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ORIGINAL PAPER

Evaluation of the Potential of Rhizobacteria in Supplying Nutrients of *Zea mays* L. Plant with a Focus on Zinc

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Abstract

Recent studies have shown that soil microorganisms have the potential to solubilize the low-soluble Zn in the soil and can increase the bioavailability of this element to plants through different mechanisms. In this study, efficient zinc-solubilizing bacteria (ZSB) were isolated and identified from the rhizosphere of different plants, and then, the plant growth-promoting traits of these isolates were evaluated under in vitro conditions. Finally, the efficiency of promising ZSB strains in supplying nutrients to maize plants especially Zn was evaluated.

Isolation of ZSB was done from rhizosphere soil samples using the serial dilution method and culturing on TMS medium containing low-soluble sources of Zn. The experiments were performed under in vitro and greenhouse conditions.

In the initial screening, 20 ZSB were obtained. The results showed that in terms of Zn solubility, there was a significant difference between the isolates and also between different Zn sources ($P < 0.01$). In the semiquantitative evaluation of Zn solubilization (halo formation), isolates ZP13, ZO11, and ZC10 with HD/CD ratios of 1.74, 1.68, and 1.61, respectively, showed the highest solubility. Also, in quantitative evaluation, ZP13, ZC10, and ZO11 had the highest solubility with an average of 24.64, 19.48, and 26.544 mg l^{-1}, respectively. In the greenhouse experiment, the isolates ZO11 and ZO14 showed good performance to increase plant growth and led to a significant increase in most of the measured parameters for morphological characteristics and uptake of elements in the plant, especially Zn. Identification of ZO11 and ZO14 showed that they belong to the genera *Acinetobacter calcoaceticus* and *Agromyces italicus*, respectively. Gluconic acid and propionic acid were the most important organic acids produced by these two isolates. In this experiment, promising rhizosphere isolates showed the ability to supply Zn for maize plants.

Keywords Microbial fertilizer · Organic acids · Plant growth promoting · Siderophore

1 Introduction

Deficiency of micronutrients especially zinc (Zn) is one of the most common nutritional problems of plants in arid and semiarid regions (Jaloud et al. 2013). Despite the high content of total Zn in the soils of these regions, signs of Zn deficiency appear in plants, due to the presence of lime in the soil and high pH (Gregory et al. 2017), and this has caused a weak correlation between total zinc concentration in soil and plant absorbable zinc (Ganeshamurthy et al. 2019).

Recent studies have shown that soil fertility has decreased due to improper use of chemical fertilizers, lack of proper management, and lack of attention to the principles of sustainable agriculture (Fes and Bandito 2018). According to some estimates, about 50% of the land under grain cultivation in the world has low amounts of absorbable Zn

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for plants which lead to a decrease in plant growth and yield (Fei et al. 2016). The analysis of agricultural soil results in Iran also confirms that Zn deficiency in these soils is spread due to several reasons such as high lime, high pH, high bicarbonate ion present in irrigation water, and excessive use of phosphorus fertilizers (Shahbazi and Besharati 2013). Among these characteristics, soil pH is the most important factor influencing the usability of Zn for plant roots (Khoshru et al. 2020a). Increasing soil pH intensifies the stabilization of Zn on the surface of soil particles, such as clay minerals and metal oxides. Surface fixation of Zn reduces its solubility and consequently decreases the Zn availability to the plant (Rutkowska et al. 2014).

It has been reported that soil microorganisms, as soil active organic colloids, secrete a variety of organic compounds such as low-molecular-weight organic acids which reduce rhizosphere pH compared to bulk soil (Khoshru et al. 2020b). Therefore, one approach to increase the availability of nonabsorbable Zn in the soil is using the potential of plant growth-promoting bacteria (PGPR) (Gontia-Mishra et al. 2017). These bacteria can use a variety of carbon sources and, due to their diverse metabolic and functional properties, play a significant role in improving health and soil fertility. By adopting the mechanisms such as the production of phytohormones, siderophores, organic acids, and proton secretion, PGPR increases the dissolution and bioavailability of nutrients such as P, K, Fe, and Zn in the soil, which in turn increases plant resistance to biotic and abiotic stresses (Sarikhani et al. 2019a, b; Khoshru et al. 2020a, b, c b and c; Delangiz et al. 2020; Moradi et al. 2021). Previously mentioned studies have shown the significant effect of microbial activities on the bioavailability of nutrients in the soil mass. It has also been shown that microbial activities can affect the various reactions of soil nutrients such as bio-sorption, complexation, dissolution, oxidation, and reduction (Shahab and Ahmad 2008).

Among Zn-solubilizing microbes, bacteria such as *Acinetobacter*, *Azotobacter*, *Bacillus*, *Gluconacetobacter*, and *Pseudomonas* (Fasim et al. 2002) are the most important suppliers of Zn to plants. *Azotobacter* sp., *B. edaphicus*, and *B. megaterium* have been shown to increase soil Zn bioavailability (Mosa et al. 2016). It has been reported that the decrease in rhizosphere pH due to the mechanisms of organic acid production and proton secretion has been a major factor in increasing the availability of Zn for the plant (Goteti et al. 2013).

Nowadays, special attention has been paid to the use of biological potentials as eco-friendly agents and their replacement with chemical compounds (Fes and Bandito 2018; Heidarpour et al. 2019; Sarikhani et al. 2019b; Khoshmanzar et al. 2020; Khoshru et al. 2020a). To achieve sustainable agriculture and use environmentally friendly approaches, the microbial potentials of soil and their metabolites can be used to provide the necessary plant elements such as Zn. The supply of elements such as Zn required by the plant in calcareous soils due to the insolubility of Zn-containing compounds is a problem; hence, the supply of this element for plants has faced a serious challenge. Using zinc-solubilizing bacteria (ZSB) is one way to deal with this problem. Therefore, this study aimed to screen and isolate potent ZSB with other growth-promoting properties.

2 Materials and Methods

2.1 Isolation of Bacteria

Soil sampling from the rhizosphere of maize, wheat, and sunflower plants was performed in different cities of East Azerbaijan. The sampled farms had no crop rotation, and the cultivation was done by row-equipped tractors. Also, according to the farm owners' reports, only nitrogen, phosphorus, and potassium (NPK) chemical fertilizers were used, and no zinc-containing chemical fertilizers were used on those farms. Rhizosphere soils were sampled using sterile laboratory tools (including a small laboratory spade, scissors, and plastic bags), and the sampling depth was 0–20 cm. To obtain rhizosphere bacterial isolates, some plant roots were sampled along with the soil around them, and all sampling steps were performed with sterilized equipment.

Initial screening of bacterial isolates was performed on nutrient agar (NA) (NaCl 5 g, agar 15 g, peptone 5 g, yeast extract 1.5 g, and distilled water 1000 ml) and tris-minimal salts medium (TMS) (D-glucose 10 g, Tris HCl 6.06 g, NaCl 4.68 g, KCl 1.49 g, NH_4Cl 1.07 g, Na_2SO_4 0.43 g, $MgCl_2.2H_2O$ 0.2 g, $CaCl_2.2H_2O$ 30 mg, agar 15 g, distilled water 1000 ml) using the serial dilution method. NA was used as a general medium to monitor the growth of the bacteria, while TMS containing 0.1% of different sources of low-soluble Zn (Zn oxide — CAS no. 1314–13-2, Zn carbonate — CAS no. 5263–02-5, and Zn-phosphate-CAS no. 7779–90-0) was used to monitor the solubility of low-soluble Zn. It should be noted that each source of low-soluble Zn was separately used in the TMS medium. Ten grams of the collected rhizosphere soil samples was added to Erlenmeyer flasks containing 100 mL of sterile distilled water (1:10 or 10^{-1} dilution). Then it was shaken for 5–10 min, and 1 mL of this suspension was transferred to the first tube containing 9 mL of sterile distilled water (10^{-2} dilution). Sequential transferring and dilution process continued to 10^{-9} dilution, and 100 µL of final dilutions (dilutions 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6}) were used in 3 replications on NA and TMS-Zn solid culture medium, and then, the cultured plates were incubated for 48 h at 26 °C (Dinesh et al. 2015). Primary screening was based on isolates colony phenotype and morphology on the NA medium and clear halo formation around the colony on

the TMS-Zn medium. The general NA medium was also used to obtain the percentage of ZSB relative to the total rhizosphere bacteria.

