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BIOCONTROL, PLANT GROWTH PROMOTION AND CONFERRING STRESS TOLERANCE: MULTIFACETED ROLE OF *BACILLUS LICHENIFORMIS* 9555

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ABSTRACT

In the present study a bacterial strain, *Bacillus licheniformis* 9555 was isolated from the rhizopheric soil collected from the banks of Hindon river, India. The river is heavily contaminated by toxic pollutants discharged from agricultural fields and industries. *B. licheniformis* 9555, served as a biocontrol agent and plant growth promoter. The bacteria also showed efficient production of exopolysaccharides (EPS) and siderophore. The treated plants showed increased chlorophyll content as compared to control. The bacteria was able to alleviate environmental stresses in plants as witnessed by reduced levels of stress related compounds like phenol, tannins and proline in *Zea mays*. The ability of the bacteria to confer stress resistance and promote growth may be the contributing factor that helps plants to grow on polluted banks of Hindon.

KEYWORDS: Keywords: Bacteria, siderophore, PGPR, chlorophyll.

INTRODUCTION

Microbes are omnipresent and can survive in a wide range of environmental conditions and also provide a helping hand to other organisms (plants) to adapt to the stress in an environment in which they establish. Plant Growth Promoting Rhizobacteria (PGPR) are microbes specially designed by nature that harbor growth promotional benefits for host plant. Plant growth promotion and biocontrol action is widely studied in *Bacillus* genera, a common inhabitant of rhizopshere (Wahyudi *et al*., 2011). Colonization of roots by PGPR is the seed to successful plant-microbe interaction. In certain associations of microbes with plants, exopolysaccharides (EPS) have a major role that help bacteria to inhabit the root surface through specific adhesion, leading to root colonization that eventually results in biofilm formation (Michiels *et al*., 1991; Matthysse *et al*., 2005; Ramey *et al*., 2004).

An investigation by the National Institute of Hydrology, Roorke, India confirmed that the concentrations of toxic chemicals in Hindon river, Uttar Pradesh, India, discharged from agricultural fields and industries were very high than the maximum permissible limits (http://www.indiawaterportal.org). The microbes surviving in such toxic levels will have special adaptability qualities to resist or utilize the toxic substances and at the same time help host plant to adapt to the toxic environment. This present study aims to isolate rhizospheric bacteria from Hindon river bank and to analyze its biocontrol activity, plant growth promotion property and ability to confer stress tolerance to host plants.

MATERIALS AND METHODS

Bacterial strain and growth conditions

Microbes were isolated from rhizopshere soil as per the protocol described by Lu and Huang (2010). The potential bacterial isolate was deposited in Microbial type culture collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India and it was identified based on morphological, physiological and biochemical characteristics.

Analyzing bacterial isolates for Antifungal property

The agar disc method (Hinton and Bacon, 1995) was used to evaluate *in vitro* antifungal activity on PDA (Potato Dextrose Agar). The phytopathogenic fungus, *Fusarium monoliforme* (MTCC 1848) was authenticated by Microbial Type Culture Collection (MTCC). The cultures of *Fusarium oxysporum, Sclerotinia sclerotiorum*, *Alternaria solani* were procured from Department of Plant-microbe interaction, National Botanical Research Institute, Lucknow, India.

Plant growth promotion

Sterile and non-sterile agricultural field soil was used in different experimental setups. Bacterial inoculums of 9 $\log_{10}$ CFU/ml was thoroughly mixed with soil and sterilized seeds of maize were sown after 24 h. The glass house was maintained at 28 ± 2°C, 16 h light/8-hrs dark with fluorescent light intensity 1000 Lux and relative humidity 70%. Plants were irrigated with tap water on alternate days to maintain about 30% soil moisture. After a period of 30 days plant parameters were recorded and chlorophyll content was evaluated using the protocol described by Arnon (1949).

B. licheniformis 9555 was analyzed for phosphate solubilization (Mehta and Nautiyal 2001), auxin synthesis (Patten and Glick 1996) siderophore production (Bano and Musarrat 2003). EPS production was quantified from 72 h old culture grown in nutrient broth using phenol-sulphuric acid method (Titus *et al.,* 1995).

