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# Beneficial aluminium immobilizing microorganisms inhabiting the rhizosphere of pea

**Alexander Shaposhnikov, Oleg Yuzikhin, Darya Syrova, Denis Karlov, Anna Sazanova, Tatiana Azarova, Edgar Sekste, Vera Safronova, and Andrey Belimov**

All-Russia Research Institute for Agricultural Microbiology, shosse Podbel'skogo, 3, Saint Petersburg, 190608, Russian Federation

Address correspondence and requests for materials to Andrey Belimov, belimov@rambler.ru

## Abstract

Acid soils contain elevated concentrations of mobile aluminium (Al) ions which are toxic for plants. Plants form symbioses with the rhizosphere microorganisms stimulating plant growth and affecting Al availability. Here, for the first time the approach based on the ability to immobilize Al in soil was applied for initial selection of beneficial rhizosphere microorganisms. Al-Immobilizing yeast *Rhodotorula* sp. AL1 and 12 bacterial strains assigned to various genera and species were isolated from the rhizosphere of pea cultivated in acid soils. Immobilization of Al was related to the increased pH of the environment and the formation of insoluble Al phosphates in soil. The strains differed in possessing beneficial properties such as modulation of the nutrient element (Ca, Fe, K, Mg, Mn, P) concentrations in soil, production of phytohormones (auxins, abscisic and gibberellic acids, ethylene), utilization of 1-aminocyclopropane-1-carboxylic acid and organic components typical for root exudates, acetylene reduction and antifungal activities. Eight strains promoted root elongation of radish seedlings by 30 ÷ 50 % with a maximal effect exerted by *Cupriavidus basilensis* strain D39. Taking together, the selected microorganisms are promising models to study the mechanisms of plant-microbe interactions in the presence of toxic Al and improving Al tolerance of plants in acid soils.

**Keywords:** acid soil, aluminium tolerance, immobilization, pea, PGPR, rhizosphere, yeast

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**Authors' information:** Alexander Shaposhnikov, PhD, Leading Researcher, orcid.org/0000-0003-0771-5589; Oleg Yuzikhin, PhD, Senior Researcher, orcid.org/0000-0001-8865-7434; Darya Syrova, Researcher, orcid.org/0000-0002-4156-393X; Denis Karlov, PhD, Senior Researcher, orcid.org/0000-0002-9030-8820; Anna Sazanova, PhD, Senior Researcher, orcid.org/0000-0002-4808-320X; Tatiana Azarova, PhD, Senior Researcher, orcid.org/0000-0002-3134-0679; Edgar Sekste, PhD, Researcher, orcid.org/0000-0002-9753-8303; Vera Safronova, PhD, Head of Laboratory, orcid.org/0000-0003-4510-1772; Andrey Belimov, Dr. of Sci. in Biology, Head of Laboratory, orcid.org/0000-0002-9936-8678

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## Introduction

Aluminium (Al) has high mobility in acid soils and negatively affects plant growth due to inhibition of root development and photosynthesis, induction of oxidative stress and decrease in nutrient uptake (Taylor, 1991; Gupta, Gaurav, and Kumar, 2013; Kochian, Piñeros, Liu, and Magalhaes, 2015). Plants counteract phytotoxic effects of $Al^{3+}$ ions by its immobilization in the rhizosphere via increasing pH, complexation by the exuded organic acids, detoxification in plant tissues and efflux from roots (Ma, Ryan, and Delhaize, 2001; Kochian, Piñeros, Liu, and Magalhaes, 2015). Pea (*Pisum sativum* L.), being a very important agricultural crop, is a relatively Al-sensitive species (Lazof and Holland, 1999; Arunakumara, Walpola, and Yoon, 2013). Therefore attempts were made to understand the mechanisms of Al toxicity and to increase Al tolerance of this crop (Singh, Yadav, and Amist, 2011; Kichigina et al., 2017).

As a legume plant species, pea has a high symbiotic potential forming symbiosis with various types of microorganisms, including plant growth-promoting rhizobacteria (PGPR) which improve adaptation to abiotic stresses (Bukhat et al., 2020; Kumar et al., 2021). Although positive effects of PGPR on plants in the presence of toxic heavy metals (particularly Cd, Cu, Pb, and Zn) are well documented (Belimov et al., 2003; Khan, 2005; Jing, He, and Yang, 2007; Sessitsch et al., 2013),

the role of these bacteria in plant tolerance to Al toxicity and acid soils is scarcely understood. It was shown that some strains of associative nitrogen fixer *Azospirillum brasilense* had high Al tolerance and improved growth of rice (Rai, 1986) and finger miller (Rai, 1991) cultivated on an acid soil. Increased Al tolerance was observed on barley inoculated with nitrogen-fixing *Azospirillum lipoferum* 137 and auxin-producing *Flavohacterium* sp. L30 (Belimov, Kunakova, and Gruzdeva, 1998). Growth stimulation of pea inoculated with Al tolerant *Viridibacillus arenosi* IHBB7171 (Thakur et al., 2017) and of maize inoculated with *Burkholderia* sp. (Arora, Singh, and Tiwari, 2017) in the presence of toxic Al concentrations was described. However, mechanisms of anti-stress effects of these bacteria were not investigated in details.

Further experiments with various Al-tolerant PGPR strains demonstrated the ability of bacteria to chelate Al by the produced siderophores and alleviate Al toxicity for ryegrass grown in soil probably due to the formation of $Al3^{+}$-siderophore complexes (Mora et al., 2017). Increased biomass production and decreased Al accumulation in mung bean inoculated with *Bacillus megaterium* CAM12 and *Pantoea agglomerans* CAH6 (Silambarasan, Logeswari, Cornejo, and Kannan, 2019c), as well as in lettuce inoculated with PGPR *Curtobacterium herbarum* CAH5 (Silambarasan, Logeswari, Valentine, and Cornejo, 2019a) or yeast *Rhodotorula mucilaginosa* CAM4 (Silambarasan et al., 2019b) were also described. The contribution of other beneficial traits of these microorganisms, such as phosphate solubilization, auxin production, 1-aminocyclopropane-1-carboxylate (ACC) deaminase and phosphatase activities in plant growth promotion was also discussed.

Recently we showed that PGPR *Pseudomonas fluorescens* strain SPB2137, initially selected as a biocontrol bacterium (Shaposhnikov et al., 2019), improved Al tolerance of pea due to immobilization and exclusion of this toxicant from root zone (Belimov et al., 2022). Although this strain immobilized Al, produced auxins and possessed ACC deaminase activity, it was relatively sensitive to Al, and its effects on plants were sometimes insignificant. This led us to the idea to select Al tolerant beneficial microorganisms having a high ability to immobilize this toxicant and increase pH of the environment. As a result, for the first time the approach for selection of beneficial microorganisms from plant rhizosphere based on their ability to immobilize Al and alkalize the environment has been successfully applied. This report describes the selection procedure and properties of such microorganisms.

## Materials and methods

**Soil characteristics.** The following soils were collected for isolation of Al-immobilizing microorganisms and named as: soil A — Umbric Albeluvisols Abruptic, Leningrad region, vicinity of the village Belogorka, soil horizon 5 ÷ 20 cm; soil B — Haplic Albeluvisols Abruptic, Leningrad region, neighborhood of the village Orekhovo, 30 ÷ 50 cm; soil C — Haplic Albeluvisols Abruptic, Leningrad region, neighborhood of the village Orekhovo, soil horizon 30 ÷ 50 cm; soil D — Haplic Leptosols, Leningrad region, neighborhood of Boksitogorsk, soil horizon 45 ÷ 60 cm; soil E — Umbric Albeluvisols Abruptic, Novgorod region, neighborhood of Holm, soil horizon 10 ÷ 30 cm; soil F — Haplic Albeluvisols Abruptic, Novgorod region, neighborhood of the village Rakovo, soil horizon 10 ÷ 30 cm. Soil type listed according to the World Reference Base for Soil Resources (https://www.fao.org/soils-portal/data-hub/soil-classification/world-reference-base/en/).