2.2 Semiquantitative and Quantitative Assessment of Zn Solubility

To investigate the semiquantitative Zn solubility by isolates, the solid TMS-Zn medium containing 0.1% low-soluble Zn sources (Zn oxide, Zn carbonate, and Zn phosphate) was used, separately. Isolates were cultured on plates and incubated at 28 °C for 12 days. For days 3, 5, 8, and 12 after primary dot culture, the ratio of halo diameter to colony diameter (HD/CD) was determined (Saravanan et al. 2007a). Ten microliters of an overnight culture of the isolates were added to TMS medium by dot culture method. Also, for quantitative investigation of the Zn solubility, TMS-Zn broth containing 0.1% low-soluble-Zn sources was used, separately. A total of 50 ml of liquid medium was poured into Erlenmeyer flasks and autoclaved. After sterilization, 100 µL of the bacterial overnight suspension was inoculated and then incubated in the shaker incubator (at 28 °C for 7 days — 120 rpm). Treatment as a control was used in in vitro assays without bacterial inoculation using equal volumes of sterile NB. Then, the samples were taken out of the incubator, and after centrifugation (6000 rpm for 10 min), the pH and Zn concentration in the supernatant were measured by atomic absorption spectrometry (Shimadzu, AA-6300) and pH meter (86,502 AZ) (Saravanan et al. 2007b).

2.3 Evaluation of Growth-Promoting Traits of Promising Isolates

The evaluation of the low-soluble mineral phosphate (from tricalcium phosphate source) solubility in Sperber media (glucose 10 g, yeast extract 0.5 g, $CaCl_2$ 0.1 g, $MgSO_4.7H_2O$ 0.25 g, $Ca_3(PO_4)_2$ 2.5 g, distilled water 1000 ml) (Sperber 1958), assessment of potassium release from mica minerals in Aleksandrov medium (glucose 5 g, $Ca_3(PO_4)_2$ 2 g, $MgSO_4.7H_2O$ 0.5 g, $FeCl_3$ 0.005 g, $CaCO_3$ 0.1 g, mica 2 g, and distilled water 1000 ml) containing white (muscovite) and black (biotite) mica (Sarikhani et al. 2018), the auxin production of isolates in the presence and absence of L-tryptophan (100 mg l^{-1}) on Dworkin and Foster (DF) mineral medium (KH_2PO_4 2.5 g, K_2HPO_4 2.5 g, $MgSO_4.7H_2O$ 0.2 g, $(NH_4)_2HPO_4$ 1.0 g, $FeSO_4.7H2O$ 0.01 g, sucrose 10 g, $MnSO_4.7H_2O$ 0.007 g, and distilled water 1000 ml) (Brick et al. 1991), and, finally, the investigation of siderophore production using liquid succinate medium (EDTA 0.01 g, KH_2PO_4 0.6 g, K_2HPO_4 0.9 g, NH_4Cl 1 g, $MgSO_4.7H_2O$ 0.2 g, $CaCl_2.6H_2O$ 0.075 g, Na-succinate 2.2 g, yeast extract 0.1 g, distilled water 1000 mL, pH to 6.8) along with CAS reagent (Chrome Azurol S reagent: 60.5 mg 50 mL^{-1} dH_2O, $FeCl_3{\cdot}6H_2O$: 0.27 mg 10 mL^{-1} HCl 10 mM, HDTMA: 72.9 mg 40 mL^{-1} HCl 10 mM) (Schwyn and Neilands 1987; Aliasgharzad et al. 2009) were performed for promising isolates.

2.4 Determining the Type and Amount of Organic Acids Produced for Promising Isolates

The isolates were first cultured in the TMS liquid medium containing 0.1% of various sources of low-soluble Zn (separately) and incubated for 7 days. After incubation, the samples were centrifuged at 10,000 rpm for 20 min and then passed through a 0.22 µm filter. The resulted solution was stored in a freezer at − 20 °C for analysis by HPLC (high-performance liquid chromatography) and measurement of organic acids. For HPLC, the injection volume was 10 µL. Chromatographic separation was performed on the Shimadzu HPLC system (Shimadzu, Kyoto Japan), equipped with a quaternary pump (LC-20AD), an auto-sampler (SIL-20AC), and a column oven (CTO-20AC). A Vertex Plus C18 reversed-phase column (C18-AQ —5 µm, 4.6 mm I.D. × 250 mm) was used. Separation of the analytes was performed with isocratic elution. The eluent was an aqueous solution of H_2SO_4 (0.001 M) + Na_2SO_4 (0.008 M) at pH 2.8, containing 1% acetonitrile (99% for LC–MS) as an organic modifier. The flow rate was 0.9 ml min^{-1}. The peaks were detected by UV–Vis spectrophotometer at a wavelength of 210 nm. After analyzing the samples and obtaining the chromatogram of each isolate, to determine the type and amount of organic acids produced by the promising isolates, the obtained unknown peaks were compared with standard samples, and their type was determined. Then, by determining the area below the peak, the amount of organic acid produced by the isolates was determined (Pereira et al. 2010).

2.5 Selected ZSB and Their Evaluation in Promoting Plant Growth

The selection of promising ZSB was done based on the ability to solubilize different low-soluble sources containing Zn. In the following, the selected promising strains were evaluated in terms of plant growth-promoting properties under greenhouse condition.

A greenhouse experiment (under the condition of temperature: 28–30 °C, humidity: 60%, and daylight: 14 h) was performed on the sandy loam-Zn-deficient soil (hypocalcic calcareous soil). The soil used was sampled from Khalatpoushan Research Station, University of Tabriz, Iran. The soil characteristics used for the greenhouse experiment include the following texture:

sandy loam; pH: 7.56; EC: 1.9 dS m^{-1}; organic carbon: 0.17%; carbonate calcium equivalent: 2.58%; available K: 198 mg kg^{-1}; available P: 3 mg kg^{-1}; available Fe: 0.78 mg kg^{-1}, and available Zn: 0.58 mg kg^{-1}.

A total of 2.5 kg of non-sterile soil was used for each pot. Five disinfected maize (single cross 704) seeds were planted in each pot. Sodium hypochlorite (0.5%) was used for seed disinfection. After germination and initial growth, the number of plants per pot decreased to two. The experiment included 8 treatments: microbial fertilizer treatment (6 isolates) and positive and negative control treatments. The microbial fertilizer treatments included 6 promising ZSB obtained under in vitro conditions (including bagasse and perlite in equal proportions + 100 mg per pot of low-soluble-Zn sources). Microbial fertilizer was prepared separately for each bacterial isolate (containing 10^8 CFU ZSB mL^{-1}, OD: 0.8); these ZSB were used as a carrier based (bagasse: perlite, 1:1), and 1000 mg was added per pot. It should be noted that due to Zn deficiency in used soil, in microbial fertilizer treatments, only 100 mg low-soluble Zn sources per pot were used, without the addition of any source of soluble Zn (zinc chemical fertilizer). The positive control treatment included a source of Zn sulfate ((10 mg Zn kg^{-1} soil) and without bacteria)), and, finally, the negative control treatment was a lack of microbial fertilizer and without Zn fertilizer. It should be mentioned that to ignore any side effects of the used carrier in this experiment, 1000 mg of the sterile carrier was added to the negative and positive controls. N from the source of urea (300 mg of urea ~ 138 mg of N kg^{-1} soil), K from the source of potassium sulfate (155 mg of potassium sulfate ~ 65 mg of K kg^{-1} soil), P from the source of triple superphosphate (155 mg TSP ~ 8.5 mg P kg^{-1} soil), and Fe from the source of Fe-EDDHA (ethylenediamine-N, N′-bis(2-hydroxyphenyl acetic acid)) (10 mg Fe kg^{-1} soil) were added to all samples for each kg of soil according to the fertilizer recommendation (Malakouti et al. 2008). Irrigation of pots was done by weighing 0.7–0.8 FC (field capacity) during plant growth up to the beginning of flowering (107 days). At the end of the growth period, the plant growth parameters such as stem height and diameter, chlorophyll index, fresh and dry weight of shoot and root, and concentration of Zn, Fe, K, and P were measured. The dry digestion method was used to determine plant elements (Waling et al. 1989). Atomic absorption spectroscopy (Shimadzu AA-6200, Japan) was used to determine plant Zn and Fe, and the spectrophotometric method (Hach DR/2000 spectrophotometer; Hatch Company, Cambridge, UK) was used to measure the P of the samples (using the ammonium vanadate-molybdate method) (Sarikhani et al. 2016), and sample K was determined by a flame photometer (Corning Flame Photometer Model 410, Corning Inc., Corning, NY, USA) (Thomas 1982).

