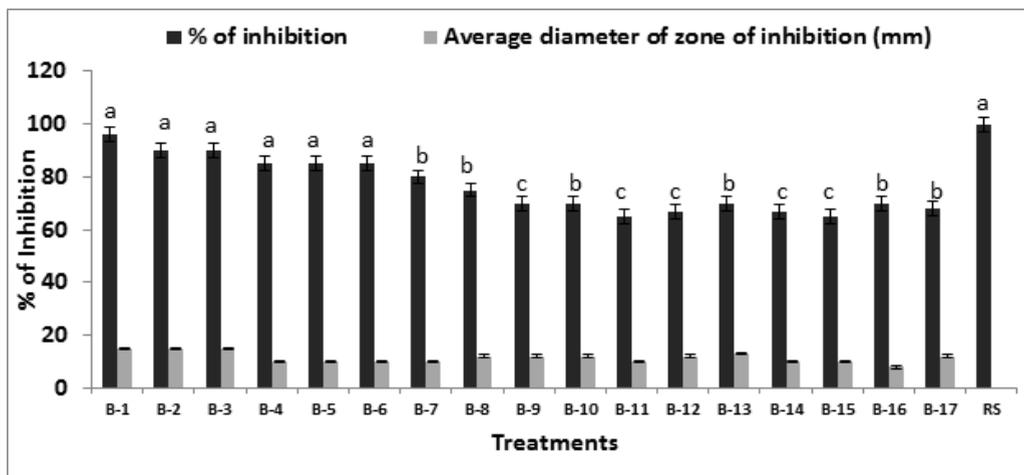
turbidity was 0.5 OD at 620 nm, then inoculate each well with 50µl of the above inoculums by surface inoculation methods and incubate at 28±2°C for 24 hr and data was interpreted as per result interpretation chart.

**Antagonistic activity of *Bacillus* sp.**

The test was carried out to see the effect of the *Bacillus* isolates on the three important rice phytopathogens *in vitro*. Pathogen inhibition test was performed by dual culture plate assay One 5-mm disc of a pure culture of the pathogen was placed at the corner of a Petri dish containing NA+PDA (Nutrient agar with Potato dextrose



**Fig. 1.** Inhibitory effect of the six bacillus isolates against *S. oryzae* in vitro. Percent of inhibition of *Bacillus* against pathogen, while SO is the growth of *Schelorantium oryzae* alone in the Petri dish. Each value is average of ten replicates. For each bar value are followed by a different lower case letters indicates LSD at p<0.05



**Fig. 2.** Inhibitory effect of the six bacillus isolates against *R. solani* in vitro. Percent of inhibition of *Bacillus* against pathogen, while RS is the growth of *Rhizoctonia solani* alone in the Petri dish. Each value is average of ten replicates. For each bar value are followed by a different lower case letters indicates LSD at p<0.05

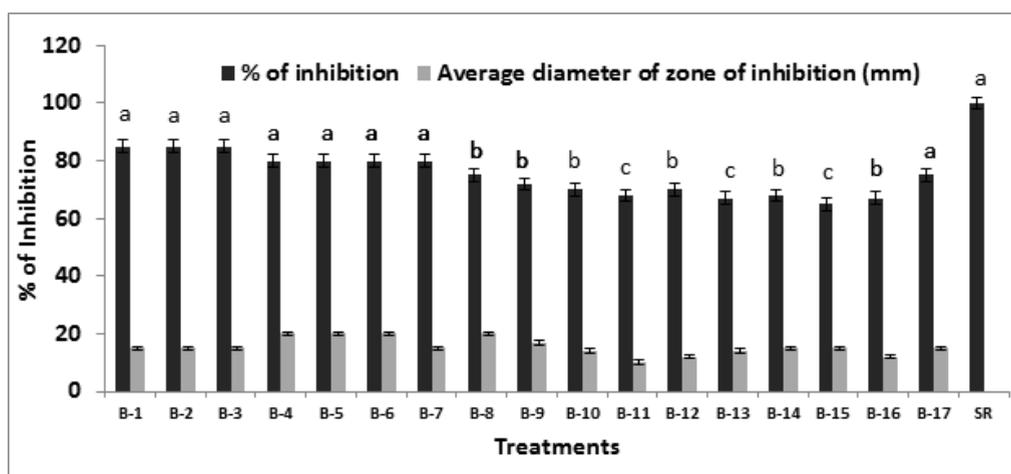
agar). A loopful of *Bacillus* culture was streaked over the surface of NA+PDA plate at opposing corners. Plates were incubated for 10 days at 28±2°C, and examined for inhibition of fungus growth by the *Bacillus* isolates. The growth diameter of the pathogen was measured and compared to control growth, where the bacterial suspension was replaced by sterile distilled water. Each experiment was used a single pathogen isolate was run in triplicate. The Results were expressed as the mean value with standard error deviation in inhibition distance between the growths of the fungus isolate and the presence of bacillus isolate tested. Percent inhibition was calculated using the following formula: % inhibition = [1-(Fungal growth/Control growth)] x100

