Plant growth promoting properties of *Serratia fonticola* ART-8 and *Pseudomonas putida* ART-9 and their effect on the growth of spring wheat (*Triticum aestivum* L.)

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ABSTRACT

Two bacterial strains, *Serratia fonticola* ART-8 and *Pseudomonas putida* ART-9, were isolated from soil sown with rye in a monoculture. Although the *S. fonticola* strain produced more chitinase than the *P. putida* strain, *P. putida* produced more cellulase, lipase, HCN, and fluorescent siderophores. *P. putida* also solubilized more phosphate, and was the only strain to produce pyoverdine. Neither bacteria produced indoleacetic acid (IAA), nor did they produce siderophores on the CAS medium. Neither of the strains was highly effective at inhibiting *Fusarium culmorum* (~5%) or *F. oxysporum* (~24%). Wheat plants inoculated with these bacterial strains had higher (5.7-10.0%) thousand kernel weight and there appeared to be a positive association between thousand kernel weight and ear length.

INTRODUCTION

Intensive farming and the use of chemicals in agriculture contribute to soil degradation, disrupt biochemical and biological processes, reduce the counts of soil-dwelling microorganisms, and lead to the loss of biodiversity (Hinsinger et al. 2009; Jacobsen and Hjelmsø 2014; van der Heijden et al. 2008). However, biodiversity protection should be one of the key goals of agriculture because soil microorganisms help transform organic matter and minerals in the biogeochemical cycle. For this reason, the popularity of organic fertilizers and crop protection products has been growing in recent years. Plant Growth Promoting Bacteria (PGPB) and Plant Growth Promoting Rhizobacteria (PGPR) improve the growth and development of crop plants. These microorganisms are present in the soil, the rhizosphere, and the rhizoplane, where they enter into symbiotic relationships with roots. They stimulate root growth by, e.g. producing phytohormones like indoleacetic acid, increase nutrient uptake by, e.g. increasing the bioavailability of phosphorus (Chen et al. 2006) and produce antibiotics, enzymes, and substances that minimize the adverse effects of biotic and abiotic stress. Selected PGPR are used as biocontrol agents to protect plants against pathogens (Ahemad and Kibret 2014; Beneduzi et al. 2012). One way in which they can do this is by producing chelating substances like siderophores, and pyoverdine (Meyer 2000), which bind and solubilize iron, thus allowing the PGPR to compete with pathogens.

Plant growth promoters and biocontrol agents include a wide variety of microorganisms, including bacteria of the genera *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*,

Enterobacter, *Erwinia*, *Flavobacterium*, *Microbacterium*, *Pseudomonas*, *Rhizobium*, and *Serratia*, and this group could be considerably expanded (Ahemad and Kibret 2014; Bhattacharyya and Jha 2012; Mendes et al. 2012).

This study evaluated the growth-promoting properties of *Serratia fonticola* ART-8 and *Pseudomonas putida* ART-9 and their effect on the thousand kernel weight and the length of ears and stalks of spring wheat. It was found that wheat plants that were inoculated with ART-8 and ART-9 had higher thousand kernel weights than control plants.

MATERIALS AND METHODS

Bacterial isolation

The experimental strains were collected from soil where rye had been grown in a monoculture for 45 years. Soil was sampled at a depth of 30cm. 10g soil samples were diluted with sterile water containing 0.85% NaCl, and 0.1mL of the solution was added to a selective medium for the isolation of *Arthrobacter* spp. (Hagedorn and Holt 1975). Two strains were chosen for further study.

Bacterial identification

DNA from pure culture was isolated with the use of a Genomic Mini AX Bacteria Spin kit (A&A Biotechnology Poland). The 16S rDNA sequences were amplified with the 27F (AGAGTTTGATCCTGGCTCAG and 1492 (GGTTACCTTGT TACGACTT) primers described by Lane (1991). The PCR reaction was carried out in $25\mu L$ solutions containing $2\mu L$ of DNA ($10ng \cdot \mu L^{-1}$), $2.5\mu L$ of 10X Buffer with Mg^{2+} (A&A Biotechnology, Poland), $0.2\mu M$ of each primer (IBB, Poland), 0.25mM of each dNTP (A&A Biotechnology, Poland), and 1U of RUN polymerase (A&A Biotechnology, Poland). Amplification was carried out in a Labcycler thermocycler (SensoQuest, Germany) as follows: initial denaturation at 95℃ for 3min; then 32 cycles at 95℃ for 30s, 55℃ for 30s, and 72℃ for 90s with final extension at 72℃ for 5min.

Sequencing was outsourced (Genomed, Poland), and the results were compared with the GenBank database using the BLAST algorithm (Altschul et al. 1990). Both sequences were

deposited in GenBank under accession numbers KY498623 and KY498624.