2.6 Identification of Selected Isolates

After various evaluations and tests on the isolated bacteria, efficient isolates (both in terms of solubilization of Zn and in terms of plant growth-promoting properties) were selected and identified. Cell motility, gram staining, oxidase, and catalase activities were determined using the methods described by Hamada et al. (2012). Thus, sequencing and analysis of the 16S rRNA encoding gene were performed to identify isolated strains (Weisberg et al. 1991). DNA of isolates was extracted by boiling method (Sambrook and Russel 2001). Two primers were used for sequencing the bacteria 16S rDNA gene, primer 8F (5′ AGAGTTTGATCCTGGCTCAG 3′) and primer 1490R (5′ GGTTACCTTGTTACGACTT 3′) (Weisburg et al. 1991). In the PCR reaction, the total volume was 50 µl, each sample containing 25 µl Taq 2 × Master Mix and 15 µl deionized water (dH_2O) and 2 µl from each forward and reverse primers and 6 µl DNA extracted from each bacterium (Loffler et al. 2000). The PCR reaction was performed in 30 cycles, initial denaturation step at 95 °C for 5 min, then 95 °C for 60 s, primer annealing at 54 °C for 45 s, primer extension at 72 °C and 2 min, and, at the end, an additional cycle with 72 °C for 10 min. For separating the PCR products, agarose gel electrophoresis (1%) in 0.5 × Tris/borate/EDTA buffer was used. For staining the products ethidium bromide, and for destaining, distilled water was used, and photographed on a gel-documentation system under UV light. PCR products are obtained, and DNA size markers (Invitrogen, USA) were used to determine the size of the PCR product (Sarikhani et al. 2018). After observing the amplificated bands, the partial sequence of the 16S rDNA gene was sequenced, and after identifying the bacteria by performing Blast-n at the NCBI site, the phylogenetic tree and the evolutionary distance for them were built by MEGA 7 software (Boratyn 2013).

2.7 Statistical Analysis

The experiment was performed in a completely randomized design by three replications either in in vitro tests or in greenhouse conditions. It should be mentioned that in the case of in vitro tests such as potassium-releasing ability of the bacteria and auxin production, the experimental design was in the form of a factorial factors included in the type of mica and the presence or absence of the amino acid L-tryptophan. Data were analyzed using SPSS statistical software (version 26.0, IBM SPSS Inc.), and graphs were drawn using Excel software (Microsoft Office 2013, 64-bit). Mean comparisons were performed with Duncan's multiple range test at the 5% probability level.

3 Results

3.1 Isolation of Bacteria

The isolation results of ZSB from rhizosphere soils of maize, wheat, and sunflower farms in different cities of East Azerbaijan are given in Table 1. According to Table 1, a total of 20 bacteria isolates were obtained from different rhizosphere soils. These bacterial isolates grew in NA media and were able to produce a clear halo in TMS-Zn media containing low-soluble sources of Zn (Fig. 1A).

3.2 Evaluation of Zn Solubility by Isolates

3.2.1 Semiquantitative Zn Solubility Test

A total of 20 isolates obtained from the initial screening step were tested for Zn solubility in solid TMS media containing low-soluble Zn from various sources (Fig. 1B). Among different sources in terms of solubilization (clear halo formation), ZP13 isolate in Zn-phosphate source, ZO11 in Zn-oxide source, and ZC10 in Zn-carbonate source with HD/CD ratio of 1.74, 1.68, and 1.61, respectively, had the greatest solubility (Table 2).

According to the results of this part of the experiment, Zn solubilization efficiency (ZSE) for promising isolates was 73.1% for ZP13 (from Zn-PO_4 source), 67.2% for ZO11 (from Zn–O source), and 60.1% for ZC10 (from Zn-CO_3 source).

3.2.2 Quantitative Assessment of Zn Solubility

Zn Phosphate A significant difference was observed between the isolates in terms of the solubilization potential of zinc from the low-soluble source of Zn phosphate ($P < 0.01$). The highest solubility of Zn phosphate was obtained for the ZP13 isolate with a value of 24.64 mg l^{-1}, which showed 347.1% Zn solubilization compared to the control treatment. ZP15 and ZO11 were next with 22.41 and

Table 1 Bacterial isolates obtained from rhizosphere soils of different cities of East Azerbaijan Province

	Location (city)	Coordinates	Rhizosphere of the plant	NA media (10^6 CFU g^{-1})	TMS media (10^6 CFU g^{-1})	ZSB/total rhizosphere bacteria (%)	ZSB isolate
1	Ajabshir	N45.89475200; E37.47692043	Maize	15.8	2.7	17	ZP3
2	Maragheh	N46.22665214; E37.39414134	Maize	20.2	4.5	22.2	ZP5, ZC5
3	Maragheh	N46.22665214; E37.39414134	Sunflower	11	1.3	11.8	ZP6, ZO6
4	Maragheh	N46.22665214; E37.39414134	Wheat	8.6	0.7	8.1	ZP7
5	Varzeghan	N46.64552254; E38.50652428	Maize	9.8	1.6	16.8	ZP9, ZO9
6	Ahar	N47.03313732; E38.86335879	Maize	19.5	3.1	15.8	ZP10, ZO10, ZC10
7	Ahar	N47.06489468; E38.46970732	Sunflower	9.7	1.1	11.3	ZO11
8	Heris	N47.12103796; E38.25455456	Maize	11.4	1.5	13.1	ZP13, ZO13-1, ZO13-2, ZO13-3, ZO13-4
9	Sarab	N47.55103970; E37.94337397	Maize	14.8	2.7	18.2	ZP14, ZO14
10	Bostan Abad	N46.84087658; E37.85058333	Maize	25.1	3.4	13.5	ZP15

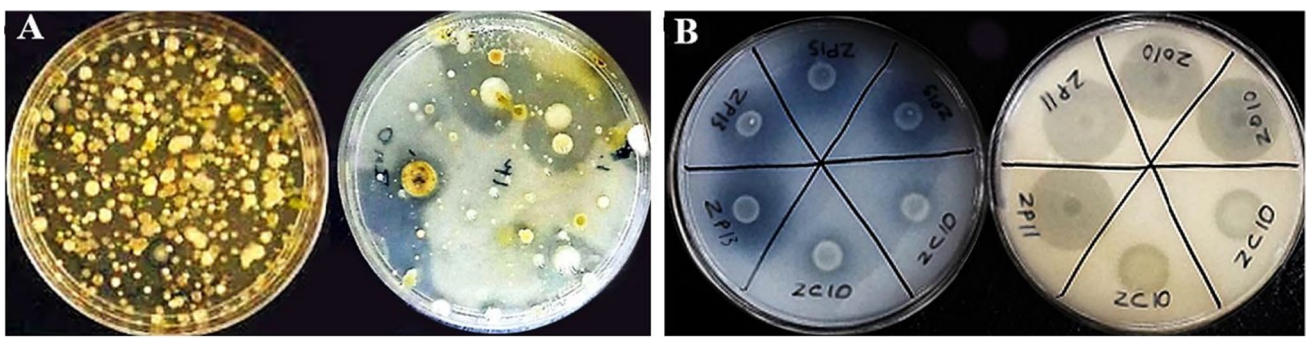

Fig. 1 **A** Primary screening of ZSB, NA culture medium (left), and TMS medium (right). **B** Clear halo formation around bacterial colony indicated Zn solubilization (left: Zn-PO_4; right: Zn–O)

Table 2 The assessment of Zn solubility (HD/CD) in the presence of different low-soluble Zn sources at 3, 5, 8, and 12 days after primary dot culture + zinc solubilization efficiency (ZSE) on the 12th day