Control experiment with only growth of *Bacillus* isolates and each pathogen in each separate Petridishes of three replicates were observed.

**Pot experiment for Efficacy of *Bacillus* on biomass and yield**

Healthy seeds of rice were treated with different treatments of *Bacillus* isolates and directly sown into 24cm-diameter pots. A loopful of bacterial isolate (starter culture) was transferred to a 500 ml flask containing sterile liquid medium and grown aerobically in a rotary shaker at 120-160 rpm for 48h at 28±2°C (Lamsal *et al.* 2013; Sethi and Adhikary 2009a,b; Sethi and Adhikary 2014; Sethi and Adhikary 2015). The bacterial culture suspension was diluted to become 10<sup>9</sup> CFU/ml

as per method described<sup>23</sup>. Seeds were surface sterilized for 30 min in 20% sodium hypochloride, followed by rinse with 70% ethanol before sown in earthen pot (4 cm diameter with hole). Each pot contained sterile garden soil. The outlined of the experimental design using 10 replicates (each replicates comprised of 3 plants) for each of the *Bacillus* isolates, a negative control with no fungus and a positive control with inoculation of fungus. Seeds were treated with bacterial suspension (containing 10<sup>8</sup> CFU/ml) of each *Bacillus* sp. in the sterile trays 24h before showing in nursery bed. Seedlings were transplanted in to 30cm diameter pots each pots contain sterile soil. The different treatments with *Bacillus* isolates were applied as outlined in the experimental design using seven trials where each trial composed with ten replicates and a negative control with no pathogens. Plants watered daily in the net house at for 8 weeks. At the end of 8 weeks, 10 plants (one plant of each replicates) were harvested to assess the effect of the various *Bacillus* isolates on the different vegetative growth parameters. Significant difference was assessed from the mean of each of the different treatments. The experiment was repeated twice. The growth parameters assessed include: length of shoot and root length, biomass and randomly selected seedlings were used to determine each parameter per treatment. Chlorophyll content was also measured with a chlorophyll meter (SPAD meter, USA)



**Fig. 3.** Inhibitory effect of the six bacillus isolates against *S. rolfsii* in vitro. Percent of inhibition of *Bacillus* against pathogen, while SR is the growth of *Schelortium rolfsi* alone in the Petri dish. Each. value is average of ten replicates. For each bar value are followed by a different lower case letters indicates LSD at p<0.05

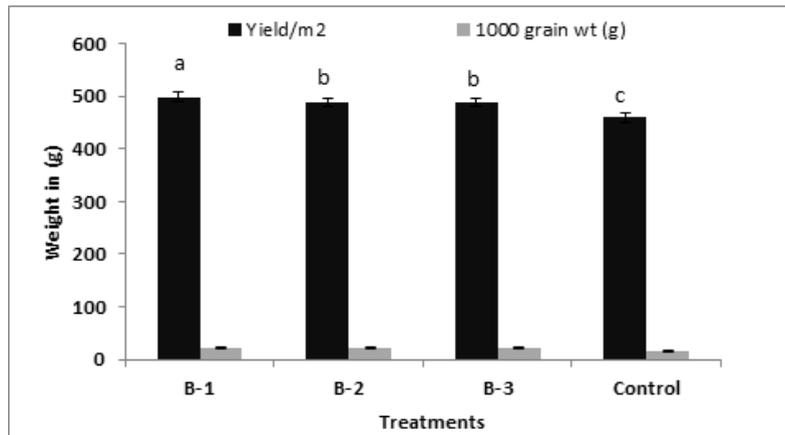


Fig. 4.

