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RESEARCH ARTICLE

Antibiotic sensitivity of phosphate-mobilizing rhizobacteria of wheat rhizosphere

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Abstract

Antibiotic sensitivity is a serious problem, especially relevant when using bacterial biofertilizers. Many strains of rhizobacteria used as bacterial biofertilizers and biofungicides demonstrate resistance to several antibiotics or contain antibiotic resistance genes. In this study, we investigated the antibiotic sensitivity of 12 strains of phosphate-mobilizing rhizobacteria isolated from the rhizosphere of wheat grown on irrigated lands of the Sirdarya, Tashkent andijan and Kashkadarya regions of Uzbekistan in 2021, belonging to the genera *Enterobacter*, *Rahnella*, *Bacillus*, *Pantoea* and *Pseudomonas*, to seven antibiotics of different classes: erythromycin, streptomycin, gentamicin, chloramphenicol, amikacin, tetracycline and cephalexin. Most strains showed high sensitivity to aminoglycosides, chloramphenicol and tetracycline, while some strains showed resistance to erythromycin, streptomycin and cephalexin. Moderate resistance to streptomycin, tetracycline and cephalexin was noted, especially among strains of the genus *Enterobacter*. Strains of the genus *Rahnella* showed moderate resistance to streptomycin, gentamicin, tetracycline and cephalexin. Strains of the genus *Bacillus* showed moderate resistance to chloramphenicol, tetracycline and cephalexin; the diameter of the zones of growth inhibition was 15 mm to 17 mm. The strain *P. agglomerans* 19 showed moderate resistance only to tetracycline, while remaining sensitive to other antibiotics, but this result requires confirmation through molecular genetic studies in the future. The data revealed are important for assessing the safety of phosphate-mobilising rhizobacteria when used as bacterial fertilisers, taking into account their resistance, to minimise the risk of spreading antibiotic resistance genes (ARGs) in agroecosystems.

Keywords: agroecosystems; antibiotic resistance; microbial biofertilizers; phosphate-mobilising rhizobacteria; wheat

Introduction

Plant growth-promoting bacteria (PGPB) have a number of beneficial properties that make them widely used in sustainable agriculture. The use of these microorganisms helps to reduce dependence on chemical fertilisers, increases soil fertility and improves overall plant health (1-3). An important concern is the presence of antibiotic resistance genes (ARGs) in many PGPB strains. Introducing multiresistant bioinoculants into agroecosystems may facilitate gene transfer to soil microbes and, ultimately, to animals and humans. Large-scale use of those strains could therefore contribute to the global antibiotic resistance crisis, as ARGs may persist in soils and even enter the food chain (4). In the context of bacterial biofertilizer applications, the presence of antibiotic resistance genes in PGPB represents a potential risk, as these genes may be innate or acquired through horizontal transfer (5). Careful monitoring of agricultural microbial strains is essential to assess their safety and role in resistance spread. In PGPR, resistance has a dual role, serving both as a biosafety concern and as a marker for tracking strain viability in laboratory and field conditions, while also giving strains a competitive advantage in complex microbial communities (6, 7). The application of these strains as bioinoculants in soil systems may pose a risk of horizontal transfer of antibiotic resistance genes to coexisting microorganisms (3). This requires a balanced approach to the selection of bacterial agents for use in agriculture.

Humans may directly or indirectly introduce antibiotic resistance genes into plant-associated microbial communities. Risk assessment of PGPB strains carrying ARGs remains limited, despite their continued promotion as biofertilizers. The presence of ARGs may further enhance their survival in antibiotic-contaminated soils (8). The large-scale use of biofertilizers carrying ARGs may extend antibiotic resistance from agricultural soils to natural habitats, aquatic systems, animals and humans. Thus, while resistant strains

can support sustainable farming, they also raise serious concerns about the global spread of antimicrobial resistance and its threat to public health (4).

Although there is evidence for the presence of antibiotic resistance genes in some PGPB strains, the extent of the risk of their transmission to other soil microorganisms and their possible impact on human pathogenic bacteria are still poorly understood (4). Antibiotics create selection pressure by promoting the spread of resistant forms in the environment. This problem is of particular relevance due to the uncontrolled use of antibiotics in medicine and veterinary medicine, which, in turn, leads to an increase in the number of multidrug-resistant strains and a decrease in the effectiveness of antibiotic therapy, increasing the frequency of severe and fatal infections (9-11). Antibiotics enter the environment in various ways, the main ones being wastewater from medical institutions, household and animal waste and drug residues in food and feed (12, 13). The agricultural sector, where antibiotics are widely used as growth stimulants in farm animals, creates a particularly significant load on ecosystems. As a result, significant amounts of antibiotics enter the soil, forming selective pressure and promoting the spread of resistance genes in agrocenoses (14).