Isolate	Zn-PO_4				Zn-CO_3				Zn–O			
	D3	D5	D8	D12, ZSE	D3	D5	D8	D12, ZSE	D3	D5	D8	D12, ZSE
ZC5	1.10	1.21	1.28	1.35, 34.3	1.11	1.14	1.17	1.22, 22.4	1.12	1.15	1.18	1.22, 21.7
ZC10	1.10	1.17	1.26	1.29, 27.1	1.19	1.34	1.54	1.61, 60.1	1.11	1.13	1.16	1.19, 19.6
ZO6	1.13	1.15	1.16	1.18, 19.4	1.11	1.13	1.17	1.22, 2.13	1.13	1.16	1.19	1.25, 24.5
ZO9	1.10	1.00	1.00	1.00, 0.0	1.00	1.00	1.00	1.00, 0.0	1.11	1.15	1.19	1.24, 25.3
ZO10	1.00	1.14	1.19	1.30, 29.2	1.10	1.13	1.16	1.19, 19.5	1.18	1.28	1.42	1.56, 57.8
ZO11	1.19	1.23	1.31	1.37, 36.1	1.16	1.19	1.25	1.32, 33.2	1.21	1.37	1.49	1.68, 67.2
ZO13-1	1.14	1.16	1.19	1.24, 25.5	1.06	1.09	1.13	1.15, 15.2	1.12	1.15	1.19	1.24, 23.3
ZO13-2	1.13	1.19	1.24	1.31, 30.2	1.11	1.14	1.16	1.19, 20.3	1.12	1.14	1.17	1.80, 19.2
ZO13-3	1.11	1.14	1.18	1.23, 24.4	1.12	1.30	1.14	1.15, 16.3	1.12	1.14	1.16	1.18, 19.5
ZO13-4	1.11	1.13	1.14	1.16, 16.4	1.13	1.15	1.18	1.21, 22.1	1.13	1.60	1.90	1.21, 22.3
ZO14	1.17	1.26	1.34	1.45, 46.1	1.19	1.27	1.36	1.43, 44.2	1.18	1.26	1.39	1.51, 50.4
ZP3	1.00	1.13	1.19	1.25, 24.4	1.00	1.00	1.00	1.00, 0.0	1.00	1.00	1.00	1.00, 0.0
ZP5	1.13	1.22	1.34	1.42, 43.2	1.00	1.00	1.00	1.00, 0.0	1.00	1.00	1.00	1.00, 0.0
ZP6	1.12	1.15	1.18	1.20, 19.4	1.00	1.00	1.00	1.00, 0.0	1.00	1.00	1.00	1.00, 0.0
ZP7	1.00	1.00	1.13	1.14, 13.4	1.00	1.00	1.00	1.20, 20.0	1.00	1.00	1.00	1.20, 20.0
ZP9	1.13	1.17	1.24	1.29, 29.3	1.00	1.10	1.10	1.20, 20.0	1.13	1.19	1.24	1.29, 30.7
ZP10	1.00	1.12	1.14	1.15, 14.4	1.00	1.00	1.00	1.00, 0.0	1.00	1.00	1.00	1.00, 0.0
ZP13	1.19	1.38	1.59	1.74, 73.1	1.15	1.19	1.24	1.31, 31.5	1.14	1.21	1.26	1.33, 32.3
ZP14	1.19	1.24	1.29	1.38, 37.1	1.00	1.00	1.00	1.00, 0.0	1.00	1.00	1.00	1.00, 0.0
ZP15	1.25	1.43	1.54	1.70, 70.2	1.16	1.21	1.26	1.32, 32.3	1.15	1.21	1.28	1.36, 35.3

ZSE was calculated by the following formula: ZSE (%) = ((HD-CD)/CD)) × 100, where HD halo dimeter and CD colony diameter (Vazquez et al. 2000)

21.32 mg l^{-1}, respectively. The results showed that there is a negative and significant correlation between pH and Zn solubilization ($r = -0.89$, $P < 0.01$). As the pH decreased, the solubilization of Zn increased (Fig. 2A). The correlation coefficient of this test with the semiquantitative method was $r = 0.54$ ($P < 0.05$) for the 12th day.

Zn Carbonate The results showed that the isolates have different abilities to solubilize zinc from the Zn-CO_3 source ($P < 0.01$). ZC10 isolate showed the highest solubility (19.48 mg l^{-1}), which had 133.2% more solubility compared to the control treatment. Isolates ZP13, ZO14, and ZP15 were also in the next ranks (Fig. 2B). The results of this section also showed that the effect of pH changes on the solubilization of Zn carbonate was negative and significant ($r = -0.89$, $P < 0.01$). The correlation coefficient of this method with the semiquantitative method was $r = 0.84$ ($P < 0.01$) for the 12th day.

Zn Oxide The results revealed that there was a significant difference among the isolates ($P < 0.01$). ZO11 isolate with a value of 26.54 mg l^{-1} had the highest solubility, which was 319.9% higher than the control treatment. Isolates ZO14, ZO10, and ZC10 were in the next ranks, respectively (Fig. 2C). The results of the correlation between pH and solubilization of Zn oxide by isolates showed that the effect of pH changes on Zn solubilization was negative and quite significant ($r = -0.93$, $P < 0.01$). The correlation coefficient of this method with the semiquantitative method was $r = 0.51$ ($P < 0.05$) for the 12th day.

Summarizing the in vitro experiments of Zn solubility by potent isolates from three sources (Zn-PO_4, Zn–O, and Zn-CO_3), it was observed that out of 20 isolates, 6 isolates ZP13, ZO11, ZC10, ZO14, ZP15, and ZO10 were promising ZSB (Fig. 3)

3.3 Plant Growth-Promoting Traits Evaluation in Isolates

Among the 20 isolates evaluated for solubilization assessment of Zn from different sources, the promising strains (6 isolates) were selected for evaluation of other plant growth-promoting characteristics including P solubility and K releasing ability, auxin, and siderophore production. Three isolates ZP15, ZP13, and ZO14 in the phosphorus solubilization experiment, two isolates ZP13 and ZO14 in potassium release and siderophore production experiments, and two isolates ZO11 and ZO14 in the auxin production experiment had the highest averages (Table 3).