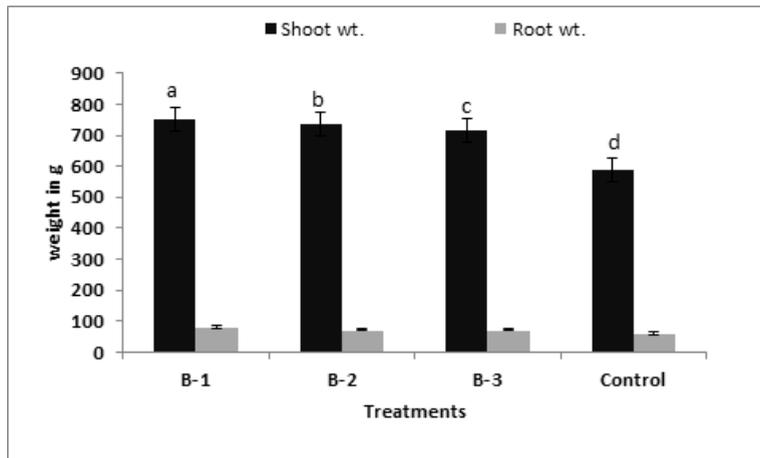


Fig. 5.

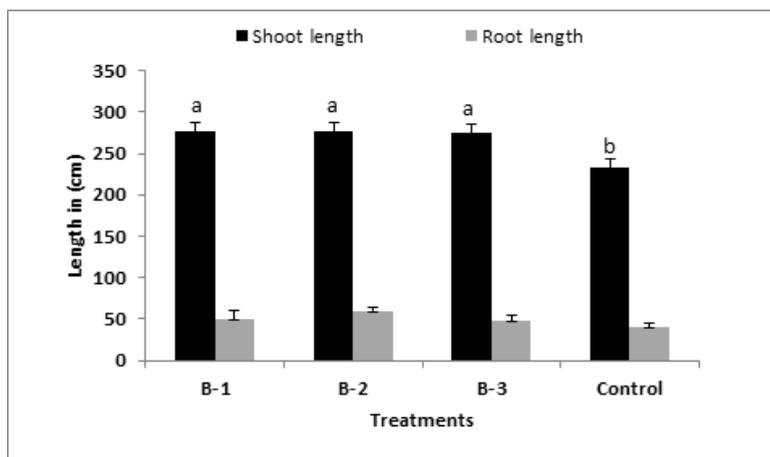


Fig. 6.

Fig. 4, 5 & 6. Plant growth promotion effect on rice of Different *Bacillus* sp. under net house trials. For each bar value are followed by a different lower case letters indicates LSD at  $p < 0.05$

**Statistical analysis**

All experiments were performed twice. Analysis of Variance and Duncan’s multiple range test (DMRT) was employed to test for significant differences between treatments at p = 0.05 (Gomez and Gomez, 1984).

**RESULTS**

**Isolation of *Bacillus* sp.**

All the isolates (six isolates) showed as gram positive, rod shaped, endospore former with white dry and fold, opaque and irregular edge colonies on nutrient agar medium. All of them are fast grower and found positive for catalase,

oxidase, beta galactosidase (ONPG) activity glucose, manitol, sucrose, arbinose, trehalose utilization, acetoin production (Voges proskauers test), nitrate reduction and all were found negative for H<sub>2</sub>S production, Arginine utilization and phenyl alanine deaminase (Table-3).

**Antagonistic effect of *Bacillus* spp. and mechanisms**

Study reveals that the six isolates of *Bacillus* sp. inhibited the growth of *R. solani*, *S. rolfsii* and *S. oryzae* significantly. The results obtained were presented in the Plate-1 (Fig-1, 2, 3). The results obtained showed that all six bacilli spp showed that inhibited the growth of these pathogens most by 90%. The mechanism of antagonism of