The large-scale use of PGPB strains requires careful evaluation of their biological and environmental traits, particularly antibiotic sensitivity. Assessing the antibiotic response of phosphate-mobilizing bacteria is therefore an essential step in determining their safety and effectiveness. This study examined the sensitivity of wheat-associated phosphate-mobilising rhizobacteria to several commonly used antibiotics.

Materials and Methods

The work studied twelve strains of phosphate-mobilizing rhizobacteria isolated from the rhizosphere of wheat and identified on the basis of morphological and physiological-biochemical characteristics. The identification was additionally confirmed using MALDI-TOF mass spectrometry. The isolates belonged to the genera *Enterobacter*, *Rahnella*, *Bacillus*, *Pantoea* and *Pseudomonas* (15-17). *Rahnella* strains were further identified based on molecular genetic characteristics as described in our previous study. The 16S rRNA gene of *Rahnella aquatilis* strain UT4 was sequenced and compared with sequences available in the National Center for Biotechnology Information database. The analysis showed 99 % identity with several *Rahnella aquatilis* sequences, including accession numbers MN826597.1, MN826570.1, MT256284.1, CP036490.1 and CP034483.1 (17).

Cultivation of rhizobacteria

Rhizobacterial strains were cultivated on a shaker at 220 rpm at a temperature of 28 ± 2 °C for 24 hours under aerobic conditions in a peptone liquid nutrient medium with the addition of glucose and NaCl, pH 7.0 ± 0.1. (18).

Study the sensitivity of rhizobacteria to antibiotics

Antibiotic sensitivity of rhizobacteria was evaluated by the disk-diffusion method according to the standard of Clinical and Laboratory Standards Institute (CLSI), 30th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2020 (19). The antibiotics used were erythromycin (Ery, 15 µg), streptomycin (Str, 10 µg), gentamicin (Gen, 10 µg), chloramphenicol (Chl, 30 µg), amikacin (Amk, 30 and 50 µg), tetracycline (Tet, 30 µg) and cephalexin (Cep, 75 µg). The diameter of growth inhibition zones was measured in millimetres and interpreted as sensitive (19 mm-36 mm), moderately sensitive (14 mm-18 mm) or resistant (0 mm-13 mm). The diameters of growth inhibition zones around the disks were measured after 24 hr of incubation at 28±2 °C.

Statistical analysis

All experiments were performed three times to ensure the reliability and reproducibility of the results. Statistical analysis was performed using a standard licensed ANOVA software package. Differences between groups were considered statistically significant at $p \leq 0.001$.

Results and Discussion

The results of the study showed significant variability in the sensitivity of phosphate-mobilising rhizobacteria to the antibiotics studied. The data are presented in Table 1. Overall, 75 % of strains showed sensitivity to chloramphenicol and aminoglycosides (amikacin), indicating the potential for the use of these bacteria without the risk of spreading resistance to these classes of antibiotics. Moderate resistance to streptomycin, tetracycline and cephalexin was noted, especially among strains of the genus *Enterobacter*. Strain of *E. cloacae* 7 is resistant to erythromycin, tetracycline and cephalexin. Strains of the genus *Rahnella* showed moderate resistance to streptomycin, gentamicin, tetracycline and cephalexin. Strain of *R. aquatilis* UT4 showed resistance to cephalexin and the diameter of the growth inhibition zone was 5.7 ± 2.5 mm, respectively. Strains of the genus *Bacillus* showed moderate resistance to chloramphenicol, tetracycline and cephalexin; the diameter of growth inhibition zones was 15 mm to 17 mm. Strain of *B. subtilis* 24 showed resistance to gentamicin. Strain of *P. agglomerans* 19 showed moderate resistance only to tetracycline, while they were sensitive to the other antibiotics, but this result requires confirmation through molecular genetic studies in the future. The diameter of growth inhibition zones was 19 mm-30 mm. Among all the strains studied, *Pseudomonas antarctica* 23 depicted the highest level of multiple resistance. This exhibited resistance to gentamicin, amikacin, tetracycline and cephalexin (Table 1 and Fig. 1).