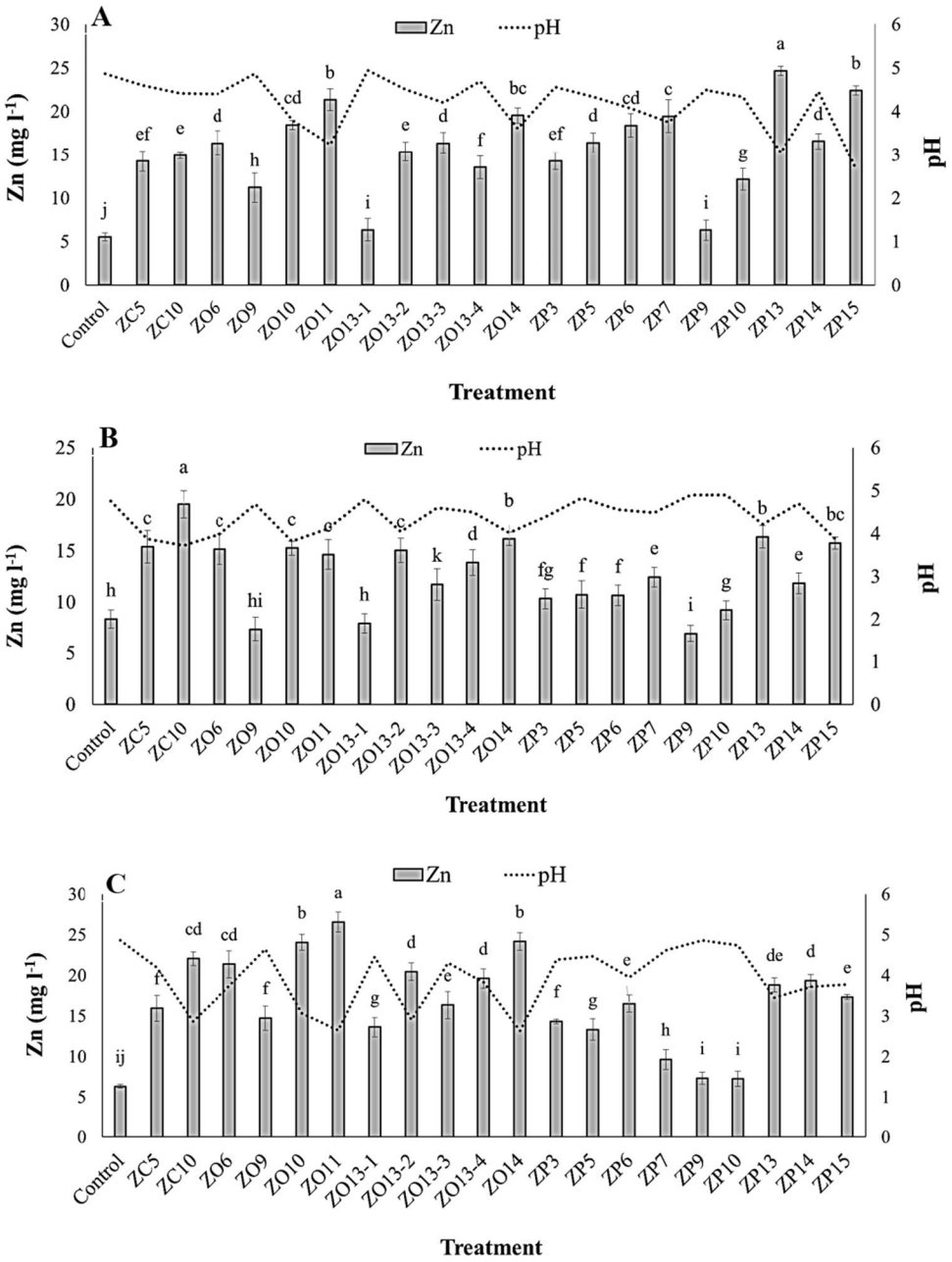

Fig. 2 Solubilization of Zn phosphate (**A**), Zn carbonate (**B**), and Zn oxide (**C**) by isolates with changes in pH of the culture medium. The letters above the columns indicate significant different at $p < 0.01$

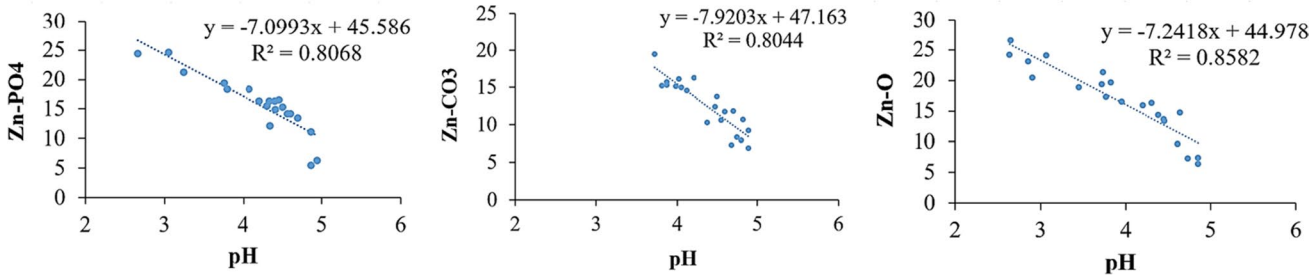

Fig. 3 Correlation diagrams between Zn solubilization from different sources by pH changes

Table 3 Evaluation of plant growth-promoting properties for selected strains

Isolate	P (mg l^{-1})	K-muscovite (mg l^{-1})	K-biotite (mg l^{-1})	Siderophore (µM)	Auxin-L (mg l^{-1})	Auxin-W (mg l^{-1})
Control	29.65 ± 2.47 h	3.52 ± 0.21 h	5.49 ± 0.43 h	0.15 ± 0.006f	6.18 ± 0.61d	6.07 ± 0.58e
ZC10	77.54 ± 4.96de	5.44 ± 0.37f	7.31 ± 0.54f	17.11 ± 1.05d	13.46 ± 1.2b	12.23 ± 1.09c
ZO10	43.23 ± 3.38ef	4.59 ± 0.28 g	6.69 ± 0.52 g	25.7 ± 1.65 cd	13.37 ± 1.26b	12.35 ± 1.15bc
ZO11	37.63 ± 2.30 g	5.85 ± 0.4de	8.31 ± 0.61e	23.31 ± 1.42c	14.39 ± 1.28a	12.12 ± 1.12c
ZO14	84.12 ± 5.4c	10.57 ± 0.71a	14.98 ± 1.13b	27.21 ± 1.63b	14.04 ± 1.15ab	13.49 ± 1.14a
ZP13	91.68 ± 6.71b	9.02 ± 0.61b	18.59 ± 1.41a	31.55 ± 1.91a	13.32 ± 1.19b	9.63 ± 0.85d
ZP15	103.7 ± 6.61a	7.48 ± 0.47 cd	10.17 ± 0.79 cd	16.47 ± 1.25e	13.09 ± 1.24bc	12.44 ± 1.16b

Auxin-L, auxin assay medium containing 100 mg l^{-1} L-tryptophan amino acid; auxin-W, auxin assay medium without L-tryptophan. The letters indicate significant difference at $P < 0.01$. The value presented ± standard error

3.4 Greenhouse Experiment Results

The results of maize inoculation with selected ZSB showed that the effect of these strains on morphological characteristics (Table 4) was significant ($P < 0.01$). Two isolates ZO11 and ZP15 had good averages for the measured indices, so they increased chlorophyll index (93.9%, 83.75%), shoot fresh weight (26.82%, 26.58%), shoot dry weight (26.7%, 26.37%), root fresh weight (41.11%, 26.42%), root dry weight (36.13%, 24.7%), stem height (23.6%, 24.91%), stem diameter (32.97%, 23.86%), and root volume (107.97%, 106.34%), respectively, compared with negative control. Subsequently, the isolate ZO14 also led to an increase in parameters such as root fresh and dry weight (37.26%, 32.51%), root volume (104.76%), stem height, and diameter (30.49%, 25.73%) compared to the negative control treatment. In fresh and dry weight of plant shoot and root, isolate ZP13 was better than the negative control treatment. Two isolates ZO10 and ZC10 had lower averages than the positive control and other isolates (Table 4).

The inoculation effect of selected bacteria on Zn uptake was significant ($P < 0.01$). There was also a significant difference between bacterial treatments for the uptake of other elements such as phosphorus, potassium, and iron (Table 5). ZO11 bacterial treatment had the highest Zn uptake on the roots and shoots of maize, which was not statistically significant with the positive control treatment (chemical fertilizer). ZO11 bacteria increased the uptake of Zn in the roots and shoots of the plant by 177.68% and 173.95%, respectively, compared to the negative control treatment. ZO14 bacteria was next in the order. ZP15 isolates in phosphorus and ZO14 in potassium uptake of roots and shoots of plants had the highest performance. ZO11 and ZO14 bacteria, in addition to the ZP13 isolate, were also highly effective in iron uptake in the roots and shoots of the plant (Table 5).

3.5 HPLC Analysis Results for Promising ZSB

According to the results of the in vitro experiment, isolates ZO11 (from zinc oxide source), ZC10 (from zinc-carbonate source), and ZP13 (from zinc-phosphate source) had the highest solubility, and the isolates ZO14, ZP15, and ZO10 were in the next ranks. Also, according to the results of greenhouse experiments, the two isolates ZO11 and ZO14 had the best performance in increasing the growth and yield of maize.

