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Bacillus sp. BCLRB2: An efficient diazotrophic Halotolerant PGPB strain

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Abstract.
Based on a finding made by farmers who noticed a good growth and a reduced incidence of phytopathogenic infections of wheat grown between the rows of olive trees, we have screened diazotrophic endophytic PGPB associated with olive tree for plant stress tolerance improving capability. Strains were selected following a biochemical characterization of plant growth promotion activities such as ability of antimicrobial production, azote fixation,…
Among the selected strains, BCLRB2 was the strain that shown the most efficient capacity to fix atmospheric nitrogen, which is the most prominent factor of all plant growth parameters under stressful environments. The strain BCLRB2, identified as *Bacillus* sp, had ACC deaminase, and highly stimulatory effect *in vitro* associated with high production of hydrolytic enzymes, AIA, and solubilization of tricalcium phosphate. The efficiency of BCLRB2 strain was explored for *in vivo* pot plant growth. As a result, inoculated plants with *Bacillus sp*. BCLRB2, showed the best growth of durum wheat seedlings compared to a control under salt stress and natural conditions. Total length, fresh weight, and total dry weight were significantly higher in inoculated plants compared to uninoculated ones.

Keywords: PGPB, Salt stress, durum wheat, diazotrophic, Halotolerant.

Introduction

Novel agricultural technologies are required to improve food production in saline and dry soils. Many scientists have attempted to develop salt-tolerant crops through breeding, but these efforts have met with limited success due to the genetically and physiologically complexity of the salt tolerance trait (1,2).Promising measures for improving plant health in salinated soils are the use of microbial inoculants, which can ameliorate salt stress, promote plant growth (3), and control diseases (4,5,6,and 7). Despite their different ecological niches, diazotrophic free-living rhizobacteria and endophytic bacteria use some of the same mechanisms to promote plant growth and control phytopathogens (8, 9, and 10).

Results and Discussion

Potential PGP of selected strain

The potential PGPB mechanisms of the selected strains were evaluated *in vitro* based on growth on nitrogen-free medium, antimicrobial activity, lytic enzymes production, phosphate solubilization, and phytohormone (IAA) production. One selected bacteria (BCLRB2) shown a highly capacity of growth on nitrogen-free medium. The biofilm production after culture without agitation is the first character of nitrogen fixation capacity of bacteria. the selected isolate BCLRB2 has the high ability to develop a biofilm. Among selected isolates, BCLRB2 was able to produce high levels of auxin with high phosphate solubilizing ability on liquid and agar medium. BCLRB2 produce lytic enzymes, and had a highly inhibition effect against many phytopathogenic *Fusarium*. The potential of biocontrol-PGPB activity was evaluated based on *in vitro* antifungal, chitinase and protease activity, and results are summarized in Table 1. *Bacillus sp.* BCLRB2 inhibited *Fusarium* and *Sclerotinia* grown in agar plate compared with control treatment and, together with BCLRB2, showed additionally protease, beta-glucanase, and chitinase activity

In vivo pot experiment:

We expected that the growth effect on crops such as wheat and maize could be increased through the enhancement of IAA by the BCLRB2 treatment. Pot study results showed that plant grown under saline conditions had significant growth retardation. Under saline conditions total length of the plant was reduced by 42% and fresh biomass was reduced by 43%. Both shoot growth and root growth were reduced by increasing salinity. However, BCLRB2 reduced wheat sensitivity to salt (figure 3)

Table 1: PGP characteristics of *Bacillus* sp. BCLRB2.

Strain	N2 fixation	IAA	ACC deaminase	Ph. soluble	HCN	chitinase	Protease	strong antimicrobial against phytopathogens
B sp. BCLRB2	++	++	+++	++	-	+	+++	*Fusarium graminarum;F. oxysporum Sclerotinia*

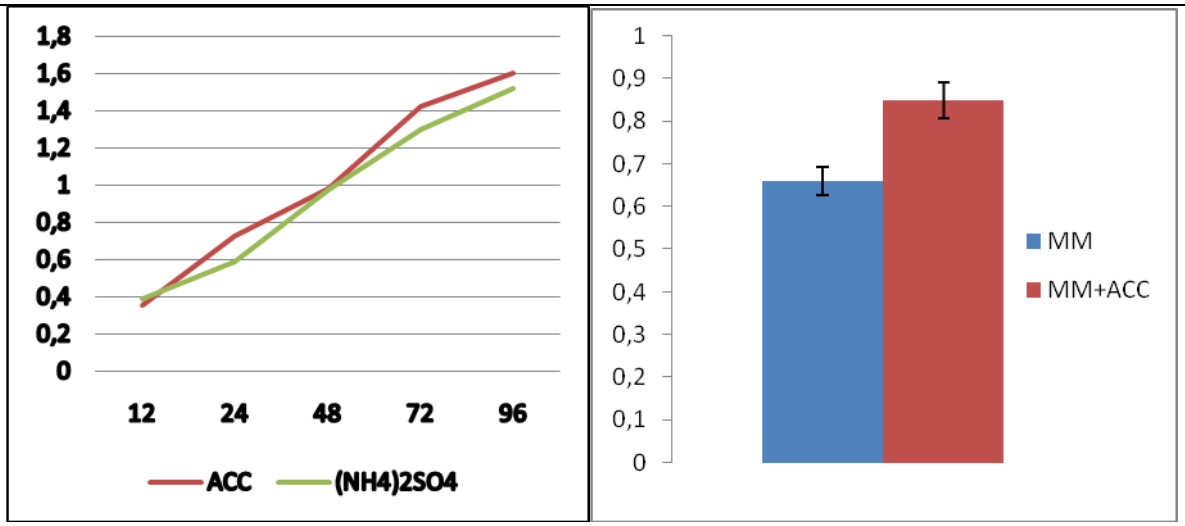

Fig.1 : (1) Expression fo ACC deaminase activity under minimal medium containing ACC and $(NH_4)_2SO_4$ and (2) ability of growth of Bacillus sp. BCLRB2 under minimal medium with and without ACC .

