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Effect of rhizobacterial consortia from undisturbed arid- and agro-ecosystems on wheat growth under differing conditions

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Significance and Impact of Study:
Wheat seedling inoculated with rhizobacterial consortia obtained from an undisturbed Chilean
arid-ecosystems showed improved growth in phosphorus-poor and partly dry soil. Arid
ecosystems should be considered in further studies as an alternative source of microbial
inoculants for agro-ecosystems subjected to stressful conditions by low nutrients and/or adverse
climatic events.

Abstract

Plant-growth promoting rhizobacteria (PGPR) are studied as complements/alternatives to
chemical fertilization in agriculture. Poor information however, exists on the potential of PGPR
from undisturbed ecosystems. Here, we evaluated the plant growth-promoting (PGP) effect of
rhizobacterial consortia from undisturbed Chilean arid-ecosystems (Consortium C1) and agro-
ecosystems (Consortium C2) on plant biomass production. The PGP effects of C1 and C2 were
assayed in wheat seedlings (Triticum aestivum L.) grown in pots under growth chamber
conditions and in pots placed in an open greenhouse under natural conditions, using two different
Chilean Andisols (Piedras Negras and Freire series) kept either at 30% or 60% of their maximum
water holding capacity (MWHC). PGP effects depended on the soil type, MWHC and the growth
conditions tested. Although both consortia showed PGP effects in artificial soils relative to
controls in growth chambers, only C1 provoked a PGP effect at 60% MWHC in phosphorus-
poor soil of the ‘Piedras Negras’ series. At natural conditions, however, only C1 exhibited statistically significant PGP effects at 30% MWHC in ‘Piedras Negras’, yet and most importantly allowed to maintain similar plant biomass as at 60% MWHC. Our results support possible applications of rhizobacterial consortia from arid ecosystems to improve wheat growth in Chilean Andisols under water shortage conditions.

**Keywords:** arid environments, biofertilizers, cereals crops, microbial inoculants, rhizosphere.

**Introduction**

The plant microbiome harbors an innumerable wealth of bacterial taxa that promote the stress tolerance and growth of plants, suppress plant diseases, degrade xenobiotic compounds, or positively influence the crop yields (Berg *et al.* 2014; El Amrani *et al.* 2015). Soil bacteria providing benefits to plants are defined as plant growth-promoting rhizobacteria (PGPR) (Glick *et al.* 2015; Martínez *et al.* 2010). The PGPR have been found to occupy spaces otherwise available to phytopathogens or to act as biofertilizers and/or phytostimulators by increasing the nutrient (e.g., nitrogen, phosphorus (P), potassium) availability to plants. They are also able to synthesize compounds that affect metabolism and development of plants including phytohormones (e.g., auxin, cytokinins, gibberellins) and enzymes (e.g., phosphatases, phytases, 1-aminocyclopropane-1-carboxylate [ACCD] deaminase. As a consequence, PGPR have been raised as an attractive alternative to decrease our society’s use of chemical fertilizers and plant production products in agriculture and their possible environmental impacts. However, there is still a lack of consolidated information and evidence to establish the PGPR as cost effective and
environmentally friendly alternatives to ensure predictable crop yields and sustainable agriculture, particularly under climate change scenario.

Currently, PGPR are widely studied and proposed as microbial inoculant for improving the yields of agro-ecosystems (Glick et al. 2015; Martínez et al. 2010). However, most studies have been carried out with PGPR isolated from agro-ecosystems (e.g., pastures, crops, and forests), and relatively little is known of the functionality and efficiency of PGPR isolated from the rhizosphere of native plants grown in arid ecosystems. In this sense, some studies have also isolated PGPR from natural vegetation and assessed the contribution of PGPR to the growth and stress tolerance (drought and salinity induced stress) of their hosts including cucumber, tomato and pepper plants (Jorquera et al. 2012; Mayak et al. 2004; Timmusk et al. 2011). In Chile many ecosystems of highly diverse topography and climatic conditions can be found; the bacterial community structures and the occurrence of potential PGPR in the rhizospheres of native plants across arid ecosystems (Atacama, Patagonia and Antarctica deserts) has recently been reported (Jorquera et al. 2014; Jorquera et al. 2016). The abundance and activity of bacterial alkaline phosphomonoesterases (APases) in the rhizosphere of native plants from Chilean extreme environments have also been recently studied, suggesting their possible relevance in the organic phosphorus (Po) cycling in soils from extreme environments, and therefore in P nutrition of native plants (Acuña et al. 2016). Nonetheless, very little is known on the potential of PGPR from extreme environments as soil inoculants added to traditional crops under environmental stress conditions such as drought events and nutrient-poor soils by erosion or degradation of soils. We hypothesize that microbial inoculants derived from arid ecosystems, such as deserts, may contribute to plant tolerance against environmental stress conditions such as water shortage.
by drought events or nutrient-poor soils by erosion or degradation of soils. Here we hence evaluated the effect of PGPR consortia isolated from either arid- and agro-ecosystems on biomass production of wheat (*Triticum aestivum* L.) seedlings growing in Chilean Andisols, which are characterized by high contents of Po but low availability for plants. Bacterial consortia were based on previously isolated PGPR and their plant growth promoting effects studied in pot experiments under growth chamber conditions and in pot experiments placed in an open greenhouse under natural conditions.