Analysis of biocontrol efficiency in protecting plants

One set of cups with sterile soil was inoculated with *B. licheniformis* 9555 and two other sets served as control, one set was for uninoculated sterile soil and the other for *Fusarium moniliforme* treatment alone. Four seedlings per cup were planted and for *B. licheniformis* treatment the seeds were soaked in small volume of bacterial culture prior to planting. After 24 h *F. moniliforme* spore suspension with an inoculums size of 3 x10^6 conidia /ml was used for spraying on seedling. The uninoculated sterile soil control set was left unsprayed. Germination percentages were recorded and plant parameters, chlorophyll content were analyzed after 30 days.

Estimation of Tannin, Phenol and Proline

Tannin and phenol estimation were conducted based on the protocol by Bray and Thorpe (1954). The approximate quantity of phenols and tannins were determined by using standard curves of gallic acid and tannic acid respectively. Proline content was determined by the method of Bate *et al.*(1973).

Statistical analysis

The data procured was subjected to statistical analysis by least significant difference (LSD) described by Fisher and Yates (1963) . The differences were considered significant at $P \leq 0.01$and 0.05 levels.

RESULTS AND DISCUSSION

Screening and identification of biocontrol agent

The selection of potential biocontrol agent involved screening of over 100 bacterial isolates from rhizosphere soil. One isolate designated NB2 showed effective antifungal action against all the tested phytopathogenic fungi (Table I). NB2 was gram positive, catalase positive, motile, non-endospore forming, rod shaped and aerobic in nature. The bacterium was identified as *Bacillus licheniformis* based on morphological, physiological and biochemical characteristics by MTCC, with accession number MTCC 9555.

TABLE I: Zone of inhibition by *B. licheniformis* 9555.

Phytopathogenic fungi	Zone of inhibition (mm)
Fusarium monoliforme	10
Fusarium oxysporum	10
Alternaria solani	12
Sclerotinia sclerotiorum	9

TABLE II: Plant growth promotion of *Zea mays* by *B. licheniformis* 9555 after 30 days in sterile soil and non-sterile soil.

Parameters	Control	*B.licheniformis* 9555 treatment	LSD at 1%	Control	*B .licheniformis* 9555 treatment	LSD at 1%
	Sterile soil			Non-sterile soil		
Shoot length (cm)	29.18±1.19	37.38±0.80	2.661	30.55±1.18	37.23±0.97	2.862
Root length (cm)	17.14±0.73	19.66±0.59	1.759	18.58±0.46	20.64±0.68	1.525
Leaf length (cm)	10.98±0.40	13.22±0.53	1.239	11.48±0.31	13.89±0.36	0.899
Lateral roots	9.20±0.33	12.92±0.38	1.207	9.03±0.38	11.35±0.44	1.086
Stem dry weight(g)	0.093±0.009	0.147±0.002	0.0038	0.094±0.004	0.141±0.003	0.010
Root dry weight (g)	0.0622±0.001	0.075±0.002	0.0047	0.065±0.002	0.075±0.002	0.0058

Data was calculated for each parameter as mean ± standard error (n = 60).

Plant growth promotion

The plant growth promotion property of the bacteria was accomplished in both sterile and non-sterile soil setup. Stem dry weight was increased by 36.73% and 33.33% over their corresponding controls in sterile and non-sterile soil respectively (Table II). A significant increase of root dry weight biomass was also observed in both soil setup. The non-sterile agricultural field soil experimental setup gave an idea of possible rhizosphere competence achieved by the microbe in establishing successful plant association and survival over indigenous microflora.

B. licheniformis 9555 showed effective siderophore production. Rhizospheric bacterial isolate, *B. megaterium* produced siderophore that served dual function of efficient plant growth promotion and disease resistance to host plant (Chakraborty *et al.,* 2006). *B. cereus* UW 85 isolated from soil produced siderophore that were efficient growth promoters of crop plants (Husen 2003). Thus the plant growth promoting efficiency and biocontrol property of *B. licheniformis* 9555 can be equated to the microbe's siderophore production capability, but at the same time other mechanism should still be explored. *B. licheniformis* 9555 treated plants showed significant increase in chlorophyll content when compared to the untreated control plant (Table III).

Extracellular polysaccharide (EPS) production by *B. licheniformis* 9555 was assayed to be 746 µg/ml, after 72 h of incubation. While in related studies EPS production of *B. cereus* was 498.04 µg/ml (Bragadeeswaran *et al*., 2011) and for *B. subtilis* NCIM 2063 it was 206 µg/ml (Francis *et al.,* 2009). In another study the biofilm formation of *Bacillus subtilis* was correlated to its biocontrol efficiency (Bais *et al*., 2004). Efficient production of EPS by *B. licheniformis* 9555 may have a significant role in plant- microbe interaction, biocontrol and tolerance against environmental stress caused by toxic pollutants.

