

Abstract

Background

The ‘Microphenotron’ is an automated screening platform that uses 96-well microtiter plates to test the response of seedlings to natural products. This system allows monitoring the phenotypic effect of a large number of small molecules. Here, this model system was used to study the effect of phytohormones produced by plant growth-promoting rhizobacteria (PGPR) on the growth of wild-type and mutant lines of *Arabidopsis thaliana*.

Methods

In the present study, high-throughput screening based on ‘Microphenotron’ was used to screen PGPRs. Rhizobacteria were isolated from the rhizosphere of *Acacia Arabica*, which was growing in saline habitats. The phylogeny of these rhizobacteria was determined by 16S rRNA gene sequencing. Strains were screened for plant growth-promoting traits such as auxin production, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, and phosphate solubilization. Ultra-Performance Liquid Chromatography (UPLC) was used to detect the presence of different indolic compounds. Finally, PGPR were evaluated to enhance the growth of *A. thaliana* in the ‘Microphenotron’ system and pot trials.

Results

~~We found that s~~Selected PGPR-rhizobacteria strains showed positive results for multiple plant-growth promoting traits. For instance, a-strain (S-6) of *Bacillus endophyticus* exhibited the highest ACC-deaminase activity. UPLC analysis indicated the presence of different indolic compounds in bacterial extracts that included indole lactic acid (ILA), indole carboxylic acid (ICA), and indole-3-acetic acid (IAA). Two strains (S-7 and S-11) of *Psychrobacter alimentarius* produced the most IAA, ICA and ILA. Screening bioassay through 96-well microtiter plates with wild-type Col. N6000 showed an increase in root growth and proliferation. The highest increase of 2-fold was recorded in root growth with *B. thuringiensis* S-26 and *B. thuringiensis* S-50. In pot trials, mutant lines of *A. thaliana* impaired for auxin signaling showed that *B. endophyticus* S-6, *Psy. alimenterius* S-11, *Enterobacter asburiae* S-24 and *B. thuringiensis* S-26 used auxin signaling for plant growth promotion. Similarly, for ethylene insensitive mutant lines (*ein2.5* and *etr1*), *Prolinoborus fasciculus* S-3, *B. endophyticus* S-6, *Psy. alimenterius* S-7, *E. asburiae* S-24, and *B. thuringiensis* S-26 showed the involvement of ethylene signaling. However, the growth promotion pattern for most of the strains indicated the involvement of other mechanisms in

enhancing plant growth. The result of [Microphenotron assays generally agreed with pot trials](#), with [mutant](#) and wild type *A. thaliana* varieties. [Bacterial strains that showed-induced the highest growth response by these cultivars in that the ‘Microphenotron’ based screening is very effectivepromoted plant growth in pot trials. This suggests that the Microphenotron can accelerate evaluation of to select](#) PGPR for agricultural applications.

Keywords: Microphenotyping system, IAA, *Arabidopsis thaliana*, Rhizobacteria, ACC-deaminase, Mutant lines

Introduction

Plant growth-promoting rhizobacteria (PGPR) play a very critical role in soil fertility– [and can increase plant growth and agricultural productivity. The numbers of bacterial strains of genus *Pseudomonas*, *Bacillus*, *Enterobacter*, *Prolinoborus*, *Psychrobacter*, and *Brevibacterium* promote plant growth. The application of these rhizobacteria significantly increases the growth and yield of agriculturally important crops \(Fahad et al., 2015; Nie et al., 2015; Zahid et al., 2015; Araújo et al., 2020\). Therefore, the application of PGPR may help to minimize the use of chemical fertilizers that can also reduce the production cost and environmental risks \(Lucy et al., 2004; Khalid et al., 2004\). However, an incomplete understanding of the mechanisms of plant growth promotion hinders the PGPR applications in the field. Natural variations in bacterial traits and abiotic factors can also influence microbial performance in the soil \(Joshi et al., 2019\).](#)

[PGPR can be characterized based on beneficial biochemical attributes, such as phytohormones production and enzymes like 1-amino cyclopropane-1-carboxylate \(ACC\) deaminase \(Haque et al., 2020\). Exogenous application of IAA of microbial origin can also trigger growth response in inoculated plants \(Ali et al., 2010\). Microorganisms can produce auxin in pure culture and in the rhizosphere \(Barea et al., 2005; Rajmanickma et al., 2018\). Auxin production in the rhizosphere could provoke a physiological response in the host plant. For agricultural applications, screening of rhizobacteria based on auxin production has provided a reliable tool for the selection of PGPR \(Ali et al., 2009\). Previously we also reported the plant growth enhancement by auxin producing PGPR \(Aslam and Ali, 2018; Raheem et al., 2018\).](#)