**Results and Discussion**

In an attempt to study the PGP effect of rhizobacterial consortia from arid- (C1) and agro-ecosystems (C2), we quantified the biomass production of wheat seedlings growing in unsterile substrate (3:1 perlite:peat) and two different Chilean Andisols mixed with perlite (3:1 soil:perlite) under chamber (20°C, 60% humidity and 8:16 h light:dark cycle) and natural conditions for 30 days.

Figure 1 reveals the PGP effect of rhizobacterial C1 and C2 in wheat seedlings grown in unsterile substrate under growth chamber conditions. The results revealed a positive effect on plant biomass by consortia inoculation, with C2 exhibiting a statistically significant (*P* ≤ 0.05; Tukey’s test) higher biomass than the uninoculated control. The positive effect of the C2 is in good agreement with earlier observations with individual inoculation strain effect (Acuña *et al.* 2013, Jorquera *et al.* 2013; Martínez *et al.* 2015) and hence confirms the performance of C2 as possible biofertilizer in wheat crops. Interestingly, addition of inocula from arid ecosystems (C1) also resulted in higher biomass compared with uninoculated control, but not statistically significant. The biomass-promoting effect of the C2 might be related to their higher auxin
production and ACCD activity, as observed by Jorquera et al. (2014) and Parra et al. (2016) for isolates strains from agro-ecosystems compared with isolates strains from arid ecosystems. In addition, the strains of C1 were isolated from arid ecosystems with low nutrient availability (e.g., organic carbon and nitrogen) (Jorquera et al. 2014; Neilson et al. 2012) and, hence, might be rather adapted to lower metabolic activities than typically found with agro-ecosystems. The latter are periodically fertilized (e.g. composted with organic residues) or are subject to tillage.

In a further experiment, PGP effects of C1 and C2 were compared in a soil:substrate mixture under controlled conditions using two different Andisols. Figure 2 and 3 reveals clear differences between the two Andisol series analyzed. Wheat seedlings inoculated with consortium C1 showed a significant \( P \leq 0.05; \) Tukey’s test) higher biomass in Piedras Negras series in growth chamber experiments at 60% maximum water holding capacity (MWHC) as compared to uninoculated controls (Fig. 2a). In Freire series, the inoculation did not promote growth of the seedlings at any of the combinations tested (Fig. 2b). Varying PGP impact of PGPR consortia inoculated to different soils has also been observed by others (Krey et al. 2011; Ramírez and Kloeppe 2010).

Under natural conditions a higher plant biomass (5~12 g pot\(^{-1}\)) (Fig. 3) than in growth chamber experiments (0.5~2.5 g pot\(^{-1}\)) (Fig. 2) was obtained. The higher biomass production may have been induced by environmental factors such as improved exposure to sunlight relative to artificial light at growth chamber conditions. Surprisingly, at 60% MWHC in Piedras Negras series, PGP effects by consortia inoculation was not observed in assays under natural conditions (Fig. 3a and 3b). Currently, the major limitation of PGPR as alternative (or complement) to chemical fertilizer in agriculture is the lack of consistent effects under natural conditions. This is likely due to competition with the autochthonous rhizosphere microbiome and environmental factors.
factors (e.g., soil properties) that either limit the population size or activity of the PGPR (Martínez et al. 2010; Glick et al. 2015).