Antibiotic-resistant bacteria in biofertilizers may reach surface and groundwater after soil application, transferring resistance genes to local microbial communities and contributing to the accumulation of ARGs in environmental reservoirs (20). Moreover, strains able to colonize plants as endophytes can potentially transmit resistance genes to consumers through plant-derived products (21). The results obtained are consistent with the literature data, according to which Gram-positive bacteria of the genus *Bacillus* usually show high sensitivity to most antibiotics, while Gram-negative representatives, especially *Pseudomonas*, are more resistant due to their multistage defense mechanisms, notably active release of antibiotics (22, 23).

Many strains of rhizobacteria used as PGPB inoculants and biocontrol agents, in particular representatives of the genera *Pseudomonas* and *Bacillus*, demonstrate resistance to several antibiotics and/or contain antibiotic resistance genes (24-26). Thus, research indicates that phosphate-mobilising bacteria were isolated and their resistance was tested against various antibiotics, including kanamycin, streptomycin, tetracycline, ampicillin, chloramphenicol and spectinomycin (13). As a result, 11 out of 13 strains were found to be resistant to at least one of these drugs (27). Similarly, research

Table 1. Sensitivity of phosphate-mobilising rhizobacteria to antibiotics inhibitory zone diameter (mm)

Shtamm	Ery 15	Str 10	Gen 10	Chl 30	Amk 30	Amk 50	Tet 30	Cep 75
E. cloacae 7	12.0 ± 1.5	17.3 ± 3.5	17.7 ± 2.5	18.0 ± 2.0	18.7 ± 2.5	18.3 ± 2.1	0.0 ± 0.0	12.3 ± 2.5
***E. cloacae* 12**	30.3 ± 2.5	28.7 ± 4.6	29.7 ± 3.1	21.7 ± 3.1	31.0 ± 3.6	29.7 ± 3.1	16.7 ± 2.1	14.3 ± 2.5
E. cloacae 18	17.7 ± 2.5	17.0 ± 2.0	19.7 ± 3.1	25.3 ± 3.1	22.0 ± 2.0	20.0 ± 3.0	20.3 ± 2.5	17.7 ± 1.5
R. aquatilis UT4	35.0 ± 2.6	17.0 ± 2.0	17.0 ± 2.0	20.3 ± 2.5	20.0 ± 2.0	20.3 ± 2.5	15.7 ± 4.7	5.7 ± 2.5
R. aquatilis 14	17.0 ± 1.0	21.0 ± 2.0	21.3 ± 2.1	23.0 ± 2.6	28.0 ± 2.6	23.0 ± 3.6	18.0 ± 3.0	13.3 ± 1.5
B. cereus 22	27.3 ± 2.1	30.3 ± 3.1	30.3 ± 2.5	16.0 ± 1.5	32.0 ± 3.0	29.3 ± 4.0	19.0 ± 2.0	17.1 ± 2.1
B. subtilis 24	32.3 ± 2.1	31.0 ± 2.0	0.0 ± 0.0	32.7 ± 2.9	27.3 ± 2.1	27.0 ± 2.6	16.0 ± 2.0	20.0 ± 5.0
B. megaterium 29	32.3 ± 2.5	30.0 ± 3.6	32.7 ± 2.5	30.3 ± 2.5	30.0 ± 3.6	26.7 ± 8.1	15.0 ± 1.5	17.0 ± 2.0
P. agglomerans 19	19.0 ± 2.0	20.3 ± 2.5	24.0 ± 3.0	30.0 ± 2.5	24.0 ± 2.0	22.0 ± 3.0	14.0 ± 2.5	20.7 ± 3.5
P. agglomerans 20	22.7 ± 2.5	20.3 ± 4.0	20.7 ± 2.5	32.0 ± 3.0	26.0 ± 3.0	22.3 ± 3.2	22.0 ± 3.0	20.7 ± 2.1
P. kilonensis 30	36.0 ± 3.0	32.7 ± 2.5	32.3 ± 2.5	27.0 ± 2.0	33.0 ± 2.6	32.3 ± 3.5	17.0 ± 2.0	18.0 ± 3.0
P. antarctica 23	15.3 ± 2.5	18.0 ± 3.0	0.0 ± 0.0	27.7 ± 1.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