The results of the liquid chromatographic analysis to determine the type and amount of organic acids produced by the promising ZSB (4 isolates ZO11, ZC10, ZP13, and

Table 4 The effect of selected bacterial isolates on the growth and morphological characteristics of maize

Treatment	Chlorophyll index (SPAD)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Stem diameter (cm)	Stem height (cm)	Root volume (cm^3)
Control –	6.57 ± 0.54d	229.2 ± 19.3c	133.73 ± 9.55d	42.01 ± 2.32d	28.60 ± 1.54b	1.24 ± 0.08c	101.67 ± 7.53c	107.1 ± 7.51e
Control +	9.60 ± 0.87b	259.51 ± 21.4b	178.90 ± 13.3ab	47.51 ± 2.4bc	36.30 ± 2.78a	1.54 ± 0.11a	130.12 ± 9.12a	176.8 ± 13.5b
ZC10	6.00 ± 0.59d	256.1 ± 20.5b	140.07 ± 11.2 cd	46.89 ± 2.59bc	29.20 ± 1.63b	1.27 ± 0.08bc	123.21 ± 8.43b	156.4 ± 11.5c
ZO10	7.70 ± 0.64c	242.26 ± 19.2bc	148.73 ± 10.2c	44.37 ± 3.79 cd	31.6 ± 1.65b	1.31 ± 0.09bc	116.34 ± 7.58b	173.4 ± 12.6b
ZO11	12.07 ± 1.14a	290.20 ± 22.5a	188.73 ± 13.4a	53.09 ± 4.2a	38.93 ± 3.42a	1.65 ± 0.11a	125.67 ± 8.61ab	222.7 ± 16.4a
ZO14	9.10 ± 0.95b	262.32 ± 19.1b	183.57 ± 12.4a	48.02 ± 3.87bc	37.90 ± 2.34a	1.56 ± 0.1a	132.61 ± 9.23a	219.3 ± 14.5a
ZP13	6.83 ± 0.53 cd	265.08 ± 19.8b	167.40 ± 11.5b	48.52 ± 3.94b	35.33 ± 2.95a	1.37 ± 0.09b	114.34 ± 7.76b	142.1 ± 9.43d
ZP15	12.73 ± 0.98a	290.75 ± 23.3a	169.07 ± 10.8b	53.19 ± 4.53a	35.67 ± 3.12ab	1.54 ± 0.11a	127.23 ± 8.23ab	221 ± 15.7a

The letters indicate significant difference at $P < 0.01$. The value presented ± standard error

Table 5 The effect of selected bacterial isolates on the uptake of Zn, P, K, and Fe of maize

Treatment	P uptake (mg plant^{-1})		K uptake (mg plant^{-1})		Fe uptake (mg plant^{-1})		Zn uptake (mg plant^{-1})	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Control −	7.13 ± 0.53f	12.3 ± 0.88e	119.4 ± 8.4ef	633.4 ± 46.1f	33.1 ± 2.48d	2.81 ± 0.22c	2.61 ± 0.18de	1.4 ± 0.11f
Control +	19.5 ± 1.4 cd	26.4 ± 1.95d	217.7 ± 15.8bc	814.5 ± 54.1 cd	60.1 ± 4.04a	6.01 ± 0.42a	7.4 ± 0.4 a	3.97 ± 0.26a
ZC10	15.2 ± 1.09e	12.9 ± 0.91e	150.4 ± 10.5de	712.9 ± 50.9e	37.8 ± 2.41 cd	3.17 ± 0.22c	1.99 ± 0.11e	1.8 ± 0.13e
ZO10	21.8 ± 1.61bc	13.9 ± 1.01e	106.2 ± 7.5f	780 ± 56.3d	48.9 ± 4.69b	4.62 ± 0.36b	4.19 ± 0.28c	2.3 ± 0.17 cd
ZO11	23.4 ± 1.82b	30.2 ± 2.35bc	256.8 ± 18.25b	898.1 ± 59.9ab	68.6 ± 4.63a	6.57 ± 0.46a	7.2 ± 0.46a	3.99 ± 0.26a
ZO14	25.6 ± 1.84ab	31.8 ± 2.84b	300.5 ± 21.39a	955.3 ± 67.6a	65.2 ± 3.17ab	6.31 ± 0.45a	5.37 ± 0.32b	3.61 ± 0.26b
ZP13	23.6 ± 1.78 b	31.1 ± 2.25b	183.9 ± 13.8 cd	855.1 ± 55.8bc	66.5 ± 4.78a	3.48 ± 0.37bc	3.5 ± 0.2 cd	2.62 ± 0.19c
ZP15	26.3 ± 1.29a	39.9 ± 2.02a	263.6 ± 15.5b	842.2 ± 55.8bc	48.5 ± 2.97b	3.23 ± 0.21c	2.01 ± 0.13e	2.11 ± 0.13d

The letters indicate significant difference at $P < 0.01$. The value presented ± standard error

ZO14) showed that the amount and type of organic acids produced by the isolates were different.

In the case of ZO11 isolate, 3 organic acids including gluconic acid (197.54 µg ml^{-1}), acetic acid (18.53 µg ml^{-1}), and propionic acid (121.94 µg ml^{-1}) were characterized (Fig. 4A; Table 6). In this experiment, the ZO14 and ZC10 isolates produced a wide range of organic acids. The ZO14 isolate by the production of 8 organic acids including alpha-ketoglutaric acid (29.42 µg ml^{-1}), pyruvic acid (75.54 µg ml^{-1}), citric acid (11.22 µg ml^{-1}), gluconic acid (93.07 µg ml^{-1}), succinic acid (148.85 µg ml^{-1}), formic acid (38.08 µg ml^{-1}), acetic acid (19.73 µg ml^{-1}), and propionic acid (146.14 µg ml^{-1}) (Fig. 4B; Table 6) was one of them. Also the isolate ZC10 produced 8 acids including oxalic acid (148.13 µg ml^{-1}), 2-ketogluconic acid (55.01 µg ml^{-1}), citric acid (51.05 µg ml^{-1}), gluconic acid (202.52 µg ml^{-1}) malic acid (39.56 µg ml^{-1}), succinic acid (28.92 µg ml^{-1}), formic acid (67.03 µg ml^{-1}), and acetic acid (118.30 µg ml^{-1}) (Fig. 4C; Table 6). Our HPLC results revealed that perhaps ZP13 isolate with the production of 4 organic acids including alpha-ketoglutaric acid (57.78 µg ml^{-1}), pyruvic acid (22.62 µg ml^{-1}), gluconic acid (168.30 µg ml^{-1}), and propionic acid (98.59 µg ml^{-1}) (Fig. 4D; Table 6) has the role in Zn solubilization from low-soluble resources.

3.6 Identification Results of Isolates

The results of biochemical and molecular identification of promising ZSB are shown in Table 7 and Fig. 5.

4 Discussion

In this experiment, ZSB were isolated from the rhizosphere of maize, wheat, and sunflower. The results showed that the microbial population and diversity in the rhizosphere of maize were more than that of the wheat and sunflower, so the percentage of ZSB in the rhizosphere of the studied can be ordered as maize > sunflower > wheat, respectively. One of the reasons for the increase in the number and diversity of the microbial population of the rhizosphere plant (regardless of the intrinsic properties of soil such as texture, organic matter, pH, percentage of lime) is the rate of secretion of photosynthetic and carbonic compounds of the plant in the rhizosphere that is a kind of investment by the plant to attract beneficial root microbes (Olanrewaju et al. 2019).

In the semiquantitative experiment of Zn solubilization based on the formation of a transparent halo around the colony in the TSM solid medium containing low-soluble Zn sources, 6 isolates were selected as the promising ZSB. The selection criterion for these isolates was HD/CD > 1.5 or ZSE > 50% (Hussain et al. 2015). The range of halo formation was 1.3–3.8 mm. Obaidullah et al. (2011) reported that 9 out of 50 isolates obtained from soils of different parts of Pakistan were identified as ZSB. The best ZSB among the isolates belonged to *Serratia* sp.

In the quantitative evaluation of zinc solubilization, the highest Zn released from the three sources was ordered as Zn oxide (26.54 mg l^{-1}) > Zn phosphate (24.64 mg l^{-1}) > Zn carbonate (19.48 mg l^{-1}). Also, in the semiquantitative method, the HD/CD parameter was ordered as Zn phosphate (1.74) > Zn oxide (1.68) > Zn carbonate (1.61). Through the Pearson correlation coefficient, the linear relationship between the two variables pH and Zn solubilization was significant and inverse. Also, it can be said that more than 80% of zinc dissolution can be explained by pH reduction.