In both Andisol series reduced irrigation (30% of MWHC) drastically decreased the plant biomass by 65-138% and 32-170% in uninoculated controls and plants inoculated with consortium C2 (Fig. 3). When seedlings were inoculated with consortium C1 their biomass however was not affected by lowered irrigation in Piedras Negras series. In addition, wheat seedlings inoculated with consortium C1 showed a significant ($P \leq 0.05$; Tukey’s test) higher biomass in Piedras Negras series at 30% of MWHC, compared with uninoculated controls (Fig. 3a). The consortium C1 containing strains with auxin production and ACCD activity isolated from arid ecosystems. Inoculation of plants with bacteria with auxin and ACCD activity often has a positive effect on growth and alleviation of water stress in plants (Barnawal et al. 2012; Glick et al. 2015). Plant inoculation with individual bacteria isolated from arid ecosystems have shown an increase in plant biomass under greenhouse conditions (Jorquera et al. 2012; Mayak et al. 2004; Timmusk et al. 2011). In Freire series, PGP effect also was not observed at any of the combinations tested (Fig. 3b).

Our results confirm that Chilean ecosystems are a good option for native PGPR screening applicable for Chilean agriculture. Our results also give promising experimental evidence for the potential application of PGPR consortia to improve the growth of wheat in Chilean Andisols, considering arid ecosystems as an alternative source of PGPR. Interestingly, as suggested in our results, the occurrence of stressful conditions in soils (e.g., low nutrients by erosion and degradation of soils or water shortage by prolonged drought periods) might exacerbate the PGP effect of microbial inoculant from extreme environments, which are not evident when soil conditions are not so stressful. However, strategies for selecting the best PGPR strains and their
application in Chilean agriculture will require more comprehensive knowledge of the traits required for rhizosphere competence and on studies on the ecology of introduced PGPR with native soil microorganisms in the rhizosphere crops, respectively. In this context, not only the effectiveness of the PGPR consortia but also the proper application technology will be relevant (cf. Bashan et al. 2014). Hence both the design of specific inoculant formulations (liquid, organic, inorganic, polymeric, or encapsulates) and suited application procedures for the needs of farmers will be required for successful use of PGPR. Thus, the collaborative efforts between scientists, farmers and agro-supplies companies appears to be key effective PGPR technology.

Material and Methods

Rhizobacterial consortia

A rhizobacterial consortium from arid-ecosystems (C1) was formed with the strains Bacillus sp. AD-54A, Serratia sp. AD-7.6 and Bacillus sp. AC-225, which were isolated from native plants grown in undisturbed soils without any historical human disturbance. Bacillus sp. AD-54A, Serratia sp. AD-7.6 were isolated from the rhizosphere of Atriplex sp. grown in the Atacama Desert (23° 2’ 6.401” S, 68° 10’ 2.864” W) whereas Bacillus sp. AC-225 was isolated from the rhizosphere of Atriplex sp. grown in the Aconcagua Valley (32° 47’ 05” S, 70° 47’ 31” W). These strains showed efficient PGP activities, such as auxin production and ACCD activity (Jorquera et al. 2014; Parra et al. 2016); but, their effect on plant biomass had not been assayed so far. A second rhizobacterial consortium from agro-ecosystems (C2) was formed with the strains Bacillus sp. MQH-19, Enterobacter sp. B16 and Enterobacter sp. N0-29PA, which showed multiple PGP activity and separately improved wheat biomass in previous inoculation studies (Acuña et al. 2013, Jorquera et al. 2013 and 2015).
Inoculation assay 1

Inoculation assays with the bacterial consortia (C-1 and C-2) were carried out using seeds and growth chambers provided by Anasac S.A. (http://www.anasac.cl/), which is one of the major Chilean agro-supplies company. The growth chamber conditions were established in accordance to Anasac S.A. protocol for wheat plants and under supervision of technicians. The MWHC was estimated using as reference the weight of pots under field capacity of substrate/soil (100% saturated state of water that can drain freely due to the force of gravity). Briefly, 10 wheat seeds were sown in plastic pots, containing 130 g of 3:1 perlite:peat as unsterile substrate, and incubated in a growth chamber (20°C, 60% of humidity, 60% of MWHC and 8:16 h light:dark cycle). After germination (100% of seeds), the seedlings were inoculated with a 1:1:1 mixture of bacterial strains (~1×10^8 cfu pot^-1 each). A basal fertilization with nutrient solution (Taylor and Foy 1985) was applied after 15 days of incubation. The inoculation treatments were performed in ten replicates and pots were randomly replaced every 2 days within chamber. After 30 days of incubation, wheat seedlings were carefully removed from the pots, washed with distilled water to remove adhered substrate particles, and then dried at 60°C for 48 h to determine total plant biomass as dry weight (g pot^-1).