Agrochemical characteristics of soils were determined by the standard methods (Arinushkina, 1970) and presented in Table 1. Soil $pH_{KCl}$ was determined using pH meter F20 (Mettler-Toledo, Schwerzenbach, Switzerland). To determine water-soluble contents of Al, Fe and Mn (the elements being toxic in acid soils), the soil samples were incubated in deionized water (5 g soil + 25 mL water) at continuous shaking at 200 rpm for 1 h and centrifuged at 10000 g for 10 min. Supernatants were tested for metal concentrations using an inductively coupled plasma emission spectrometer ICPE-9000

**Table 1. Characteristics of the studied soils**

| Soil designation | $pH_{KCl}$ | hydrolytic acidity, mg equal $kg^{-1}$ | Total C, g $kg^{-1}$ | Nitrate nitrogen, mg N $kg^{-1}$ | Available P, mg $kg^{-1}$ | Available K, mg $kg^{-1}$ | Water soluble elements, mg $kg^{-1}$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Al | Fe | Mn |
| A | 4.6 ± 0.1 | 55 ± 7 | 22 ± 1 | 10 ± 1 | 17 ± 1 | 87 ± 7 | 7.6 ± 0.1 | 6.0 ± 0.0 | 0.07 ± 0.01 |
| B | 3.7 ± 0.3 | 266 ± 21 | 56 ± 1 | 7 ± 1 | nd | 133 ± 11 | 14.1 ± 0.4 | 2.1 ± 0.1 | 0.20 ± 0.10 |
| C | 4.4 ± 0.4 | 375 ± 2 | 6 ± 2 | 4 ± 1 | nd | 209 ± 21 | 12.0 ± 0.3 | 5.0 ± 0.1 | 0.43 ± 0.01 |
| D | 4.5 ± 0.4 | 175 ± 1 | 2 ± 1 | 4 ± 1 | 24 ± 2 | 208 ± 22 | 8.5 ± 0.5 | 21.3 ± 0.6 | 0.54 ± 0.02 |
| E | 4.7 ± 0.3 | 455 ± 4 | 22 ± 0.1 | 15 ± 3 | 22 ± 2 | 106 ± 17 | 2.6 ± 0.1 | 2.0 ± 0.1 | 0.08 ± 0.02 |
| F | 4.5 ± 0.2 | 210 ± 31 | 31 ± 2 | 9 ± 1 | 15 ± 1 | 70 ± 06 | 1.1 ± 0.1 | 2.8 ± 0.3 | 0.19 ± 0.03 |

Data are means ± SE (n = 2). nd stands for not determined.

(Shimadzu, Kyoto, Japan) following manufacturer's instructions.

**Isolation of microorganisms.** For isolation of Al tolerant microorganisms, the previously developed method was applied (Belimov et al., 1999). For this purpose, 100 g of soil samples were supplemented with 200 mg Al $kg^{-1}$ as $AlCl_3$, moistened up to 70 % of water holding capacity and incubated at 22 °C for 7 days. Seeds of pea (*Pisum sativum* L.) line SGE were surface-sterilized and scarified by treatment with 98 % $H_2SO_4$ for 10 min, rinsed with sterile water, and germinated in the prepared soils for 10 days at 22 °C with a 16 h photoperiod of 500 μmol $m^{-2}$ $s^{-1}$. Then seedling roots were gently washed and homogenized in sterile tap water. Aliquots of diluted homogenates (50 μL) were plated onto Petri dishes with the original medium composed for isolation of Al-tolerant rhizosphere microorganisms (ATRM) medium containing (g $L^{-1}$): mannitol — 2; fructose — 2; sucrose — 1; sodium citrate — 1; yeast extract — 0.5; $KH_2PO_4$ — 1; $NH_4NO_3$ — 0.25; $MgSO_4$ — 0.25; $CaCl_2$ — 0.1; NaCl — 0.1; agar — 16; pH = 5.6. The ATRM medium was additionally supplemented with 15 mg Al $L^{-1}$ as $AlCl_3$. After incubation for 5 days at 28 °C, colonies varying in morphology were picked and repeatedly re-streaked on the ATRM medium until colony morphology of each isolate was homogenous. Then the isolates were tested for Al tolerance by incubating on the ARTM medium supplemented with a range of Al concentrations varying from 10 to 150 mg Al $L^{-1}$. The minimal growth inhibiting (MIC) and minimal lethal (MLC) concentrations were determined, and the most Al-tolerant isolates were selected for further study.

**Aluminium immobilization by microorganisms.** The microorganisms were cultivated for 5 days at 28 °C in a liquid the ATRM medium or in soil suspensions (1 g soil + 10 mL deionized sterile water) supplemented with 14 mg Al $L^{-1}$ as $AlCl_3$. The samples were centrifuged at 10000 g for 15 min at 4 °C and supernatants were tested for Al and nutrient element concentrations (B, Ca, Fe, K, Mg, Mn, P, S and Zn) using spectrometer ICPE-9000 (Shimadzu, Kyoto, Japan) as described above.

**Properties of microorganisms.** Microorganisms were cultivated in a liquid ARTM medium and the suspensions were centrifuged as described above. Phytohormones (indole-3-acetic, indole-3-lactic, indole-3-carboxylic, abscisic, salicylic and gibberellic ($GA_3$) acids) were extracted from the medium by ethyl acetate (pH = 3.0), separated on Waters ACQUITY UPLC BEH RP18 Shield (1.7 μm, 2.1 × 50 mm) column (Waters, USA) and determined using the UPLC system Waters ACQUITY H-Class (Waters, USA) as described previously (Belimov et al., 2015).

To determine the production of endogenous ethylene, microorganisms were cultivated for 4 days in flasks containing 5 mL of a liquid ARTM medium supplemented with the precursors of microbial ethylene biosynthesis: methionine, glutamine and asparagine (Frankenberger and Arshad, 1995) in the amount of 0.2 g $L^{-1}$. Then the flasks were sealed with rubber stopper, incubated for 1 day, and then 1 mL of a gas phase was taken for ethylene analysis. Reduction of acetylene to ethylene (nitrogen fixation assay) by the microorganisms was measured by the acetylene-reduction method (Rennie, 1981) as modified by Belimov et al. (Belimov et al., 2005). The amounts of endogenous ethylene and the fixed ethylene were determined using a gas chromatograph GC-2014 (Shimadzu, Japan) following manufacturer's instructions.

The presence of 1-aminocyclopropane-1-carboxylate (ACC) deaminase was determined visually by growing the microorganisms on agar nitrogen free Dworkin and Foster's medium (Jacobson, Pasternak, and Glick, 1994) supplemented with 250 mg $L^{-1}$ of ACC as a sole nitrogen source (Glick, Karaturovic, and Newell, 1995).

Antimicrobial activity of the studied microorganisms against phytopathogenic bacteria *Achromobacter xylosoxidans* Cm1, *Pectobacterium carotovorum* 01001, *Pseudomonas corrugate* 176, *Pseudomonas brassicacearum* 520-1, *Pseudomonas syringae* RCAM03278 and fungi *Alternaria solani* RCAM03318, *Fusarium sporotrichioides* RCAM02587, *Pythium ultimum* RCAM02498 and *Botrytis cinerea* RCAM04325 was determined by the method of wells as previously described (Kravchenko et al., 2002). Biocontrol strain *Pseudomonas fluorescens* SPB2137 (Shaposhnikov et al., 2019) was used as a positive control. Phytopathogenic strains and *Ps. fluorescens* SPB2137 were obtained from the Russian Collection of Agricultural Microorganisms (RCAM, St. Petersburg, Russian Federation, http://www.arriam.ru/kollekciya-kul-tur1/).

**Identification of microorganisms.** The selected bacterial strains were identified by determination of 16S rRNA gene and 16S-23S rDNA ITS-region sequences as described previously (Belimov et al., 2005). The yeast strain was identified by the determination of the nuclear ribosomal internal transcribed (ITS) region sequence as described previously (Brazhnikova et al., 2022). The sequences were compared to similar sequences in the NCBI database using BLAST analysis and submitted to the GenBank/DDBJ/EMBL databases. BLAST analysis was used to identify the closest relatives from the NCBI database.

The GEN III MicroPlate™ test panel with Microbial Identification Systems software OmniLog® Data Collection (Biolog, Inc., Hayward, USA) was used to obtain a phenotypic pattern and additional identification of bacteria. Utilization of some components (citric, fumaric, lactic, malic, pyruvic and succinic acids, glucose and fructose) playing an important role in Al detoxication by plant root exudates (Ma, Ryan, and Delhaize, 2001; Belimov et al., 2022), and pH of the batch cultures supplemented with these substances as a sole source of carbon, were determined as previously described (Kuzmicheva et al., 2017).