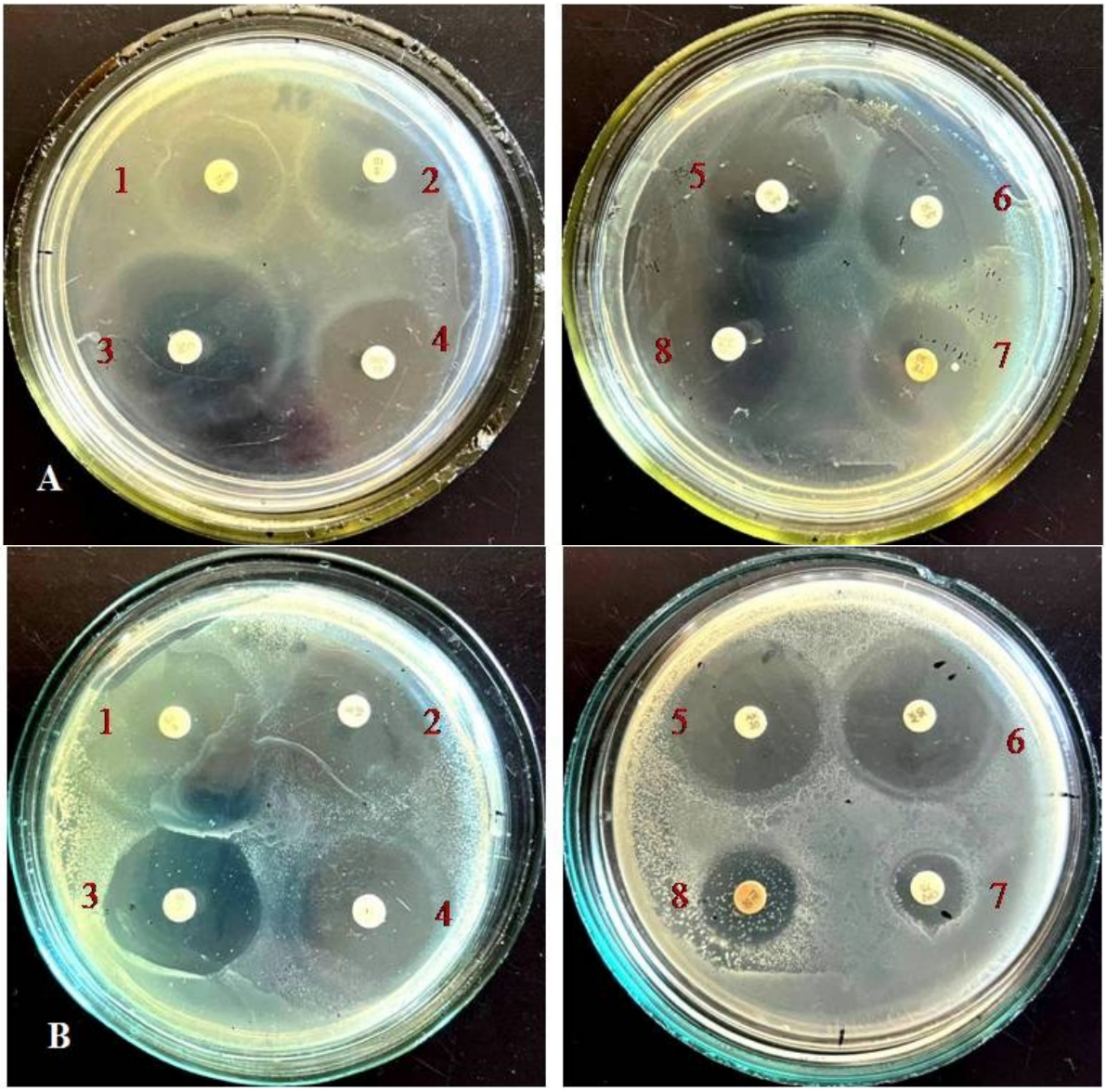

Fig. 1. Antibiotic sensitivity of phosphate-mobilising rhizobacteria *Pantoea agglomerans* 20. **A.** And *Bacillus megaterium* 29; **B.** 1 - erythromycin (Ery, 15 µg); 2 - streptomycin (Str, 10 µg); 3 - gentamicin (Gen, 10 µg); 4 - chloramphenicol (Chl, 30 µg); 5 - amikacin (Amk, 30 µg); 6 - amikacin (50 µg), 7 - tetracycline (Tet, 30 µg); 8 - cefalexin (Cep, 75 µg).

indicates that among 11 phosphate-mobilising bacteria isolated from quinoa rhizosphere, five strains exhibited multiple resistance to at least two antibiotics. It is noteworthy that even widely used biocontrol agents like *Bacillus subtilis*, officially registered in Europe for plant protection against phytophthorosis, may contain ARGs, which require special attention in the development and introduction of microbial preparations in agriculture (28).