Analysis of biochemical and physiological characteristics

The strains were cultured on tryptic soy agar (TSA, Merck) at various temperatures. Cellulase production was determined by the method proposed by Hankin et al. (1971) with carboxymethylcellulose (CMC) as the carbon source. Chitinase production was evaluated according to the method described by Roberts and Selitrennikoff (1988) and modified by Ashwini and Srividya (2014) to evaluate bacteria's ability to produce pyoverdine (Succinate Medium-SM), hydrogen cyanide (HCN), indoleacetic acid (IAA), lipase and protease with the use of the media and the procedures described by Ghodsalavi et al. (2013). The strains' ability to produce siderophores was determined on Chromasurol S (CAS) medium according to the protocol developed by Milagres et al. (1999). Phosphate-solubilising ability was tested on National Botanical Research Institute's phosphate growth medium (NBRIP) (Nautiyal 1999) with the protocol modified by reducing the time of incubation from 14 days to 7 as in Przemieniecki et al. (2015).

Dual plate assay

The ability of the bacterial strains to inhibit the growth of *Fusarium culmorum* and *F. oxysporum* was investigated on potato dextrose agar (PDA, A&A Biotechnology, Poland) according to the method described by Przemieniecki et al. (2014). In the control sample, the bacterial suspension was replaced with demineralized water. The following equation was used to calculate the percent inhibition of fungal growth with each strain:

$$\text{Inhibition } (\%) = [1 - \frac{\text{fungal growth in the presence of bacteria}}{\text{fungal growth in the control variant}}] \times 100$$

Greenhouse tests

Greenhouse tests were performed in pots containing 8kg of soil, the characteristics of which are described in Table 1. Before sowing, kernels were rinsed in a 1% CMC solution

Table 1. Characteristics of soil used in the pot experiment.

Property	Value
Textural class	sandy loam
pH (KCl)	5.8
Total nitrogen [%]	0.013
Phosphorus as P_2O_5 (mg P·100g^{-1} soil)	24.5
Potassium as K_2O (mg K·100g^{-1} soil)	20.0
Magnesium (mg Mg·100g^{-1} soil)	4.5
Nitrogen fertilization (mg N·kg^{-1} soil)	125
Potassium fertilization (mg K·kg^{-1} soil)	125
Addition of $C_3(PO_4)_2$ (mg P·kg^{-1} soil)	25

containing $5 \cdot 10^8$ CFU·mL^{-1} of bacteria. Twenty-four kernels of spring wheat cv. Bombona were sown in each pot at a depth of 1cm and there were 3 pots for each variant. The spring wheat plants were harvested in BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) stage 89. Ear and stalk length were measured in our laboratory. Thousand kernel weight and the organic matter content of ears were determined.

Statistical analyses

All experiments were performed with three replications. To test the significance of differences between ART-8, ART-9, and the control, one-way ANOVA was used followed by Duncan's test. Results were considered statistically significant at $p < 0.05$. STATISTICA 10 was used for all calculations. All results are given as mean±standard deviation.

RESULTS

The bacteria were identified as *Serratia fonticola* ART-8 and *Pseudomonas putida* ART-9 by 16S rDNA sequence analysis. The bacteria were able to grow at each of the tested temperatures. The colony growth rate was highest at 28°C, whereas at 11°C and 4°C, the colony growth rate was lower (Table 2), and the colonies were smaller.

In general, the properties of *P. putida* ART-9 indicated that it would promote plant growth better than *S. fonticola* ART-8 (Table 2). Although the *S. fonticola* strain produced more chitinase than the *P. putida* strain, *P. putida* produced more cellulase, lipase, HCN, and fluorescent siderophores. *P. putida* also solubilized more phosphate, and was the (only) strain to produce pyoverdine. Neither bacteria produced indoleacetic acid (IAA), nor did they produce siderophores on the CAS medium.

Table 2. Selected biochemical and physiological traits of *Serratia fonticola* ART-8 and *Pseudomonas putida* ART-9.