**Root elongation assay.** The plant root elongation promoting activity of the studied microorganisms was determined using the root elongation assay (Belimov et al., 2005) with several modifications. Microorganisms were cultivated on a liquid ARTM medium for 4 days at 28 °C. The obtained bacterial suspensions were diluted with sterile tap water by 10 and 100 times and used as inoculum. Six mL of the diluted bacterial suspensions or sterile ARTM medium (uninoculated controls) were added to glass Petri dishes with filter paper. The seeds of radish (*Raphanus sativus* var. *radicula* Pers.) cultivar Ranniy were surface-sterilized with a mixture of ethanol and 30 % $H_2O_2$ (1 : 1) for 20 min, washed with sterile water and placed on wetted filter paper. Two Petri dishes with 10 seeds each were prepared for each treatment. Root length of seedlings was measured after incubation of closed Petri dishes for 5 d at 25 °C in the dark.

**Statistical analysis.** Statistical analysis of the data was performed using the software Statistica 10 (TIBCO Software Inc., Palo Alto, CA, USA). ANOVA analysis with Fisher's LSD test was used to evaluate the differences between means.

## Results and discussion

Total of 65 colonies were initially isolated, the isolates were purified and tested for Al tolerance on agar ATRM medium (data not shown). Among them, 12 strains having a high level of Al tolerance and capable of immobilizing Al in batch cultures of a liquid ATRM medium and/or soil suspensions were selected for further experiments (Table 2).

The minimal growth inhibiting concentration of the selected strains varied from 15 to 35 mg Al $L^{-1}$, whereas the lethal concentration varied from 40 to 120 mg Al $L^{-1}$. The most Al-tolerant microorganism selected was the yeast strain AL1 (Table 2).

The strains should be considered as true Al-tolerant microorganisms, since their tolerance to toxicant was comparable with the most Al-tolerant microorganisms selected by other authors, namely *Azospirillum brasilense* strain $Al^r$-3 tolerant to 25 mg Al $L^{-1}$ (Rai, 1991), *Curtobacterium herbarum* strain CAH5 tolerant to 180 mg Al $L^{-1}$ (Silambarasan et al., 2019a), *Rhodotorula mucilaginosa* strain CAM4 tolerant to 60 mg Al $L^{-1}$ (Silambarasan et al., 2019b), *Bacillus megaterium* CAM12 and *Pantoea agglomerans* CAH6 tolerant to 200 mg Al $L^{-1}$ (Silambarasan et al., 2019c), *Kosakonia radicincitans* strain CABV2 tolerant to 200 mg Al $L^{-1}$ (Silambarasan et al., 2022) and strains *Klebsiella* sp. RC3, *Stenotrophomonas* sp. RC5, *Klebsiell*a sp. RCJ4, *Serratia* sp. RCJ6 and *Enterobacter* sp. RJAL6 tolerant to about 250 mg Al $L^{-1}$ (Mora et al., 2017). It should be noted that the test media for studying Al tolerance of these microorganisms significantly differed and sometimes were rich in organic compounds or phosphorus that could partially bind and chelate Al ions. Therefore, it is difficult to compare the results of these studies with high accuracy. The strains isolated here were much more tolerant to Al as compared with PGPR strains *Az. lipoferum* 137 or *Flavobac-*

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**Table 2. Tolerance to Al and immobilization of Al by the selected microorganisms**

| Strain name and treatment | Minimal growth inhibiting concentration, mg Al $L^{-1}$ | Minimal lethal concentration, mg Al $L^{-1}$ | Immobilized Al in the liquid nutrient medium, μg Al $mL^{-1}$ | Al concentration in supernatant of soil suspensions, μg Al $mL^{-1}$ | Final pH of soil suspension |
|---|---|---|---|---|---|
| Control | – | – | 2.34 ± 0.03 | 39.1 ± 0.7 | 4.33 ± 0.01 |
| **AL1** | 35 | 120 | 2.28 ± 0.05 | 9.6 ± 0.1 * | 4.62 ± 0.02 * |
| **A12** | 30 | 80 | 2.77 ± 0.04 * | 16.4 ± 6.4 * | 4.81 ± 0.01 * |
| **A35** | 20 | 50 | 2.90 ± 0.15 * | 9.6 ± 0.1 * | 4.97 ± 0.07 * |
| **A47** | 30 | 55 | 2.57 ± 0.08 | 16.1 ± 0.3 * | 4.91 ± 0.02 * |
| **B01** | 25 | 50 | 2.84 ± 0.16 * | 9.8 ± 0.1 * | 5.16 ± 0.05 * |
| **B10** | 15 | 40 | 2.76 ± 0.05 * | 23.8 ± 7.2 * | 4.80 ± 0.05 * |
| **C06** | 25 | 65 | 3.04 ± 0.03 * | 8.1 ± 0.4 * | 5.17 ± 0.07 * |
| **D60** | 20 | 45 | 2.69 ± 0.12 | 14.3 ± 4.9 * | 5.01 ± 0.04 * |
| **D31** | 15 | 40 | 3.14 ± 0.06 * | 9.3 ± 0.5 * | 4.83 ± 0.03 * |
| **D39** | 20 | 50 | 3.01 ± 0.10 * | 12.2 ± 2.9 * | 5.30 ± 0.11 * |
| **E52** | 25 | 55 | 2.65 ± 0.04 | 9.3 ± 0.2 * | 4.76 ± 0.05 * |
| **F55** | 25 | 50 | 2.62 ± 0.11 | 15.2 ± 5.5 * | 4.54 ± 0.08 * |

Data are means ± SE (n = 3). Asterisks show significant differences as compared to the control treatment (Fisher's LSD test, $P < 0.05$). The initial concentration of Al in liquid ATRM medium was 14 μg Al $ml^{-1}$. Soil suspensions were supplemented with μg Al $ml^{-1}$.

**Table 3. Concentration of nutrient elements in supernatant of soil suspensions supplemented with the selected microorganisms**

| Strain name and treatment | Concentration of elements, μg mL$^{-1}$ | | | | | |
|---|---|---|---|---|---|---|
| | Ca | Fe | K | Mg | Mn | P |
| Control | 43 ± 1 | 14 ± 1 | 3.8 ± 0.2 | 6.3 ± 0.1 | 5.3 ± 0.1 | 13.2 ± 0.3 |
| **AL1** | 44 ± 1 | 22 ± 2 * | 4.8 ± 0.1 * | 6.6 ± 0.1 | 4.6 ± 0.1 * | 11.3 ± 0.6 * |
| **A12** | 49 ± 1 * | 15 ± 1 | 4.4 ± 0.1 * | 6.9 ± 0.1 * | 5.0 ± 0.1 | 11.1 ± 0.4 * |
| **A35** | 44 ± 2 | 23 ± 1 * | 3.7 ± 0.1 | 6.4 ± 0.1 | 4.7 ± 0.1 * | 10.8 ± 0.2 * |
| **A47** | 47 ± 2 | 19 ± 1 * | 4.8 ± 0.1 * | 6.9 ± 0.2 * | 4.9 ± 0.1 | 9.9 ± 0.1 * |
| **B01** | 47 ± 1 | 17 ± 1 | 4.1 ± 0.1 | 6.7 ± 0.1 | 4.7 ± 0.1 * | 10.7 ± 0.4 * |
| **B10** | 46 ± 2 | 21 ± 2 * | 3.8 ± 0.1 | 6.6 ± 0.3 | 4.9 ± 0.2 | 11.8 ± 0.6 * |
| **C06** | 37 ± 1 * | 19 ± 1 * | 3.6 ± 0.1 | 5.7 ± 0.2 * | 3.8 ± 0.1 * | 9.5 ± 0.4 * |
| **D60** | 45 ± 2 | 18 ± 1 | 3.9 ± 0.1 | 6.5 ± 0.1 | 4.6 ± 0.2 * | 10.0 ± 0.1 * |
| **D31** | 37 ± 1 * | 20 ± 2 * | 3.7 ± 0.1 | 5.9 ± 0.1 | 3.9 ± 0.1 * | 8.4 ± 0.6 * |
| **D39** | 41 ± 4 | 20 ± 2 * | 3.8 ± 0.2 | 6.3 ± 0.4 | 4.4 ± 0.3 * | 9.1 ± 0.8 * |
| **E52** | 42 ± 1 | 19 ± 1 * | 3.8 ± 0.1 | 6.2 ± 0.2 | 4.7 ± 0.1 * | 8.8 ± 0.4 * |
| **F55** | 49 ± 2 * | 16 ± 1 | 4.4 ± 0.1 * | 6.9 ± 0.3 * | 5.0 ± 0.2 | 11.2 ± 0.3 * |

Data are means ± SE (n = 2). Asterisks show significant differences as compared to the control treatment (Fisher's LSD test, $P < 0.05$).