Based on the evaluation results of zinc solubilization (quantitative and semi-quantitative), it seems that the solubility behavior of the isolates was influenced by the kind of present anion in these sources. In some studies, the effect of the type of anion in many sparingly soluble salts on the absorption behavior of cations by bacteria has been reported. For example, Tang et al. (2009) reported that different anions affect the behavior of bacteria in binding and oxidizing cations such as iron and manganese. They reported that different anions, by affecting

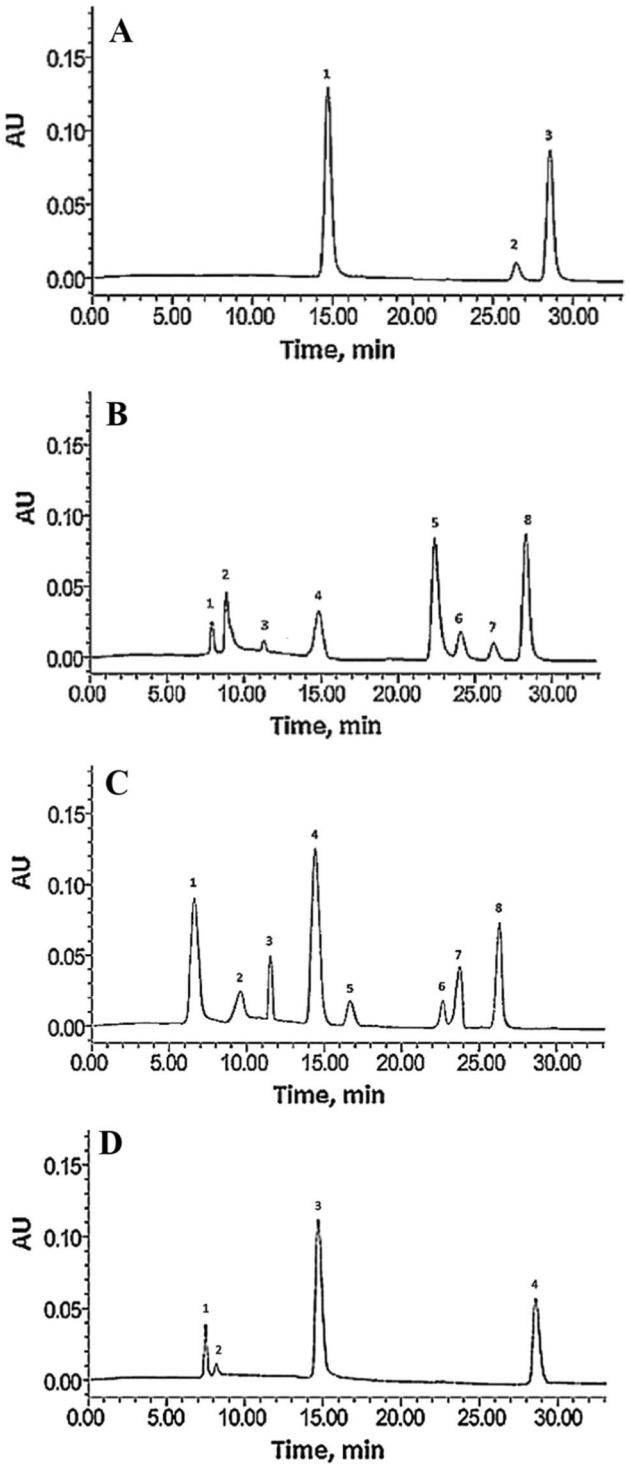

Fig. 4 HPLC analysis and determination of the type and concentration of organic acids produced by promising isolates. **A** Isolate ZO11, **B** isolate ZO14, **C** isolate ZC10, and **D** isolate ZP13

Table 6 Information related to the analysis provided in Fig. 4, HPLC analysis and determination of the type and concentration of organic acids produced by promising isolates. **A** Isolate ZO11, **B** isolate ZO14, **C** isolate ZC10, and **D** isolate ZP13

Legend	No	Retention time (min)	Peak name	Concentration (μg ml^{-1})
A	1	14.561	Gluconic acid	197.543
	2	26.493	Acetic acid	18.532
	3	27.708	Propionic acid	121.949
B	1	7.989	α-Ketoglutaric acid	29.422
	2	8.903	Pyruvic acid	75.547
	3	11.281	Citric acid	11.225
	4	14.968	Gluconic acid	93.073
	5	22.394	Succinic acid	148.852
	6	24.108	Formic acid	38.081
	7	26.117	Acetic acid	19.734
	8	28.343	Propionic acid	146.14
C	1	6.691	Oxalic acid	148.134
	2	9.873	2-Ketogluconic acid	55.015
	3	11.581	Citric acid	51.001
	4	14.389	Gluconic acid	202.523
	5	16.917	Malic acid	39.569
	6	22.820	Succinic acid	28.927
	7	23.865	Formic acid	67.037
	8	26.234	Acetic acid	118.306
D	1	7.469	α-Ketoglutaric acid	57.787
	2	8.118	Pyruvic acid	22.621
	3	14.929	Gluconic acid	168.302
	4	28.802	Propionic acid	98.598

ligand–metal arrangements, could alter the ligand–metal complex's formation or dissociation constant (KF and KD), thereby affecting the binding and oxidation of manganese and iron cations at bacterial reaction centers. Also, these researchers reported that anions could also affect the dependence of cation dissolution on pH changes.

The differences in the results of Zn solubilization could be attributed to the nature of the experimental methodologies adopted for quantitative and semiquantitative estimation (Sarikhani et al. 2019b). It seems that the evaluation of Zn solubilization in the solid medium is closer to the natural conditions of the rhizosphere, and on the other hand, the evaluation of Zn solubilization in a liquid medium can reveal their maximum solubilization potential (Khoshru et al. 2019). The bacterial ability of Zn solubilization from Zn oxide, Zn carbonate, and Zn sulfide compounds has been reported in soil by Tariq et al. (2007) and in vitro conditions (in liquid media) by Saravanan et al. (2007a, b). Saravanan et al. (2004) reported that the bacterial species *Pseudomonas* sp. and *Bacillus* sp. could solubilize many compounds containing low-soluble Zn in a liquid medium. Inoculation of *Gluconacetobacter diazotrophicus* PA15 in broth medium was reported to solubilize low-soluble Zn after 48 h (Saravanan et al. 2007a, b). Fasim et al. (2002) reported that *P. aeruginosa* has shown

Table 7 Results of bacterial identification

Strain	Morphology	GT	CA	OA	*M*	NCBI code	BS (%)	Identification
ZC10	Coccobacilli	+	−	−	−	MZ618725	99.55	*Agromyces* sp.
ZO11	Coccobacilli	−	+	+	−	MZ618723	99.36	*Acinetobacter calcoaceticus*
ZO14	Rod like to cocci	+	+	−	−	OQ076273	99.50	*Agromyces italicus*
ZP13	Coccobacilli	−	+	−	−	OQ076550	99.61	*Pantoea agglomerans*
ZP15	*Bacilli*	−	−	+	+	OQ076696	99.66	*Stenotrophomonas rhizophila*

GT gram type, CA catalase activity, OA oxidase activity, M motility, BS BLAST similarity

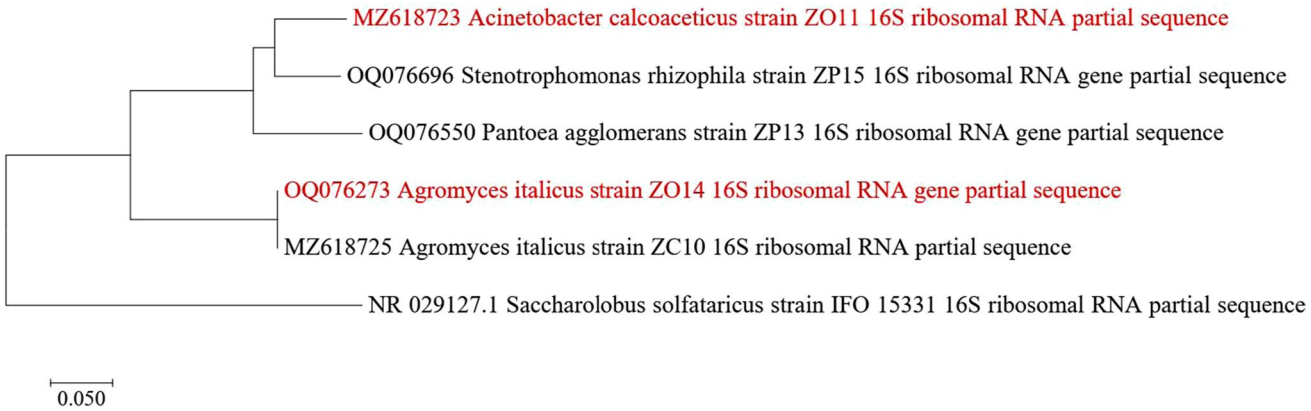

Fig. 5 Phylogenetic tree with accession numbers (analyses were performed using MEGA version 7.0.14) based on 16S rRNA sequences shows the position of isolated strains with other obtained strains (the promising isolates are distinguished by red). The tree was constructed using the neighbor-joining method with the bootstrap analyses of 1000 cycles. *Saccharolobus solfataricus* is presented as an out-group. The 16S rRNA sequences of the *S. solfataricus* strain were downloaded from NCBI GenBank database

the ability to Zn solubilize from a source of Zn oxide in a broth medium. Dinesh et al. (2018) reported in a study that ZSB optimally dissolves both low-soluble compounds of Zn (Zn oxide and carbonate), although the rate of solubilization in Zn oxide was higher than that of Zn carbonate. ZSB3 isolate showed the highest zinc solubilization potential (62.48 mg l^{-1}). A positive correlation was obtained between Zn solubilization and decreasing pH.