83 ~~Therefore, we need to screen a greater number of potential PGPR candidates for agricultural~~
84 ~~applications.~~ A variety of techniques have been used to screen ~~rhizobacteria for traits associated~~
85 ~~with~~ PGPR that do not consistently predict a desirable outcome for plant growth and
86 productivity. Similarly, the application of potential PGPR that performs well in a greenhouse
87 often fails to deliver expected results in field settings (Basu et al., 2021). ~~Screening and selection~~
88 ~~of suitable PGPR candidates in pot trials is a time taking process and an incomplete~~
89 ~~understanding of the mechanisms of plant growth promotion hinders the PGPR applications in~~
90 ~~the field. Natural variations in bacterial traits and abiotic factors can also influence microbial~~
91 ~~performance in the soil (Joshi et al., 2019).~~ Therefore, we need reliable high throughput and low-
92 cost bioassays to screen a large number of bacteria ~~for plant inoculation to identify effective~~
93 ~~PGPR.~~

94 The Microphenotron is an automated screening platform that has been used to evaluate the effect
95 of different chemical treatments on *A. thaliana* (Burrell et al., 2017). ~~This system uses 96-well~~
96 ~~microtitre plates to deliver chemical treatments to seedlings (Forde et al., 2013) and It can~~
97 ~~readily demonstrate the effect of metabolites by triggering major changes in root architecture in~~
98 ~~*A. thaliana* (Forde et al., 2013). Moreover, mutant lines of *A. thaliana* can be used to study~~
99 ~~different signaling pathways (Caviglia et al., 2018). For example, the GUS reporter gene has~~
100 ~~been used to study the α -glucuronidase induced by microbial indole-3-acetic acid (IAA) in~~
101 ~~histochemical analysis. Knockouts of the functional version of a gene provide another way to~~
102 ~~look deep at the molecular level which facilitates by *A. thaliana* or tomato mutant lines (Jiang et~~
103 ~~al., 2013; Martínez et al., 2020). It can readily demonstrate the effect of metabolites by triggering~~
104 ~~major changes in root architecture in *A. thaliana* (Forde et al., 2013). Microorganisms also~~
105 ~~produce a variety of metabolites that can influence plant growth and productivity.~~ This system
106 may also be used to screen PGPR as it allows direct interaction of selected microorganisms with
107 the root system of plants. ~~but ‘Microphenotron’ has not been evaluated as a method to select~~
108 ~~effective PGPR. The Microphenotron, a model system based on *Arabidopsis thaliana* L. has~~
109 ~~been used to study the effect of wide concentrations of nutrient substrates on root and shoot~~
110 ~~development (Burrell et al., 2017). Screening of rhizobacteria is an established corner of~~
111 ~~rhizobiology but still needs improvement.~~

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Ryu et al. (2005) used *A. thaliana* for screening well characterized PGPR strains to study signal transduction pathways for plant growth and development. Bacterial volatile compounds (BVCs) are important mediators of plant growth promotion. Screening of PGPR using *A. thaliana* showed that bacteria release a blend of chemicals and volatiles that have a beneficial effect on plant growth (Ryu et al., 2003; Sharifi and Ryu, 2018).

Commented [LMG3]: Not related to topi

To the best of our knowledge, we are first time reporting the use of 'Microphenotron' to screen PGPR for plant ~~inoculation~~ growth promotion. In the current study, ~~we have evaluated this method to screen beneficial rhizobacteria for agricultural applications.~~ Rhizobacteria were isolated from the rhizosphere of halotolerant plants growing in Pothohar salt rang, Pakistan. Strains were tested with *in vitro* assays for traits associated with plant growth promotion. Finally, the ~~strains were tested in both the~~ 'Microphenotron' ~~tested strains were evaluated in system ad~~ pot trials ~~by using with~~ wild type and knockout mutants of *A. thaliana*.

Materials & Methods

Isolation of rhizobacteria

Bacterial strains were isolated from the rhizospheric soil of *Acacia Arabica* (L.) that inhabited the saline areas of Pothohar salt range, Pakistan. The rhizospheric soil samples were collected in clean bags. Samples were processed within 24 h by making serial dilutions in sterilized distilled water and inoculated on L-agar (Luria-Bertani) plates supplemented with four different NaCl concentrations i.e., 0.25 M, 0.5 M, 0.75 M, and 1 M. Pure cultures were obtained by quadrant streaking after picking distinct colonies from inoculated plates.

16S rRNA gene Sequencing

The taxonomic status of bacterial strains was confirmed by 16S rRNA gene sequencing. DNA was isolated from freshly grown bacterial cultures by using the Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's instructions. A fragment (1.5-kb) of the 16S rRNA gene was amplified by using forward and reverse primers (Johnson, 1994). PCR amplification was accomplished in Thermocycler Primus 96 (PeQLab, Erlangen, Germany) as described previously (Raheem et al., 2018). The amplified PCR products were purified by using the QIAquick Gel Extraction Kit (Venlo, Netherland). Samples were sequenced by using 27f and 1522r primers by sending purified PCR products to Eurofins, United Kingdom (UK). For final