*terium* sp. L30 (MIC and MLC equal to 5 and 30 mg Al L$^{-1}$, respectively) improving Al tolerance of barley plants (Belimov, Kunakova, and Gruzdeva, 1998) and *Ps. fluorescens* SPB2137 (MIC equal to 5 mg Al L$^{-1}$) improving Al tolerance of pea plants (Belimov et al., 2022).

Seven strains were able to immobilize Al during cultivation in the liquid nutrient medium, whereas all the strains decreased Al concentration in the supernatant of soil suspensions suggesting Al immobilization in soil (Table 2). Microorganisms increased pH of soil suspension (Table 2), and the final Al concentration in soil suspension negatively correlated with pH (r = –0.62; $P = 0.025$; n = 13). This suggested that microbial alkalization of the environment was involved into the immobilization of Al. Experiments with batch cultures showed that all the strains, except AL1, utilized organic acids resulting in alkalization of the medium, whereas sugars were utilized resulting in acidification. Strain AL1 didn't utilize lactic and pyruvic acids, whereas strain D31 did not utilize fructose. Cultivation of AL1 on organic acids did not change pH with exception of alkalization of the medium in the presence of malic acid (data not shown). This suggested possible involvement of organic acids (that could be present in soil or exuded by plant roots) in the alkalization process in soil suspensions and in the plant rhizosphere.

Experiments with soil suspension also showed that microorganisms changed the mobility of nutrient elements (Table 3). Particularly, all the strains decreased concentration of P in supernatants, and this parameter negatively correlated with pH (r = –0.61; $P = 0.026$; n = 13) but positively correlated with Al concentration (r = +0.75; $P = 0.003$; n = 13). It is possible that Al immobilization occurred due to the formation of insoluble phosphates accompanied by alkalization of the medium. Generally, microorganisms increased concentrations of Fe, K and Mg, but decreased concentrations of Mn (Table 4). Concentration of Ca were increased by the strains A12 and F55, but decreased by the strains C06 and D31. Concentrations of other nutrients (B, S and Zn) were not affected by microorganisms (data not shown). The results are in line with repeatedly documented effects of various rhizobacteria on the availability of nutrient elements for plants (Pii et al., 2015; Meena et al., 2017; Belimov et al., 2020). It should be interesting to estimate the effects of the studied microorganisms on the plant nutrient uptake from acid soil. However, based on the obtained results, we propose that such effects may be complex and vary depending on both the strain and nutrient element.

All the strains produced auxins, strains A12 and D31 produced abscisic acid, strains B01, D31 and F55 produced $GA_3$, and 7 strains produced ethylene (Table 4). Salicylic acid was not detected in supernatants. Capability to utilize ACC was found in 8 strains with B10, C06 being the most active utilizers (Table 4). Acetylene reduction (nitrogen fixing) activity was detected in 7 strains. Among them, the strains C06 and E52 had maximum values of the reduced acetylene (Table 4) comparable with the activity of known associative ni-

**Table 4. Production of phytohormones, utilization of ACC and acetylene reduction by the selected microorganisms**

| Strain name | Phytohormone production | | | | | | ACC utilization as a nitrogen source | Acetylene reduction, nmol $C_2H_4$ $mL^{-1}$ $h^{-1}$ |
|---|---|---|---|---|---|---|---|---|
| | Indole-3-acetic acid, ng $mL^{-1}$ | Indole-3-lactic acid, ng $mL^{-1}$ | Indole-3-carboxylic acid, ng $mL^{-1}$ | Abscisic acid, ng $mL^{-1}$ | Gibberellic acid ($GA_3$), ng $mL^{-1}$ | Ethylene, pmol $C_2H_4$ $mL^{-1}$ $d^{-1}$ | | |
| **AL1** | 32 ± 1 | 19 ± 1 | 194 ± 4 | nd | nd | nd | – | nd |
| **A12** | 68 ± 2 | 52 ± 1 | 393 ± 7 | 14 ± 1 | nd | 0.6 ± 0.2 | – | 0.59 ± 0.02 |
| **A35** | 108 ± 3 | 38 ± 1 | 48 ± 1 | nd | nd | nd | + | nd |
| **A47** | 524 ± 12 | 294 ± 2 | 80 ± 2 | nd | nd | 0.9 ± 0.1 | – | 0.31 ± 0.05 |
| **B01** | 63 ± 2 | 38 ± 1 | 67 ± 1 | nd | 16 ± 2 | 0.5 ± 0.1 | + | nd |
| **B10** | 3 ± 1 | 399 ± 3 | 44 ± 1 | nd | nd | 0.5 ± 0.1 | ++ | nd |
| **C06** | 9 ± 1 | 4 ± 1 | 33 ± 2 | nd | nd | nd | ++ | 0.87 ± 0.03 |
| **D60** | 7 ± 1 | 457 ± 3 | 11 ± 1 | nd | nd | 0.8 ± 0.3 | + | 0.33 ± 0.08 |
| **D31** | 96 ± 2 | 542 ± 4 | 22 ± 2 | 23 ± 2 | 91 ± 3 | nd | + | nd |
| **D39** | 82 ± 2 | 64 ± 1 | 31 ± 3 | nd | nd | 0.8 ± 0.1 | + | 0.26 ± 0.02 |
| **E52** | 312 ± 7 | 498 ± 4 | 45 ± 2 | nd | nd | nd | + | 0.77 ± 0.03 |
| **F55** | 313 ± 7 | 24 ± 1 | 31 ± 1 | nd | 12 ± 1 | 0.9 ± 0.2 | – | 0.27 ± 0.04 |

Data are means ± SE (n = 3). ACC stands for 1-aminocyclopropane-1-carboxylic acid. nd stands for not determined. Growth on ACC: active (++), middle (+) or no growth (–).



trogen fixers belonging to *Azospirillum* and other genera (Cavalcante and Dobereiner, 1988; Belimov et al., 1995; Banik, Mukhopadhaya, and Dangar, 2016).

Strain F55 showed antifungal activity against *F. sporotrichioides*, *A. alternata* and *P. ultimum*, and strain A12 inhibited growth only of *A. alternata*. The example of the results of such test is presented in Fig. 1. The selected strains possessed no antibacterial activity against the test-strains (data not shown).

Eight strains promoted root elongation of radish seedlings after inoculation with microbial cultures diluted by 10 times (Fig. 2A), and four strains did it in the presence of microbial culture diluted by 100 times (Fig. 2B). In both cases a maximum positive effect was evident after inoculation with strains D31, D39 and E52. These strains could be considered as PGPR. Strain A12 completely inhibited seed germination at both concentrations of the inoculum, suggesting that this strain is a phytotoxic or phytopathogenic bacterium. Information about the effects of Al-tolerant and/or Al-immobilizing microorganisms on plants is limited. The Al-tolerant strain *Viridibacillus arenosi* IHBB7171 promoted pea plants probably due to the production of auxins and the activity of ACC deaminase (Thakur et al., 2017). PGPR strain *Burkholderia* sp.