According to the results, promising ZSB isolates had different potentials in terms of PGP traits. Isolate ZO11 could produce auxin; isolate ZO14 had multi-PGP properties including auxin production, siderophore production, phosphorus, and potassium solubilization; ZP15 isolate had auxin production, phosphorus, and potassium solubility; and isolate ZP13 produced siderophore and auxin and had phosphorus and potassium solubility. Two isolates ZC10 and ZO10 produced poor performance in terms of in vitro PGP characteristics.

The fact that rhizosphere bacteria may have several plant growth-stimulating behaviors has been shown in numerous reports (Eshaghi et al. 2019; Khoshru et al. 2020b), and this microbial behavior is a very high advantage compared to one-dimensional chemical fertilizers (Khoshru et al. 2020b).

In the greenhouse experiment results, two isolates, ZC10 and ZO10, showed poor performance (although they had good Zn solubilization performance from Zn-CO_3 and Zn–O, respectively, under in vitro conditions). Isolate ZP13 did not perform well in greenhouse conditions in terms of PGP properties, although it had good performance under the in vitro condition. This isolate had the highest production of siderophore under in vitro conditions and in greenhouse conditions; it was able to provide the highest iron uptake for plant roots (isolate ZO11 also had the same conditions). However, this isolate has not been successful in Zn uptaking for the plant.

Zn bioavailability increasing in the rhizosphere has been reported by chelating compounds (Obrador et al. 2003). Chelating compounds reduce the reactivity of Zn in soil. Zn chelated has high accessibility for plant roots. Through the production of siderophore and other organic ligands, microbes can supply the zinc required by the plant (Ghavami et al. 2016). For example, *Microbacterium saperdae*, *Enterobacter cancerogenesis*, and *Pseudomonas monteilii* are bacteria that have improved Zn bioavailability to plants by producing siderophores (Whiting et al. 2001). It has been reported that the bacteria *Pseudomonas* sp. (96–51), *Agrobacterium* sp. (Ca-18), and *Azospirillum lipoferum* (JCM-1270, ER-20) have provided the Zn required to the rice by producing chelators (Tariq et al. 2007).

The high potential of low-soluble phosphate solubilization by isolate ZP13 (also isolate ZP15) may be one of the possible reasons for the low uptake of Zn by plants treated with these isolates. It is possible that the phosphate dissolved by these isolates had a negative effect on the Zn uptaking by the plant because the Zn-phosphate complex has low solubility and easily precipitates in the soil and further decreases its accessibility for plant roots (Xie et al. 2019).

In terms of PGP properties under in vitro conditions, the ZP15 isolate had a moderate performance compared to other promising ZSB, but this bacterium in greenhouse condition showed a good performance in promoting plant growth. This isolate, although equal to ZO11 and ZO14 isolates in terms of plant morphological characteristics, performed relatively moderately in uptaking elements for the plant. Isolate ZO11 well performed under in vitro, only in the solubilization of Zn from the source of Zn oxide and also in the production of auxin (dependent on the presence of the amino acid L-tryptophan), while isolate ZO14 produced a relatively good performance in the solubilization of Zn from all three sources. Also, this isolate had high averages in the parameters of P solubilization from the TCP source, K release from mica, siderophore production, and auxin production (with and without L-tryptophan).

The promising strains in this experiment certainly were ZO11 and ZO14, but it seems that ZO11 only helped the plant through the production of auxin and changes in the root architecture, but ZO14 isolate has had all its beneficial effects on the plant. There are various reports that inoculation of ZSB on plants has also increased the plant Zn content (Biari et al. 2008; Tariq et al. 2007). Whiting et al. (2001) reported that the bioavailability of Zn in the rhizosphere increased by about 0.45-fold due to inoculation with ZSB. An 18% increase in Zn content in the wheat plant has been reported due to inoculation with two bacteria, *Azotobacter* and *Azospirillum* (Eleiwa et al. 2012). Similarly, inoculation of these two bacteria in corn plants has led to a significant increase in Zn content in corn grain (Biari et al. 2008). Mishra et al. (2012) reported that inoculation of a consortium of two bacteria *Pseudomonas* sp. and *Rhizobium leguminosarum* PR1 improved Zn uptake in the shoot part of rice and increased the Zn content of rice grain by 133% compared to uninoculated control treatment. Signs of Zn deficiency in wheat and barley have been corrected by inoculation with *Bacillus* M-13 and *P. aeruginosa* 7NSK2 (Sadaghiani et al. 2009).

According to the HPLC results, the most important organic acids produced by ZO11 isolate were gluconic acid and propionic acid; for ZO14 isolate succinic acid and propionic acid; for ZC10 isolate gluconic, oxalic, and acetic acids; and for ZP13 isolate gluconic acid and propionic acid. In general, by literature reviewing, it seems that the two organic acids, gluconic and propionic acid, are the most important organic acids produced by ZSB in the process of solubilizing zinc from various low-soluble sources. Various studies have reported that gluconic acid is the most important organic acid involved in the process of solubilization of low-soluble zinc sources (Yasmin et al. 2021). However, in this study, it was observed that propionic acid also has an influential role in the Zn solubilization process.

It has been reported the solubilization of Zn and its bioavailability has increased due to the production of organic acids in *Pseudomonas* sp. and *Bacillus* sp. (Saravanan et al. 2004). Fasim et al. (2002) reported that the production of many organic acids such as 2-ketogluconic acid along with proton secretion was the mechanisms involved in Zn solubilization. Some of the organic acids produced by ZSB bacteria include gluconic acid, cinnamic acid, ferulic acid, syringe acid, gallic acid, caffeic acid, and chlorogenic acid (Saravanan et al. 2007b; Fasim et al. 2002; Tariq et al. 2007).

Identification of promising isolates under in vitro experiments showed that they belong to the genera *Agromyces*, *Acinetobacter*, *Bacillus*, and *Pantoea*. Among the above bacteria (6 isolates), isolates ZO11 and ZO14 and ZP15 had significant results in increasing the yield of maize, but in ZP15 despite stimulation of plant growth and development, signs of Zn deficiency were observed in the plant treated with this isolate, while in isolates ZO11 and ZO14, no signs of zinc deficiency were observed. According to the identification results, these two bacteria belonged to *Acinetobacter calcoaceticus* and *Agromyces italicus*, respectively.

5 Conclusion

According to the results of this research, the promising isolates ZO11 and ZO14 belonging to the genera *Acinetobacter* and *Agromyces* showed the ability to eliminate Zn deficiency in maize plants. The results of this experiment showed that these rhizospheric isolated bacteria can be used as an eco-friendly approach to supply Zn required by plants from various low-soluble sources, which will lead to a reduction in the use of Zn chemical fertilizers and ensure human food security. Before using these isolates in the formulation of biofertilizers and their commercialization, field tests are necessary to confirm their efficiency in zinc supply to plants.

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Author Contribution All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Bahman Khoshru, Mohammad Reza Sarikhani, Shahin Oustan, Adel Reyhanitabar, and Mohammad Ali Malboobi. The first draft of the manuscript was written by Bahman Khoshru, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability This manuscript contains the data obtained from the doctoral thesis. All the data used in this manuscript are completely transparent and available and can be provided at the request of the respected editor of the journal.

Code Availability Software application or custom code — phylogenetic tree analyses were performed using MEGA version 7.0.14.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Conflict of Interest The authors declare no competing interests.

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