| Trait	Result	
	ART-8	**ART-9**
16SrDNA sequence analysis	*Serratia fonticola* (99%)	*Pseudomonas putida* (100%)
Temperature tolerance		
grown in 28°C	yes (good)	yes (good)
grown in 11°C	yes (average)	yes (average)
grown in 4°C	yes (average)	yes (average)
Plate test		
cellulase production	yes (poor)	yes (average)
chitinase production	yes (poor)	no
lipase production	yes (average)	yes (good)
fluorescent siderophore production	no	yes (average)
phosphate solubilisation	yes (poor)	yes (average)
HCN production (qualitative)	no	yes
protease production	yes (good)	yes (good)
siderophore production	no	no
Indoleacetic acid (IAA) production	no	no
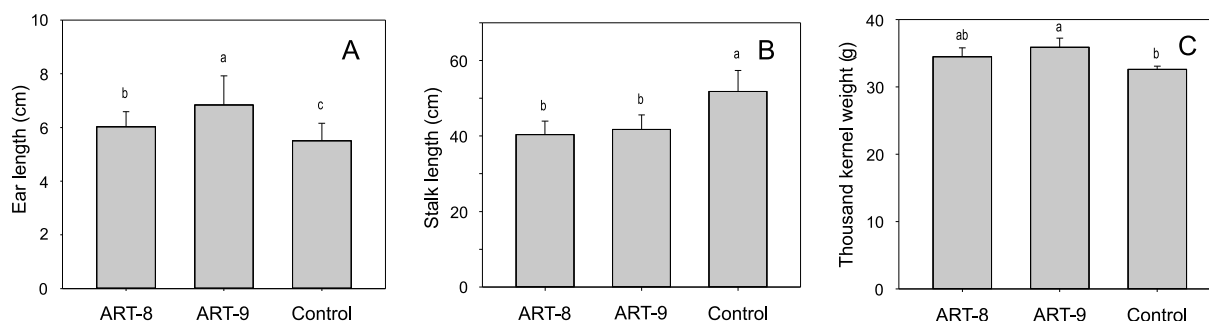
Figure 1. Ear length (A), stalk length (B), and thousand kernel weight (C) of spring wheat after inoculation with *Serratia fonticola* ART-8 and *Pseudomonas putida* ART-9. Graphs show mean and standard deviation. Different superscripts (a, b, c) indicate that variants differed significantly from each other ($p < 0.05$).

The results of the dual plate test were nearly identical for both strains. Both bacteria exerted only a small and non-significant inhibitory effect on the growth of *Fusarium culmorum* (*S. fonticola* ART-8, 6.7±2.3%; *P. putida* ART-9, 4.0±0.0%), and although both strains inhibited the growth of *F. oxysporum*, neither strain was particularly effective at this (*S. fonticola* ART-8, 24.4±1.9%; *P. putida* ART-9, 23.3±6.7%).

In the greenhouse test, treating kernels with *S. fonticola* ART-8 and *P. putida* ART-9 improved the characteristics of spring wheat, and the improvement with *P. putida* was more than with *S. fonticola*. Plants that were inoculated with ART-9 had significantly higher thousand kernel weight that control plants (10% higher). Although plants that were inoculated with ART-8 also had higher thousand kernel weight than control plants (5.7% higher), this difference was not statistically significant.

There appeared to be a positive association between thousand kernel weight and ear length. The mean ear lengths of the two variants that were inoculated were significantly longer than that of the control variant. Although inoculated plants had significantly shorter stalks than control plants, there was less variation in their stalk lengths (ART-8, 40.4±3.5; ART-9, 41.7±3.9; control, 51.8±5.6).

The mean thousand kernel weight of plants inoculated with ART-9 was significantly higher than that of the control plants (approximately 10% higher). Although the thousand kernel weight of plants inoculated with ART-8 was also higher than that of the control plants (approximately 5.7%), this difference was not statistically significant (Figure 1).

DISCUSSION

The results of this study indicate that *Pseudomonas putida* ART-9 promotes the growth of wheat plants to a greater extent than *Serratia fonticola* ART-8. In addition, the characteristics of *P. putida* ART-9 indicated that it would exert more positive effects on agricultural ecosystems than *S. fonticola* ART-8. The high potential of *P. putida* as a PGPR and a biocontrol agent has been demonstrated by numerous studies (Ahemad and Khan 2011, 2012). Our results also confirmed the findings of Bhattacharyya and Jha (2012) and Ahemad and Kibret (2014). It should also be noted that several authors have suggested that *S. fonticola* strains could be pathogenic for humans (Aljorayid et al. 2016; Tasic et al. 2013), which is another reason to prefer *P. putida* over *S. fonticola*.

The plant growth promoting properties of the bacterial strains examined in the present study are not as potent as those of other strains and species. For example, Przemieniecki et al. (2015) found that the phosphorus solubilization capability of *P. putida* strain SP0113 is nearly twice as high as that of strain ART-9. In addition, Ashwini and Srividya (2014) found that *Bacillus subtilis* inhibits the growth of *Fusarium solani* and *F. oxysporum* by around 40%, nearly twice as much as the two strains tested in the present study.

In summary, *P. putida* ART-9 and *S. fonticola* ART-8 improved the growth of spring wheat. Both bacterial strains had various characteristics that should promote plant growth. However, *P. putida* is more suitable for use as a PGPB because not only does it have more positive effects on the growth of spring wheat, but also because *S. fonticola* may cause opportunistic infections in humans.

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