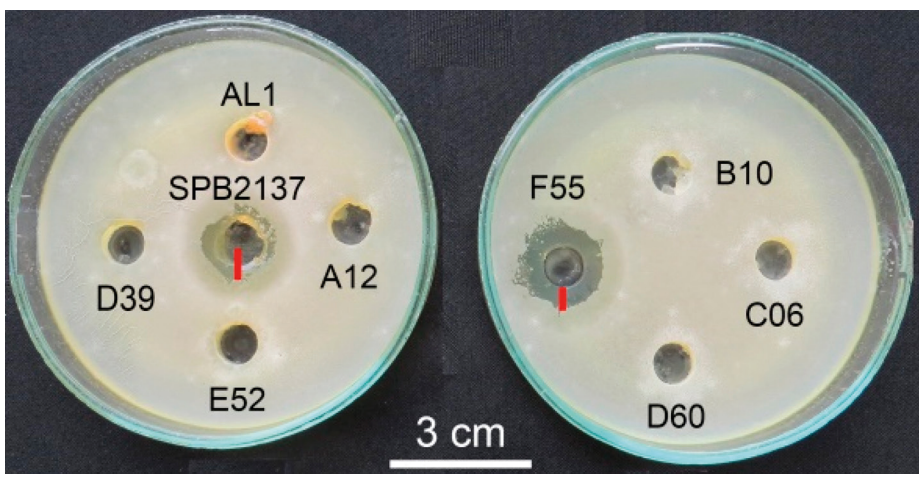


**Fig. 1.** Antifungal activity of the studied microorganisms against *Pythium ultimum* RCAM02498. Strain names are shown by black text. Red bars show radius of the fungal growth inhibition zone. Biocontrol strain *Pseudomonas fluorescens* SPB2137 is used as a positive control.

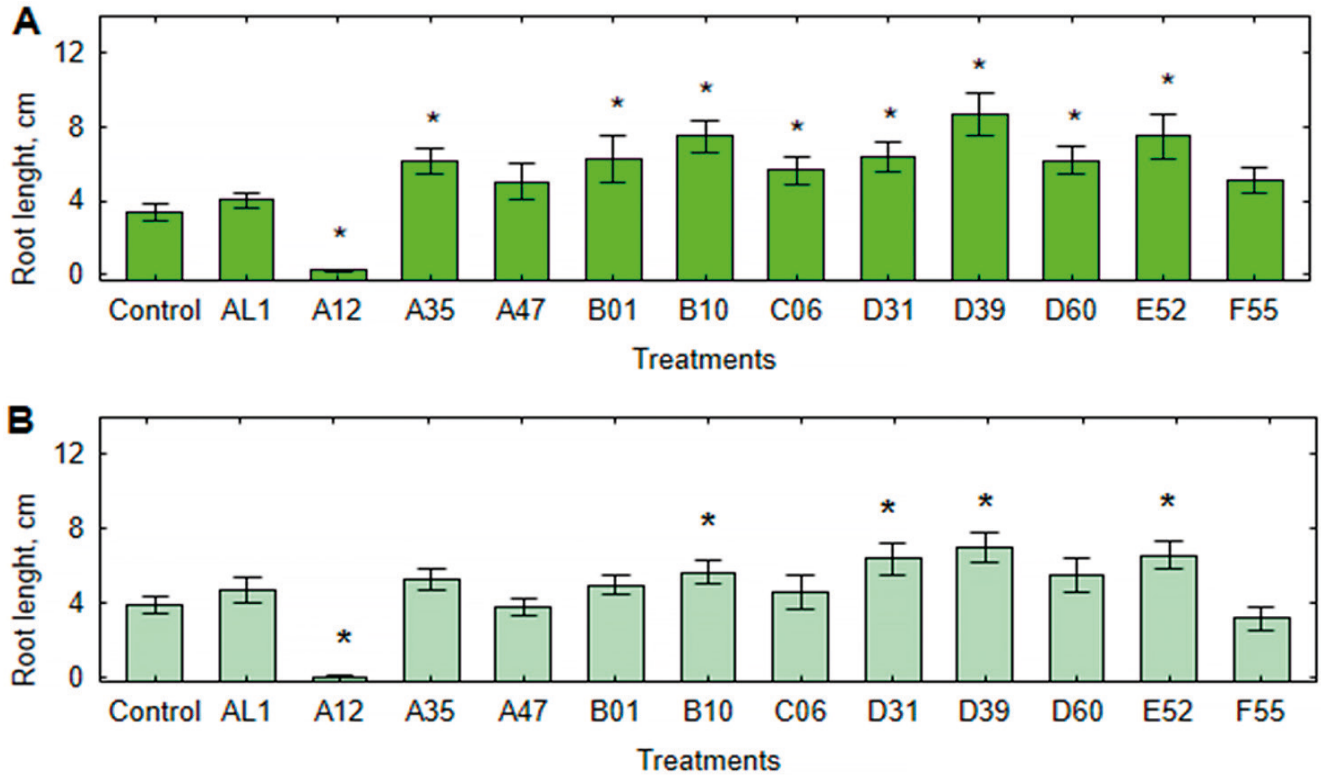


**Fig. 2.** Effect of the studied microorganisms on root elongation of radish seedlings in sterile assay. Seeds were treated with supernatants of batch cultures diluted with sterile water by 10 (A) and 100 (B) times. Vertical bars show SE. Asterisks show significant differences as compared to the control treatment (Fisher's LSD test, $P < 0.05$, n = 20).

increased root length of maize and decreased Al accumulation in roots as a result of binding Al ions by the phosphates (Arora, Singh, and Tiwari, 2017). Inoculation with nitrogen-fixing *Azospirillum lipoferum* 137 and auxin-producing *Flavobacterium* sp. L30 stimulated the growth of barley cultivated in acid soil and decreased accumulation of proline induced by Al toxicity in roots (Belimov et al., 1998). Al-tolerant strains of *Azospirillum brasilense* improved the growth of rice (Rai, 1986) and finger miller (Rai, 1991) cultivated in acid soil. Positive effects of symbiotic nodule bacteria on nitrogen fixation and adaptation to Al toxicity of mung bean (Munns et al., 1979) and soybean (Munns, Hohenberg, Righetti, and Lauter, 1981) were also registered. However, the mechanisms of positive bacterial effects on plants were not studied in these reports. Later, several PGPR strains belonging to *Klebsiella* sp., *Stenotrophomonas* sp., *Serratia* sp. and *Enterobacter* sp. and possessing multiple beneficial traits (P solubilization, auxin production, ACC deaminase and phosphatase activity, exudation of organic acids and siderophores) immobilized Al and increased biomass of ryegrass grown in a volcanic soil rich in aluminum (Mora et al., 2017).

**Table 5. Taxonomic position of the studied microorganisms**

| Strain sign | Defined taxonomic position of the studied strain | Type strains of the closest relatives in the NCBI database | Sequence similarity with the type strain, % | | Sequence accession number in the NCBI database |
|---|---|---|---|---|---|
| | | | 16S DNA | ITS | |
| AL1 | *Rhodotorula* sp. | *Rhodotorula mucilaginosa* CBS316T | nd | 99.08 | OP596320 |
| A35 | *Paraburkholderia* sp. | *Paraburkholderia phytofirmans* PsJNT | 98.55 | | OP596319 |
| | | *Paraburkholderia megapolitana* LMG 23650T | | 89.71 | OP598098 |
| C06 | *Paraburkholderia sediminicola* | *Paraburkholderia sediminicola* HU2-65WT | 99.86 | nd | OP596318 |
| D39 | *Cupriavidus basilensis* | *Cupriavidus basilensis* DSM 11853T | 99.90 | nd | OP596316 |
| E52 | *Herbaspirillum* sp. | *Herbaspirillum rhizosphaerae* UMS-37T | 99.10 | | OP596317 |
| | | *Herbaspirillum hiltneri* N3T | | 93.87 | OP598099 |

nd stands for not determined.