Inoculation assay 2

Inoculation assays were carried out under growth chamber and natural conditions with wheat seeds coated with bacterial consortia as described by Parra et al. (2016). Briefly, bacterial consortia were suspended in 10% skim milk as cryoprotective agent and freeze-dried (-80°C for 24 h) using a FreeZone Freeze Dry Systems (Labconco) (Schwab et al. 2007). Disinfected (70% ethanol for 5 min and NaClO for 20 min) wheat seeds were coated with a mixture of adhesive solution (arabic gum) and dolomite as coating material (Cartes et al. 2011), together with a
suspension of lyophilized bacterial consortia at $10^8$ cfu g$^{-1}$ of seed. Consortia coated seeds were sown in two Chilean andisols, Piedras Negras and Freire series, and mixed with perlite in a proportion 3:1 (soil:perlite). In order to focus on effect of the inocula on plant biomass, the soils (60% of MWHC) were put in plastic bags and subjected to heat treatments using a microwave (10 min at 2,450 MHz) for three consecutive days (Borie and Rubio 1999). Chemical properties of Andisol pre- and post-heat treatment were determined and shown in the Table 1. Twenty coated seeds of each selected consortium were sown in pots, each containing 1 kg of substrate. The pots in quadruplicate were maintained under controlled and uncontrolled conditions: for controlled conditions the pots were placed in a growth chamber (22°C, 80% of relative humidity and 16:8 h day:night cycle); for uncontrolled conditions the pots were transported to the field (Vilcún, 38°39'S; 72°14'W), placed into open greenhouse, and subjected to changing environmental conditions during summer with an average of temperature of 16.2°C and a precipitation of 18.4 mm (INE 2015). During incubation in open greenhouse under natural conditions, the pots were irrigated with distilled water when required to maintain 60% of MWHC. Additional pot sets were subjected to 50% less irrigation and maintained to 30% of MWHC. After 30 days of incubation, plants were carefully removed, washed and dried (60°C for 48 h) to determine biomass as dry weight (g pot$^{-1}$).

Statistical design and analysis

The statistical design was completely randomized with four replicates having ten plants per pot in inoculation assay 1 and twenty plants per pot in inoculation assay 2. In each treatment, data of plant biomass in soils at 60% and 30% of MWHC were separately subjected to one-way analysis of variance (ANOVA) and means were compared by the Tuckey’s test for multiple comparisons.
Difference at $P \leq 0.05$ was considered as significant between treatments, using the statistical IBM SPSS 21 software.

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Conflict of Interest
The authors have no conflict of interest to declare.

References


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### Table 1. Chemical properties of the Chilean andisols from Piedras Negras and Freire.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Piedras Negras Before heat treatment</th>
<th>Piedras Negras After heat treatment</th>
<th>Freire Before heat treatment</th>
<th>Freire After heat treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (mg kg(^{-1}))</td>
<td>21</td>
<td>45</td>
<td>30</td>
<td>59</td>
</tr>
<tr>
<td>Pi (mg kg(^{-1}))</td>
<td>6</td>
<td>6</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>K (mg kg(^{-1}))</td>
<td>51</td>
<td>55</td>
<td>50</td>
<td>106</td>
</tr>
<tr>
<td>pH(_{\text{H}_2\text{O}})</td>
<td>5.7</td>
<td>5.5</td>
<td>5.2</td>
<td>5.5</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>12</td>
<td>15</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>K (cmol(_{(+)}) kg(^{-1}))</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Na (cmol(_{(+)}) kg(^{-1}))</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Ca (cmol(_{(+)}) kg(^{-1}))</td>
<td>1.5</td>
<td>2.5</td>
<td>3.8</td>
<td>4.9</td>
</tr>
<tr>
<td>Mg (cmol(_{(+)}) kg(^{-1}))</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Al (cmol(_{(+)}) kg(^{-1}))</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>CEC(^a)</td>
<td>1.9</td>
<td>3.2</td>
<td>4.8</td>
<td>6.3</td>
</tr>
<tr>
<td>Al saturation (%)(^b)</td>
<td>2.6</td>
<td>2.2</td>
<td>3.4</td>
<td>3</td>
</tr>
</tbody>
</table>

\(^a\)CEC: cation exchange capacity [\(\Sigma (K, Ca, Mg, Na\) and Al)]; \(^b\) calculated as: Al/CEC \(\times 100\)

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