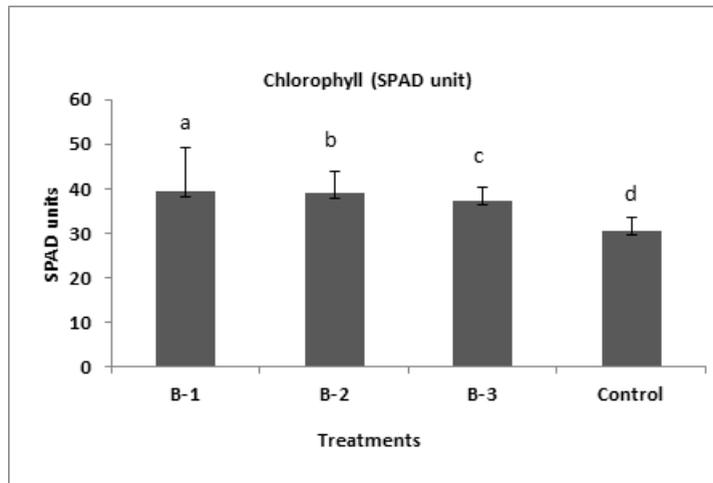


Fig. 7. chlorophyll estimation of leaf samples after treated with *Bacillus* sp. and un-inoculated control. For each bar value are followed by a different lower case letters indicates LSD at p<0.05

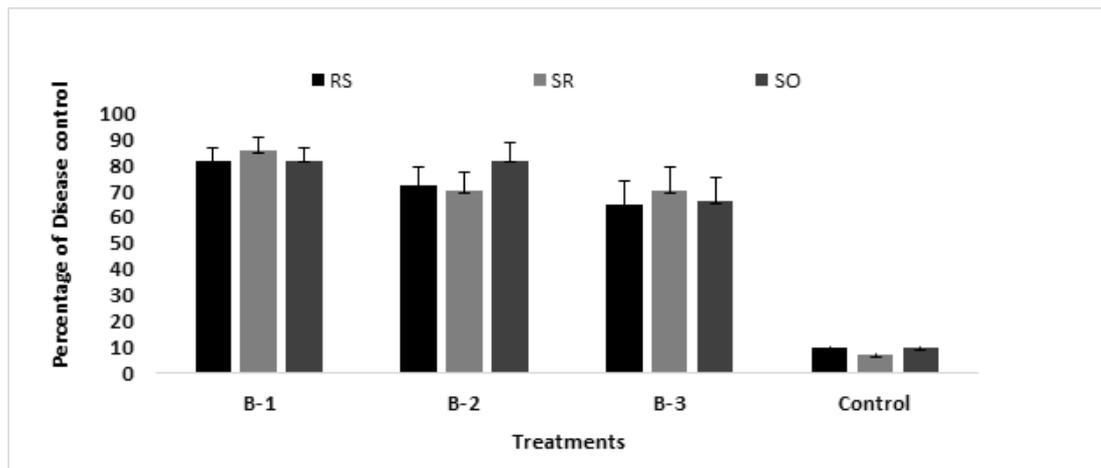


Fig. 8. *Bacillus* sp. treated response to diseases control against pathogens, while RS, SR and SO represents the Rhizoctonia solani, Schelortium rolfsi, Schelortium oryzae. Each value is average of ten replicates

the six *Bacillus* isolates was investigated and the mechanism results are presented in table-1.

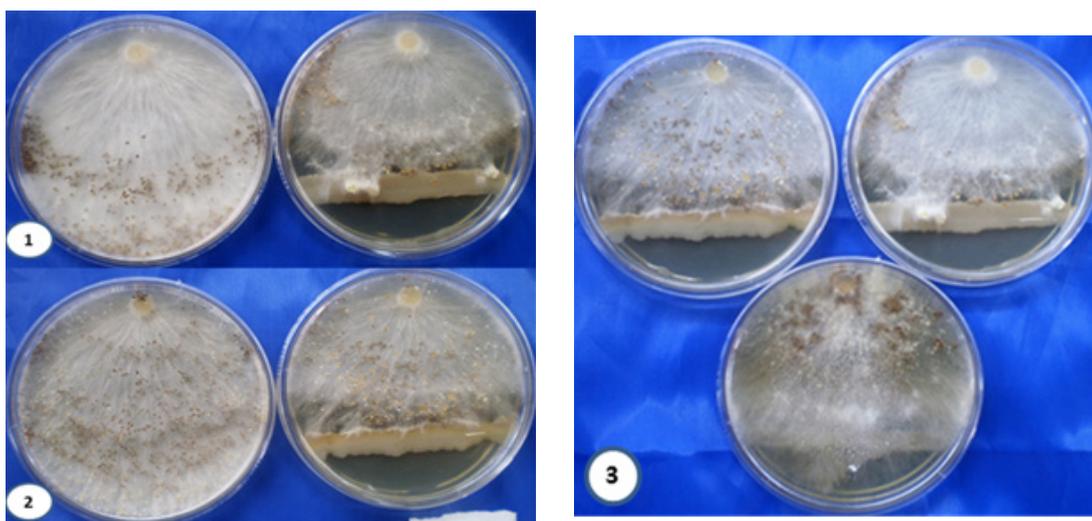
**Phosphate solubilisation, HCN and IAA production**