**Table 6. Utilization of carbon sources and resistance to chemicals as assayed by the Biolog GEN III MicroPlate method**

| Carbon source | Strain | | | |
|---|---|---|---|---|
| | A35 | C06 | D39 | E52 |
| Dextrin | – | ± | – | – |
| D-Maltose | – | – | – | – |
| D-Trehalose | – | – | – | – |
| D-Cellobiose | – | – | – | – |
| Gentiobiose | – | – | – | – |
| Sucrose | – | – | – | – |
| D-Turanose | – | – | – | – |
| Stachyose | – | – | – | – |
| Growth at pH 6 | + | + | + | + |
| Growth at pH 5 | + | + | ± | ± |
| D-Raffinose | – | – | – | – |
| α-D-Lactose | – | – | – | – |
| D-Melibiose | – | – | – | – |
| β-Methyl-D-Glucoside | – | ± | – | – |
| D-Salicin | – | ± | – | – |
| N-Acetyl-DGlucosamine | + | + | – | + |
| N-Acetyl-β-DMannosamine | ± | ± | – | – |
| N-Acetyl-D-Galactosamine | ± | + | – | – |
| N-Acetyl Neuraminic Acid | – | – | – | – |
| Growth at 1 % NaCl | – | – | ± | ± |
| Growth at 4 % NaCl | – | – | – | – |
| Growth at 8 % NaCl | – | – | – | – |
| α-D-Glucose | + | + | – | + |
| D-Mannose | ± | + | – | ± |
| D-Fructose | + | + | ± | ± |
| D-Galactose | ± | + | – | + |
| 3-Methyl-Glucose | – | – | – | – |
| D-Fucose | ± | ± | – | + |
| L-Fucose | + | + | – | + |
| L-Rhamnose | ± | ± | – | – |
| Inosine | – | ± | – | – |
| 1 % Sodium Lactate | – | + | ± | + |
| Fusidic Acid | – | – | – | ± |
| D-Sorbitol | ± | + | – | – |
| D-Mannitol | + | + | – | + |
| D-Arabitol | + | + | – | + |
| myo-Inositol | ± | ± | – | – |

| Carbon source | Strain | | | |
|---|---|---|---|---|
| | A35 | C06 | D39 | E52 |
| L-Histidine | + | ± | + | – |
| L-Pyroglutamic Acid | + | + | + | + |
| L-Serine | + | ± | ± | – |
| Pectin | – | – | ± | – |
| D-Galacturonic Acid | ± | ± | ± | + |
| L-Galactonic Acid Lactone | ± | ± | – | + |
| D-Gluconic Acid | ± | + | + | + |
| D-Glucuronic Acid | ± | ± | ± | + |
| Glucuronamide | ± | ± | ± | ± |
| Mucic Acid | + | + | + | + |
| Quinic Acid | ± | + | + | + |
| D-Saccharic Acid | ± | + | + | + |
| p-Hydroxy-Phenylacetic Acid | + | + | + | – |
| Methyl Pyruvate | + | + | + | + |
| D-Lactic Acid Methyl Ester | ± | ± | – | – |
| L-Lactic Acid | ± | + | + | + |
| Citric Acid | – | ± | + | + |
| α-Keto-Glutaric Acid | ± | + | ± | + |
| D-Malic Acid | ± | ± | + | ± |
| L-Malic Acid | ± | + | + | + |
| Bromo-Succinic Acid | + | + | + | + |
| Tween 40 | + | + | ± | – |
| γ-Amino-Butryric Acid | + | + | – | ± |
| α-Hydroxy-Butyric Acid | ± | + | ± | + |
| β-Hydroxy-D,LButyric Acid | + | + | + | + |
| α-Keto-Butyric Acid | + | + | – | + |
| Acetoacetic Acid | – | – | ± | ± |
| Propionic Acid | – | – | + | + |
| Acetic Acid | – | + | + | + |
| Formic Acid | – | + | + | + |
| **Resistance to chemicals:** | | | | |
| Troleandomycin | – | ± | – | – |
| Rifamycin SV | ± | + | + | + |
| Minocycline | – | – | – | – |
| Lincomycin | ± | + | + | + |
| Guanidine HCl | – | – | ± | – |

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*End of the Table 6*

| Carbon source | Strain | | | | Carbon source | Strain | | | |
|---|---|---|---|---|---|---|---|---|---|
| | A35 | C06 | D39 | E52 | | A35 | C06 | D39 | E52 |
| Glycerol | ± | + | ± | + | Niaproof 4 | – | – | – | – |
| D-Glucose-6-$PO_4$ | ± | ± | – | – | Vancomycin | – | ± | + | + |
| D-Fructose-6-$PO_4$ | ± | ± | ± | – | Tetrazolium Violet | ± | ± | + | + |
| D-Aspartic Acid | + | + | + | + | Tetrazolium Blue | ± | ± | + | + |
| D-Serine | – | – | – | – | Nalidixic Acid | – | – | + | – |
| Gelatin | ± | – | – | – | Lithium Chloride | – | – | – | – |
| Glycyl-L-Proline | + | + | + | + | Potassium Tellurite | – | – | + | + |
| L-Alanine | + | + | ± | + | Aztreonam | – | – | + | ± |
| L-Arginine | + | + | – | – | Sodium Butyrate | – | – | – | – |
| L-Aspartic Acid | + | + | + | + | Sodium Bromate | – | – | – | – |
| L-Glutamic Acid | + | + | + | + | D-serine | – | – | – | – |

Note: '–' — negative reaction; '+' — positive reaction; '±' — uncertain reaction.

A consortium of aluminum tolerant strains *Bacillus megaterium* CAM12 and *Pantoea agglomerans* CAH6 stimulated growth and reduced Al uptake by mung bean plants (Silambarasan et al., 2019c). Similar results were obtained after inoculation of lettuce with *Curtobacterium herbarum* CAH5 (Silambarasan et al., 2019a) and *Rhodotorula mucilaginosa* CAM4 (Silambarasan et al., 2019b). It might be speculated that the selected Al-immobilizing microorganisms, particularly the strains having root elongation promoting activity, contribute to counteracting negative effects of Al on plants.

Assessing the obtained results, five the most interesting bacterial strains (AL1, A35, C06, D39 and E52) were genetically identified by sequencing of 16S rRNA gene and 16S-23S rDNA ITS-region. The strain AL1 was identified as yeast *Rhodotorula* sp. closely related to species *Rhodotorula mucilaginosa*, however the level of sequence similarity with the type strain was not enough to assign AL1 to this species (Table 5). Strains C06 and D39 had very high sequence similarity with type strains of *Paraburkholderia sediminicola* and *Cupriavidus basilensis* and were assigned to these species, respectively. Strains C06 and E52 had low similarity of both 16S rRNA gene and 16S-23S rDNA ITS-region sequences with several type strains of genera *Paraburkholderia* and *Herbaspirillum*. Therefore, these stains were identified on a genus level (Table 5). The taxonomic positions of the studied strains suggested their relation to the typical inhabitants of the plant rhizosphere, namely representatives of genera *Paraburkholderia*, *Cupriavidus* and *Herbaspirillum* (Döbereiner, 1988; Estrada-De Los Santos, Bustillos-Cristales, and Caballero-Mellado, 2001; Jurelevicius et al., 2010). Yeast *Rhodotorula mucilaginosa* was described as an Al-tolerant and Al-immobilizing microorganism exerting positive effects on plants subjected to toxic Al concentrations (Silambarasan et al., 2019b). Bacterium *Cupriavidus basilensis* and other species of this genus nodulated tropical legume plants (Andam, Mondo, and Parker, 2007; Jurelevicius et al., 2010; Marchetti, Catrice, Batut, and Masson-Boivin, 2011).

The studied bacterial strains were also tested for a more detailed characterization of phenotype and taxonomic identification using the GEN III MicroPlate™ test panel. Phenotype profiles of these strains are presented in Table 6. Strain A35 was not identified by this method on a species level, but had the closest relative species *Paraburkholderia phenazinium* with very low similarity level (SL) of 0.221. Strain C06 was closely related to *Paraburkholderia phenazinium* (SL = 0.589). Strains D39 and E52 were identified as *Cupriavidus campinensis* (SL = 0.795) and *Herbaspirillum huttiense* ss. *Huttiense* (SL = 0.697), respectively. In general, the results of 16S DNA sequencing and the phenotyping test were similar, but some disagreement between them was probably due to the interspecies phenotypic variability or the absence of some species in the OmniLog® Data Collection. A more detailed study needs to be performed to identify *Paraburkholderia* sp. C06 and *Herbaspirillum* sp. E52 up to the species level. It is possible that these strains belong to new species. All the studied microorganisms were deposited to the RCAM collection.

## Conclusion

Taking together, Al-tolerant microorganisms (bacteria and yeasts) are present in acid soils and colonize plant rhizosphere. Some of them immobilize Al in nutrient me-

dium and in soil suspensions, probably due to increasing pH of the environment and binding Al ions with phosphates. These microorganisms could also change mobility of nutrient elements in soil. A number of beneficial properties, such as phytohormone production, utilization of ACC and nitrogen fixation, were detected in the selected Al-immobilizing microorganisms. Consequently, several bacterial strains stimulated plant root elongation and could be considered as PGPR. Thus, the proposed original approach for the selection of growth-promoting microorganisms among Al-resistant and Al-immobilizing inhabitants of the rhizosphere made it possible to detect strains that have a high potential for increasing the resistance of plants to toxic Al. However, the bacteria deleterious for plants were also presented among Al-tolerant and Al-immobilizing microorganisms. Further study will be aimed at understanding the mechanisms of interaction between these microorganisms and plants in the presence of toxic Al and in acid soils.