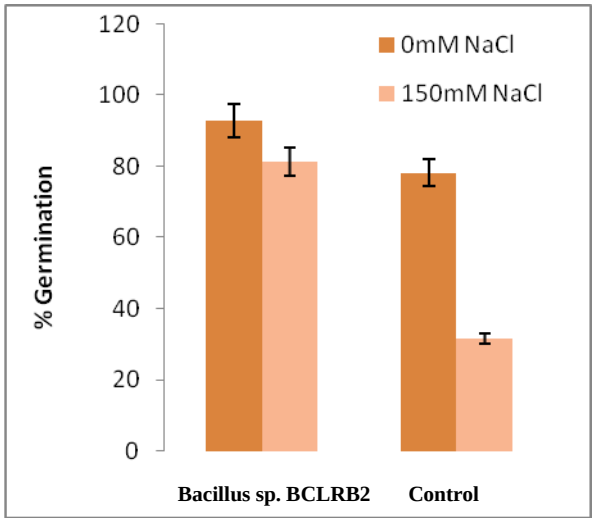

Fig. 2: Effect of germination rate after inoculation seed of durum wheat with Bacillus sp. BCLRB2 under 0 and 150 mM NaCl

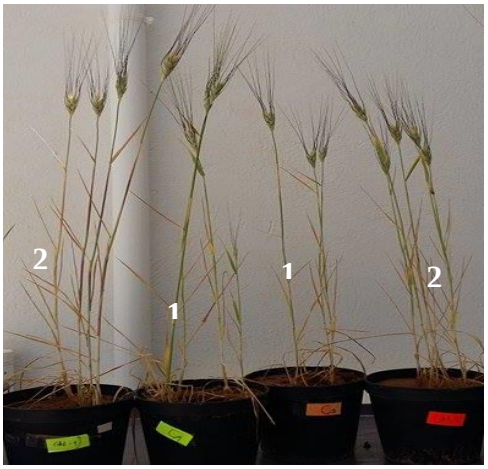

Fig. 3: Five month-old durum wheat seedlings grown in agricultural soil in the presence of PGPB strain Bacillus sp. BCLRB2 without NaCl (Yellow) and with 150 mM NaCl (Red).**1**: uninoculated plant; **2**: inoculated plant.

Materials and Methods

Diazotrophic potential of the isolates

Bacterial isolates were examined for their nitrogen-fixation (diazotrophic) potential first by testing their growth on the liquid and solid mineral nitrogen free medium removing NaNO3 with oil as a sole source of carbon and energy. Isolates were then tested for nitrogenase, using the method of Quantification of N fixing capacity.

Quantification of N fixing capacity

The isolates which showed positive and predominant growth during the first 3 to 4 days were selected for quantification using the Kjeldahl N digestion and distillation system (Kelplus system, Classic Dx[VA]).The selected isolates were incubated in 10 ml of Jensen's broth in a rotary shaker at 150 rpm for 5 days at 28° C. From the quantification result, one potent N fixing isolates having the highest N fixing ability was selected for further characterization.

Utilization of ACC as sole nitrogen source:

The ability of bacterial strain BCLRB2 to use ACC as a nitrogen source was tested in 5 mL DF salt minimal medium (Penrose & Glick, 2003)(11) containing 30 µL of 0.5 M ACC.

The ACC-deaminase activity was stimulated by constant shaking of the bacterial culture for 24 h at 28±2^{o}C. ACC-deaminase activity was also checked at 200 mM salt concentration by growing bacterium in DF medium supplemented with ACC. The ability of a strain to utilize ACC was verified by maintaining the same strain in a control in the absence of any nitrogen source.

Plant Growth Promoting Attributes

Bacteria was checked for plant growth promotion (PGP) attributes including phosphate solubilization, indol acetic acid (IAA) production, HCN production, antimicrobial molecules production, chitinase and protease activities, under saline conditions.

In Vivo effect on PGP potentiel Activity

Experiment was conducted in small plastic pots (24 * 12 * 12 cm) under controlled conditions (temperature 22 ± 2°C, humidity 70–80 %) in greenhouse chamber. Wheat (*triticum durum*) seeds pre-disinfected were dipped for 10 min in a cell suspension of isolate SA21 (OD 610 = 0.1) and dried overnight in aseptic conditions. A germination essay was conducted before pot experiment under two conditions 0 and 150 mM NaCl. The experiment was conducted in sterilized salinated soil in similar sets of treatments as in planta study, taking five replicates. The effect of treatments was determined under negative control (uninoculated seeds received only irrigation solution), positive control (uninoculated in sterilized agricultural soil) conditions and inoculated seeds with strain SA21. Five plants from each set were taken randomly to determine plant weight, shoot and root length, shoot and root dry weight and shoot/root ratio.

Conclusion

Bacillus sp. BCLRB2 isolate had a multiple plant growth capabilities such as IAA production, phosphate solubilization and chitinase, protease production and antifungal activity. *Bacillus sp.* strain BCLRB2 showed most prominent PGP traits *in vitro.*

An in vivo pot experiment was performed to test the growth promotion of wheat by *Bacillus* sp. strain BCLRB2. It showed an improvement in total plant length by 31% and 43% increase in fresh biomass under 125 mM NaCl.

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