*Bacillus* isolates were examined for their ability to solubilize phosphate as an indirect mechanism of biocontrol by modifying the environmental condition. In order to examine the *Bacillus* isolates for their ability to solubilize phosphate, a standard agar medium; (pH 6-7) containing 5 g of tricalcium phosphate (TCP) as a sole source of phosphorus was prepared and used. All *Bacillus* isolates formed clear halos around on pikovskayas medium having bromothymol blue changed its colour from blue to yellow due to

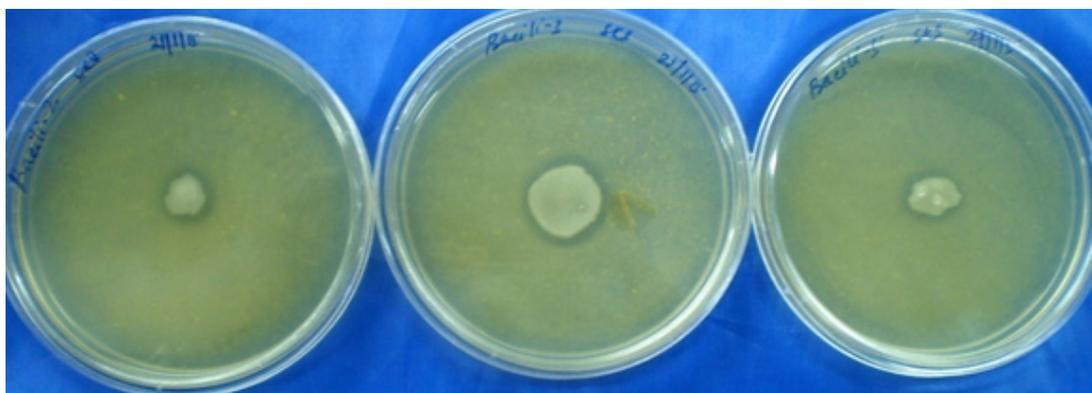
increase in pH of the growth medium. In case of IAA production test, all the six isolates of *Bacillus* showed IAA production. Development of pink colour with and without addition of tryptophan in culture broth was also observed in to their cell free supernatant using spectrophotometer absorbance at 530 nm. Maximum IAA production was recorded in B-1, 2 and 3. It was found that none of the *Bacillus* strains found produces HCN. (Table-1)

**Cell wall degrading enzymes (chitinase and protease)**

All *Bacillus* strains showed chitinase and protease activities. Chitinase activity was confirmed due to the formation of purple colored zone. Colour intensity and diameter of the purple



**Plate 1.** Dual culture assay of *Bacillus* sp. isolated from rice rhizosphere against rice plant pathogen *S. oryzae* (1) *S. rolfsii* (2) (3) *R. solani* for growth inhibition assays



**Plate 2.** (Fig-1): Protease activities of *Bacillus* sp. showed clearance zone after 48 hr inoculation



Note that the non-inoculated control resulted in retarded shoot and root growth compared with the treatments with *Bacillus* isolates

**Fig. 9.** Growth promotion of rice plant in the pot culture experiment under net house by inoculation of different *Bacillus* isolates (B-1, B-2 and B-3 is of *Bacillus* sp. isolates and Control-non-inoculated)

colored zone were taken as the criteria to determine the chitinase activity after three to seven days of incubation. All *Bacillus* sp. which showed the protease activity and zone confirmed the production of protease enzyme (Plate-2 Fig-1). The degree of protease activities was measured based on the diameter of clearance zone.

**Efficacy of *Bacillus* sp. on pathogen control, vegetative growth and yield of rice crop**

As a result of the *in vitro* screening performances of the six isolates in antagonising the growth of these three pathogens. Out of the six isolates 3 strains were screening and studied the efficacy in the net house test on rice using RBD (randomized block design) as follows:

Rice planted without any treatment (control); Rice planted with different *Bacillus* sp.; Rice planted with each of *R. Solani*, *S. rolfsii*, *S. Oryzae*; Rice planted with both *Bacillus* and each of *R. Solani*, *S. rolfsii*, *S. Oryzae*.