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**References**

Andam, C. P., Mondo, S. J., and Parker, M. A. 2007. Monophyly of *nodA* and *nifH* genes across Texan and costa rican populations of *Cupriavidus* nodule symbionts. *Applied and Environmental Microbiology* 73(14):4686–4690. https://doi.org/10.1128/AEM. 00160-07

Arinushkina, E. V. 1970. Rukovodstvo po khimicheskomu analizu pochv. 487 pp. Lomonosov Moscow University Press. Moscow. (In Russian)

Arora, P., Singh, G., and Tiwari, A. 2017. Effect of microbial inoculation in combating the aluminium toxicity effect on growth of *Zea mays*. *Cellular and Molecular Biology* 63(6):79–82. https://doi.org/10.14715/cmb/2017.63.6.16

Arunakumara, K. K. I. U., Walpola, B. C., and Yoon, M. 2013. Aluminum toxicity and tolerance mechanism in cereals and legumes — A review. *Journal of the Korean Society for Applied Biological Chemistry* 56:1–9. https://doi.org/10.1007/s13765-012-2314-z

Banik, A., Mukhopadhaya, S. K., and Dangar, T. K. 2016. Characterization of $N_2$-fixing plant growth promoting endophytic and epiphytic bacterial community of Indian cultivated and wild rice (*Oryza* spp.) genotypes. *Planta* 243(3):799–812. https://doi.org/10.1007/s00425-015-2444-8

Belimov, A. A., Kozhemyakov, A. P., and Chuvarliyeva, G. V. 1995. Interaction between barley and mixed cultures of nitrogen fixing and phosphate solubilizing bacteria. *Plant and Soil* 173:29–37. https://doi.org/10.1007/BF00155515

Belimov, A. A., Kunakova, A. M., and Gruzdeva, E. V. 1998. Influence of soil pH on the interaction of associative bacteria with barley. *Microbiologiya* 67(4):463–469. (In Russian)

Belimov, A. A., Ivanchikov, A. Y., Yudkin, L. V., Khamova, O. F., Postavskaya, S. M., Popolzukhin, P. V., Shmakova, A. A., and Kozlova, G. Y. 1999. New strains of associative growth-stimulating bacteria dominating the rhizoplane of barley seedlings: characterization and introduction. *Mikrobiologiya* 68(3):337–342. (In Russian)

Belimov, A. A., Hontzeas, N., Safronova, V. I., Demchinskaya, S. V., Piluzza, G., Bullitta, S., and Glick, B. R. 2005. Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). *Soil Biology and Biochemistry* 37(2):241–250. https://doi.org/10.1016/j.soilbio.2004.07.033

Belimov, A. A., Safronova, V. I., Tsyganov, V. E., Borisov, A. Y., Kozhemyakov, A. P., Stepanok, V. V., Martenson, A. M., Gianinazzi-Pearson, V., and Tikhonovich, I. A. 2003. Genetic variability in tolerance to cadmium and accumulation of heavy metals in pea (*Pisum sativum* L.). *Euphytica* 131:25–35. https://doi.org/10.1023/A:1023048408148

Belimov, A. A., Dodd, I. C., Safronova, V. I., Shaposhnikov, A. I., Azarova, T. S., Makarova, N. M., Davies, W. J., and Tikhonovich, I. A. 2015. Rhizobacteria that produce auxins and contain ACC deaminase decrease amino acid concentrations in the rhizosphere and improve growth and yield of well-watered and water-limited potato (*Solanum tuberosum*). *Annals of Applied Biology* 167:11–25. https://doi.org/10.1111/aab.12203

Belimov, A. A., Shaposhnikov, A. I., Syrova, D. S., Kichko, A. A., Guro, P. V., Yuzikhin, O. S., Azarova, T. S., Sazanova, A. L., Sekste, E. A., Litvinskiy, V. A., Nosikov, V. V., Zavalin, A. A., Andronov, E. E., and Safronova, V. I. 2020. The role of symbiotic microorganisms, nutrient uptake and rhizosphere bacterial community in response of pea (*Pisum sativum* L.) genotypes to elevated Al concentrations in soil. *Plants* 9(12):1801. https://doi.org/10.3390/plants9121801

Belimov, A. A., Shaposhnikov, A. I., Azarova, T. S., Syrova, D. S., Kitaeva, A. B., Ulyanich, P. S., Yuzikhin, O. S., Sekste, E. A., Safronova, V. I., Vishnyakova, M. A., Tsyganov, V. E., and Tikhonovich, I. A. 2022. Rhizobacteria mitigate the negative effect of aluminum on pea growth by immobilizing the toxicant and modulating root exudation. *Plants* 11:2416. https://doi.org/10.3390/plants11182416

Brazhnikova, Y. V., Shaposhnikov, A. I., Sazanova, A. L., Belimov, A. A., Mukasheva, T. D., and Ignatova, L. V. 2022. Phosphate mobilization by culturable fungi and their capacity to increase soil P availability and promote barley growth. *Current Microbiology* 79(8):240. https://doi.org/10.1007/s00284-022-02926-1

Bukhat, S., Imran, A., Javaid, S., Shahid, M., Majeed, A., and Naqqash, T. 2020. Communication of plants with microbial world: Exploring the regulatory networks for PGPR mediated defense signaling. *Microbiological Research* 238:126486. https://doi.org/10.1016/j.micres.2020.126486

Cavalcante, V. and Dobereiner, J. 1988. A new acid-tolerant nitrogen fixing bacterium associated with sugarcane. *Plant and Soil* 108:23–31. https://doi.org/10.1007/BF02370096

Döbereiner, J. 1988. Isolation and identification of root associated diazotrophs. *Plant and Soil* 110:207–212. https://doi.org/10.1007/BF02226800

Estrada-De Los Santos, P., Bustillos-Cristales, R., and Caballero-Mellado, J. 2001. *Burkholderia*, a genus rich in plant-associated nitrogen fixers with wide environmental and geographic distribution. *Applied and Environmental Microbiology* 67(6):2790–2798. https://doi.org/10.1128/AEM. 67.6.2790-2798.2001

Frankenberger, W. T. and Arshad, M. 1995. Phytohormones in soils: production and function. 503 pp. Marcel Dekker, Inc. New York.

Glick, B. R., Karaturovic, D. M., and Newell, P. C. 1995. A novel procedure for rapid isolation of plant growth promoting pseudomonads. *Canadian Journal of Microbiology* 41(6):533–536. https://doi.org/10.1139/m95-070

Gupta, N., Gaurav, S. S., and Kumar, A. 2013. Molecular basis of aluminium toxicity in plants: A review. *American Journal of Plant Sciences* 4(12C):21–37. https://doi.org/10.4236/ajps.2013.412A3004

Jacobson, C. B., Pasternak, J. J., and Glick, B. R. 1994. Partial purification and characterization of 1-aminocyclopropane-1-carboxylate deaminase from the plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2. *Canadian Journal of Microbiology* 40(12):1019–1025. https://doi.org/10.1139/m94-162

Jing, Y.-D., He, Z. L., and Yang, X.-E. 2007. Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. *Journal of Zhejiang University SCIENCE B* 8(3):192–207. https://doi.org/10.1631/jzus.2007.B0192

Jurelevicius, D., Korenblum, E., Casella, R., Vital, R. L., and Seldin, L. 2010. Polyphasic analysis of the bacterial community in the rhizosphere and roots of *Cyperus rotundus* L. grown in a petroleum-contaminated soil. *Journal of Microbiology and Biotechnology* 20(5):862–870. https://doi.org/10.4014/jmb.0910.10012

Khan, A. G. 2005. Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. *Journal of Trace Elements in Medicine and Biology* 18(4):355–364. https://doi.org/10.1016/j.jtemb.2005.02.006