TABLE III. Estimation of chlorophyll, tannin, phenol and proline content after 30 days.

Parameter	Controls		Treatment with B.licheniformis 9555		LSD at 1%
	Plant alone	*F.monoliforme* Treated	Plant	Plant + *F.monoliforme*	
Total chlorophyll(mg/g)	22.33±0.450	15.83±0.372	45.11±0.264	42.37±0.303	1.079
Chlorophyll a (mg/g)	19.38±0.380	14.31±0.521	35.81±0.156	33.75±0.375	1.113
Chlorophyll b(mg/g)	2.866±0.127	1.67±0.064	9.09±0.209	8.63±0.367	0.591
Phenol (µg/g)	207.59±2.17	215.43±2.41	72.17±2.257	113.31±1.680	6.62
Tannic acid (µg/g)	178.45±1.48	185.39±1.69	54.55±2.418	89.34±2.857	6.55
Proline (µm/g)	59.52±2.71	60.28±2.15	21.57±1.209	42.48±1.575	5.94

Data was calculated for each parameter as mean ± standard error (n = 12).

TABLE IV: Biocontrol efficiency of *B.licheniformis* 9555 in protecting plants against *F. monoliforme*

Parameters	Controls		F. monoliforme +B.licheniformis 9555 Treated	LSD at 1%
	F. monoliforme Treated	Plant alone		
Shoot length (cm)	10.86±1.94	29.52±1.30	29.71±0.72	3.53
Root length (cm)	8.18±1.31	17.07±0.78	19.30±0.33	2.15
Leaf length (cm)	7.95±0.95	11.21±0.45	13.02±0.25	1.78
Lateral roots	6.09±0.94	9.42±0.34	11.80±0.46	1.56
Stem dry weight(g)	0.053±0.009	0.088±0.001	0.099±0.007	0.016
Root dry weight (g)	0.040±0.006	0.0610±0.009	0.0688±0.008	0.0072

Data was calculated for each parameter as mean ± standard error (n = 54).

Biocontrol efficiency in protecting plants

In the control set treated with *F. moniliforme* 55% of seeds germinated, while the dual treated (*F. moniliforme* + *B. licheniformis* 9555) and uninoculated control recorded 100% germination. Shoot length, stem dry weight and root dry weight of the dual treated plant showed a significant increase of 63.44%, 46.46% and 41.17% respectively, above the fungus treated control plant (Table IV).Siderphore production is an efficient biocontrol mechanism employed by PGPRs. Study by Wahyudi *et al.* (2011) reported 12 *Bacillus* sp from the rhizosphere of soya bean plants that showed effective antifungal action through siderphore production. Significant increase in chlorophyll content was also observed in dual treated plants when correlated to control groups (Table III).

Estimation of Phenol, Tannin and Proline

B. licheniformis 9555 treated plant had the least content of stress response compounds while the *F. monoliforme* treated plant showed the highest accumulation. The dual treated (*F. monoliforme* + *B. licheniformis* 9555) plant showed 47.40% decrease in phenol content when set against the *F. moniliforme* treated control (Table III). A statistically evident decrease in tannic acid and proline content was noted in the *B. licheniformis* 9555 treated and dual treated plants when contrasted against both set of control plants (Table III). In general stress related compounds accumulate on treatment with PGPRs and functions in plant defense mechanism (Singh *et al*., 2002; Jain *et al*., 2012). *B. licheniformis* 9555 treatment did not induce production of stress related compounds and at the same time have reduced the stress caused by the green house environment on the plant. The biocontrol efficiency of *B. licheniformis* 9555 has protected the plant from phytopathogenic infection thereby resulting in low accumulation of stress related compounds. The mechanism by which *B. licheniformis* 9555 confers environmental stress tolerance to plant might be one of the factors for survival by plants in highly polluted regions like Hindon river.

CONCLUSION

B.licheniformis 9555 proved to be an efficient biocontrol agent as well as good plant growth promoter. Siderophore production by bacteria can be stated as the reason behind this bifold property of the bacteria. *B.licheniformis* 9555 is a microbe that has evolved in a stressful environment with toxic chemicals and this survival ability was conferred by associated plants in reducing environmental stress as manifested from reduced levels of stress related compound accumulation in treated *Zea mays*. The bacteria seem to have adapted a mechanism that reduces production of plant stress related defense compounds, but at the same time confers environmental stress tolerance to plants.

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