**Response to Disease control using *Bacillus* Sp. as treatments**

Disease control caused by these pathogens was achieved using *Bacillus* sp. in the net house trial with pot experiments. The treatment with highest disease control activity was significant. The

**Table 1.** Plant growth promoting attributes and Mechanism of pathogen Inhibition of *Bacillus* sp. isolated from rice rhizosphere

<i>Bacillus</i> strains	Phosphate solubilization	HCN production	Tryptophan Utilization (IAA Production)	Protease activity	Chitinase activity
B-1	+	-	+	+	+
B-2	+	-	+	+	+
B-3	+	-	+	+	+
B-4	+	-	+	+	+
B-5	+	-	+	+	+
B-6	+	-	+	+	+
B-7	+	-	+	+	+
B-8	+	-	+	+	+
B-9	+	-	+	+	+
B-10	+	-	+	+	+
B-11	+	-	+	+	+
B-12	+	-	+	+	+
B-13	+	-	+	+	+
B-14	+	-	+	+	+
B-15	+	-	+	+	+
B-16	+	-	+	+	+
B-17	+	-	+	+	+

isolate B-1 was found with 82, 86 and 82% disease control against *R. Solani*, *S. rolfsii*, *S. Oryzae* and BAC-2&3 was showed 72, 70, 82% and 65, 70 66% against *R. Solani*, *S. rolfsii*, *S. Oryzae* in. This result was quite significantly different from the control which was having no disease control.

#### Response to plant growth parameters using *Bacillus* sp. as treatments

All resulted in a significant increase in vegetative growth like length and weight of root and shoot, yield, 1000 grain weight over than plants not infected with pathogens but treated with bacillus sp. (Fig: 4-6). Among the treatments, isolate B-1 displayed the highest growth promoting activity in all of the parameters evaluated B-1 treated plants showed significant increase in shoot and root biomass respectively (Fig-5). Chlorophyll content was measured active tillering and panicle initiation stage and found the highest in *Bacillus*

treated plants with a significant difference from the non-inoculated control. There was no significant variation in the chlorophyll content of rice plants among the bacterial treatments (Fig-7) however, non-inoculated (control) plants were found less chlorophyll (Fig-7).

## DISCUSSION

The application of *Bacillus* sp. which was already reported that act as PGPR (plant growth promoting rhizobacteria) is a potentially attractive approach to disease management and improved crop productivity in sustainable agriculture (Kloepper *et al.* 2004; Lamsal *et al.* 2013).

It has been well reported that the isolation of bacillus sp. from different cultivated lands cultivating different crops including cereals and vegetables having their plant growth promoting activities (IAA, Siderophore production, Phosphate solubilization) and biocontrol efficiency such as chitinase, protease, ACC deaminase, oxalate oxidase has been well reported (Kumar *et al.* 2012; Mehta *et al.* 2010; Singh *et al.* 2008; Chung *et al.* 2008). The results showed that all six strains isolated from rice rhizosphere showed good plant growth promoting activities as well as better antagonistic effects against three pathogens like *R. solani*, *S.rolfsii* and *S. oryzae* respectively.

*Bacillus* strains were identified based on morphological, phenotypic characters and

**Table 2 & 3.** Morphological and biochemical characters of *Bacillus* sp. isolated from rice rhizosphere

Colony shape	Round
Color	White
Appearance	Rough
Margin	lobate
H <sub>2</sub> S production	Negative
Phenyl alanine deaminase	Negative
Gram reaction	+ ve

**Table 3.** Different Carbon Utilization Test (Hi carbokit)

Test	Original colour of the medium	Colour of the medium change	Reaction
Malonate	Bluish green	No change	negative
Voges proskauer's	Light yellow	Pinkish red	positive
Citrate utilizing	Light green	Dark blue	positive
ONPG (Detects Beta galactosidase)	Colourless	Yellow	positive
Nitrate Reduction	Light yellow	Pinkish red	positive
Catalase activity	Colourless	Effervescence coming out from the loop	positive
Arginine utilization	Light purple	No colour change	negative
Sucrose utilization	Red	Yellow	positive
Mannitol utilization	Red	Yellow	Positive
Glucose utilization	Red	Yellow	Positive
Arabinose utilization	Red	Yellow	Positive
Trehalose utilization	Red	Yellow	Positive
Maltose utilization	Red	Yellow	Positive

biochemical parameters as per Bergys manual of systematic Bacteriology. It was grown and maintained on NA medium at 30°C. The isolate upon gram staining was gram positive, rod shaped, spore forming, motile and biochemical feature such as starch hydrolysis, utilization of phosphates and production of cell wall specific extracellular enzymes (such as chitinase, protease). In vitro inhibition of various phytopathogens by *Bacillus* sp. has also been reported (Chung *et al.* 2008; Ashwini and Srividya 2012). In vitro IAA production by *Bacillus* spp. in significant amount has also been reported by (Mehta *et al.* 2010; Singh *et al.* 2008). Production of IAA with or without tryptophan by all *Bacillus* isolates is similar to above finding.