Kichigina, N. E., Puhalsky, J. V., Shaposhnikov, A. I., Azarova, T. S., Makarova, N. M., Loskutov, S. I., Safronova, V. I., Tikhonovich, I. A., Vishnyakova, M. A., Semenova, E. V., Kosareva, I. A., and Belimov, A. A. 2017. Aluminum exclusion from root zone and maintenance of nutrient uptake are principal mechanisms of Al tolerance in *Pisum sativum* L. *Physiology and Molecular Biology of Plants* 23(4):851–863. https://doi.org/10.1007/s12298-017-0469-0

Kochian, L. V., Piñeros, M. A., Liu, J., and Magalhaes, J. V. 2015. Plant adaptation to acid soils: The molecular basis for crop aluminum resistance. *Annual Review of Plant Biology* 66:571–598. https://doi.org/10.1146/annurev-arplant-043014-114822

Kravchenko, L. V., Makarova, N. M., Azarova, T. S., Provorov, N. A., and Tikhonovich, I. A. 2002. Isolation and phenotypic characterization of plant growth-promoting rhizobacteria with high antiphytopathogenic activity and root-colonizing ability. *Microbiology* 71(4):444–448. https://doi.org/10.1023/A:1019849711782

Kumar, M., Giri, V. P., Pandey, S., Gupta, A., Patel, M. K., Bajpai, A. B., Jenkins, S., and Siddique, K. H. M. 2021. Plant-Growth-Promoting Rhizobacteria emerging as an effective bioinoculant to improve the growth, production, and stress tolerance of vegetable crops. *International Journal of Molecular Sciences* 22(22):12245. https://doi.org/10.3390/ijms222212245

Kuzmicheva, Y. V., Shaposhnikov, A. I., Petrova, S. N., Makarova, N. M., Tychinskaya, I. L., Puhalsky, J. V., Parahin, N. V., Tikhonovich, I. A., and Belimov, A. A. 2017. Variety specific relationships between effects of rhizobacteria on root exudation, growth and nutrient uptake of soybean. *Plant and Soil* 419:83–96. https://doi.org/10.1007/s11104-017-3320-z

Lazof, D. B. and Holland, M. J. 1999. Evaluation of the aluminium-induced root growth inhibition in isolation from low pH effects in *Glycine max, Pisum sativum* and *Phaseolus vulgaris*. *Australian Journal of Plant Physiology* 26(2):147–157. https://doi.org/10.1071/PP98072

Ma, J. F., Ryan, P. R., and Delhaize, E. 2001. Aluminium tolerance in plants and the complexing role of organic acids. *Trends in Plant Science* 6(6):273–278. https://doi.org/10.1016/S1360-1385(01)01961-6

Marchetti, M., Catrice, O., Batut, J., and Masson-Boivin, C. 2011. *Cupriavidus taiwanensis* bacteroids in *Mimosa pudica* indeterminate nodules are not terminally differentiated. *Applied and Environmental Microbiology* 77(6):2161–2164. https://doi.org/10.1128/AEM. 02358-10

Meena, V., Meena, S. K., Verma, J. P., Kumar, A., Aeron, A., Mishra, P. K., Bisht, J. K., Pattanayak, A., Naveed, M., and Dotaniya, M. L. 2017. Plant beneficial rhizospheric microorganism (PBRM) strategies to improve nutrients use efficiency: A review. *Ecological Engineering* 107:8–32. https://doi.org/10.1016/j.ecoleng.2017.06.058

Mora, M. L., Demanet, R., Acuna, J. J., Viscardi, S., Jorquera, M., Rengel, Z., and Duran, P. 2017. Aluminum-tolerant bacteria improve the plant growth and phosphorus content in ryegrass grown in a volcanic soil amended with cattle dung manure. *Applied Soil Ecology* 115:19–26. https://doi.org/10.1016/j.apsoil.2017.03.013

Munns, D., Keyser, H., Fogle, V., Hohenberg, J., Righetti, T., Lauter, D., Zaroug, M., Clarkin, K., and Whitacre, K. 1979. Tolerance of soil acidity in symbioses of mung bean with rhizobia. *Agronomy Journal* 71(2):256–260. https://doi.org/10.2134/agronj1979.00021962007100020010x

Munns, D. N., Hohenberg, J. S., Righetti, T. L., and Lauter, D. J. 1981. Soil acidity tolerance of symbiotic and nitrogen-fertilized soybeans. *Agronomy Journal* 73(3):407–410. https://doi.org/10.2134/agronj1981.00021962007300030006x

Pii, Y., Mimmo, T., Tomasi, N., Terzano, R., Cesco, S., and Crecchio, C. 2015. Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. *Biology and Fertility of Soils* 51:403–415. https://doi.org/10.1007/s00374-015-0996-1

Rai, R. 1986. Manganese-mediated resistance to aluminium and antibiotics in strains of *Azospirillum brasilense* and their interaction with rice genotypes in acid soil. *The Journal of Agricultural Science* 106(2):279–285. https://doi.org/10.1017/S0021859600063863

Rai, R. 1991. Aluminium-tolerant strains of *Azospirillum brasilense* and their associative nitrogen fixation with finger miller (*Eleusine coracana*) genotypes in an acid soil. *Journal of General and Applied Microbiology* 37:9–24. https://doi.org/10.2323/jgam.37.9

Rennie, R. J. 1981. A single medium for the isolation of acetylene-reducing (nitrogen-fixing) bacteria from soils. *Canadian Journal of Microbiology* 27(1):8–14. https://doi.org/10.1139/m81-002

Sessitsch, A., Kuffner, M., Kidd, P., Vangronsveld, J., Wenzel, W. W., Fallmann, K., and Puschenreiter, M. 2013. The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. *Soil Biology and Biochemistry* 60:182–194. https://doi.org/10.1016/j.soilbio.2013.01.012

Shaposhnikov, A. I., Vishnevskaya, N. A., Shakhnazarova, V. Y., Belimov, A. A., and Strunnikova, O. K. 2019. The role of barley root exudates as a food source in the relationship between *Fusarium culmorum* and *Pseudomonas fluorescens*. *Mycologiya i Phytopathologiya* 53(5):311–318. https://doi.org/10.1134/S0026364819050052 (In Russian)

Silambarasan, S., Logeswari, P., Valentine, A., and Cornejo, P. 2019a. Role of *Curtobacterium herbarum* strain CAH5 on aluminum bioaccumulation and enhancement of *Lactuca sativa* growth under aluminum and drought stress-

es. *Ecotoxicology and Environmental Safety* 183:109573. https://doi.org/10.1016/j.ecoenv.2019.109573

Silambarasan, S., Logeswari, P., Cornejo, P., Abraham, J., and Valentine, A. 2019b. Simultaneous mitigation of aluminum, salinity and drought stress in *Lactuca sativa* growth via formulated plant growth promoting *Rhodotorula mucilaginosa* CAM4. *Ecotoxicology and Environmental Safety* 180:63–72. https://doi.org/10.1016/j.ecoenv.2019.05.006

Silambarasan, S., Logeswari, P., Cornejo, P., and Kannan, V. R. 2019c. Role of plant growth-promoting rhizobacterial consortium in improving the *Vigna radiata* growth and alleviation of aluminum and drought stresses. *Environmental Science and Pollution Research* 26(27):27647–27659. https://doi.org/10.1007/s11356-019-05939-9

Silambarasan, S., Logeswari, P., Sivaramakrishnan, R., Cornejo, P., Sipahutar, M. K., and Pugazhendhi, A. 2022. Amelioration of aluminum phytotoxicity in *Solanum lycopersicum* by co-inoculation of plant growth promoting *Kosakonia radicincitans* strain CABV2 and *Streptomyces corchorusii* strain CASL5. *Science of the Total Environment* 832:154935. https://doi.org/10.1016/j.scitotenv.2022.154935

Singh, N. B., Yadav, K., and Amist, N. 2011. Phytotoxic effects of aluminum on growth and metabolism of *Pisum sativum* L. *International Journal of Innovations in Biological and Chemical Sciences* 2:10–21.

Taylor, G. J. 1991. Current views of the aluminum stress response: The physiological basis of tolerance. C*urrent Topics in Plant Biochemistry and Physiology* 10:57–93.

Thakur, R., Sharma, K. C., Gulati, A., Sud, R. K., and Gulati, A. 2017. Stress-tolerant *Viridibacillus arenosi* strain IHB B 7171 from tea rhizosphere as a potential broad-spectrum microbial inoculant. *Indian Journal of Microbiol*ogy 57:195–200. https://doi.org/10.1007/s12088-017-0642-8