*Bacillus* sp. produced a significant amount of ACC deaminase. It has been reported earlier that rhizospheric bacteria producing  $\alpha$ -ketobutyrate show plant growth promoting activities (Penrose and Glick 2003). Some of the isolates produced mycolytic enzymes viz., chitinase which probably degrade the components of fungal cell wall such as chitin, There are many reports on the production of lytic enzymes by microorganisms (Gupta *et al.* 2006; Huang and Chen 2004).

The action of lytic enzymes BPR7 was quite effective on all six test pathogens. Therefore, it is likely that cell wall lysis would have been due to concerted action of chitinase (Chaiharn *et al.* 2008). Positive colour change of filter paper to reddish brown indicated the production of HCN. None of the six *Bacillus* isolates produced HCN which is similar to in whose research the *Bacillus* isolate were all negative for HCN (Singh *et al.* 2008). Cyanide is a toxic and dreaded chemical produced by many rhizobacteria. Some bacteria synthesize it, others excrete it and yet others metabolize it in other to avoid predation and competition (Zeller *et al.* 2007).

Formation of inhibition zones due to reduction in radial growth of target phytopathogens by *Bacillus* sp. may be due to the production of antimicrobial substances such as chitinolytic enzymes, HCN, antibiotics, siderophore and nutrient competition (Singh *et al.* 2008; Glick 1995; Chung *et al.* 2008). However, role of other inhibitory metabolites such as toxins and proteolytic enzymes in the inhibition process of fungal pathogens may not be ruled out (Hu *et al.*

2008). It may be concluded that *Bacillus* sp. B-1, 2 & 3 being as a good PGPR and a potential biocontrol agents, may be used as potential bioinoculants for rice. Further study on field experiments related to growth promotion, biocontrol and induced systemic resistance to pathogens is in progress.

It was also found that use of microorganisms to control plant diseases offers a most alternative to the use of commercially available chemicals (Emmert and Handelsman 1999; Shoda 2000; Bais *et al.* 2004). The presence of the most potential strains of microorganism in the soil and associated with plant roots which may suppress plant pathogens without producing lasting effects on the rest of the soil microbial and plant communities (Osburn *et al.*, 1995; Howarth, 1991). In accordance, the diversity of microbial communities provides a rich source of potential biocontrol agents. The method of specific isolation, characterization and their use as biocontrol agent described here, it should be possible to isolate and characterization of new *Bacillus* sps are effective in biocontrol of microbial pathogens.

## CONCLUSIONS

Different sp. of *Bacillus* were isolated from Odisha (as indigenus) from different cropping habitats were screened and compare for the antifungal activities against different soil borne pathogens like *R. Solani*, *S. Rolfsii* and *S. oryzae* of rice. Their plant growth promotion activities like IAA production, phosphate solubilisation ability, plant growth parameters like vegetative growth and yield were tested. Mechanism of antifungal activities against pathogens was also studied through Chitinase, protease activity and dual culture assays against pathogens in terms of growth inhibition of pathogens. All the strains showed positive in IAA and phosphate solubilising efficiency, biochemical characterization was showed and from above it was confirmed that all the isolates were *Bacillus subtilis*.

All the strains were showed the positive against pathogens, Strains B-1, B-2, B-3 showed strong inhibitory potential on the mycelia growth of the studied fungi and showed 90% inhibit the growth of pathogens and also showed significant in vegetative growth and yield of crops then control. The antifungal activity and IAA production and

phosphate solubilising efficiency of the indigenous isolates B-1, B-2, B-3 was further selected for plant growth promotion assays. We consider the selected indigenous bacterial strains B-1, B-2, B-3 isolated from Odisha soil are feasible to be used for development of potential biocontrol inoculants for these three pathogens may also use for potential biocontrol agent for soil borne pathogens.

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