Pseudomonas aeruginosa, frequently found in agricultural soils and belonging to the group of plant growth-promoting rhizobacteria, is a multidrug-resistant opportunistic pathogen. This bacterium is capable of carrying antibiotic resistance genes located on both the chromosome and plasmids. The resistance of *P. aeruginosa* to various antibiotics is provided due to the enhanced expression of β-lactamases, the work of efflux systems, changes in the structure of the cell membrane and the ability to modify antibiotic molecules (in particular, aminoglycosides) by chemical means (29, 30). *Pseudomonas chlororaphis* GP72, isolated from the rhizosphere of green pepper, shows resistance to antibiotics, particularly chloramphenicol and bleomycin. The resistance was noticed in the presence of *emrB* and *rarD* genes encoding proteins

involved in efflux systems (31, 32). These data indicate the ability of even environmentally relevant rhizobacteria, such as *P. chlororaphis*, to possess multidrug resistance mechanisms, which calls for careful regulation of their use in agricultural practice.

Strains of *Bacillus cereus* isolated from sunflower rhizosphere also show resistance to a wide range of antibiotics such as hygromycin B, chloramphenicol, streptomycin, penicillin, fosfomycin, bacitracin, teicoplanin and vancomycin. The resistance of these strains is due to the presence of several antibiotic resistance genes like *bl2a*, *bacA*, *bcrA*, *fos* and *vanSA* (33-35). The commercial strain of *Pantoea agglomerans* P10c used as a biocontrol agent exhibited resistance to a wide range of antibiotics, namely chloramphenicol, streptomycin, polymyxin, nalidixic acid, penicillin, bacitracin, novobiocin, fosfomycin, bicyclomycin, kasugamycin, cloxacillin, spectinomycin and fluoroquinolones. Research revealed that there was the presence of multiple antibiotic resistance genes like *acrD*, *ant3IA*, *bacA*, *bl2BE*, *catB3*, *emrD*, *emrA*, *mdtC*, *mdtB*, *mdtA*, *ksgA*, *rarD*, *tolC* and *qnrB* in genomic analysis (36). In contrast to the commercial strain of *P. agglomerans* P10c, which has multiple resistance to a wide range of antibiotics and carries a significant number of antibiotic resistance genes, our isolated strain of *P. agglomerans* 19 showed a limited resistance profile. It was found to be moderately resistant only to tetracycline, maintaining sensitivity to the other antibiotics tested. However, this result required confirmation in our future molecular genetic studies. These differences indicate the potential environmental safety of our strain when used in agrocenoses, as the risk of ARG spread into the environment is significantly lower compared to industrial strains.

Strain of *Enterobacter cloacae* 13047 shows resistance to a wide range of antibiotics, including tetracyclines, chloramphenicol, aminoglycosides, beta-lactams, glycylcyclines, macrolides, cephalosporins, sisomycin, dibecacin, penicillin, tobramycin, gentamicin, netilmicin, acriflavine, carbapenems, fluoroquinolones, trimethoprim and sulfonamides. Resistance is conferred by the presence of multiple ARGs, namely *aac3IIA*, *aac6IB*, *acrA*, *ant2IA*, *bl2F*, *bl3IMP*, *bl1AMPC*, *bl3VIM*, *dfrA17*, *catB3*, *catA2*, *qnrA*, *sul1*, *sul2* and *tetD* (37, 38). The broad spectrum of antibiotic resistance of this strain is of concern, especially given its presence in both natural, clinical and agrarian environments, indicating a high risk of ARG transmission between different ecosystems. Our strain of *E. cloacae* 7 was resistant to erythromycin, tetracycline and cephalexin and showed only moderate resistance to streptomycin. These findings highlight the importance of evaluating the safety and genetic traits of bioagents before their application in agroecosystems. Monitoring antibiotic sensitivity is essential to minimize the risk of resistance gene transfer and to ensure the safe development of rhizobacterial inoculants.

Conclusion

This study underscores the importance of evaluating phosphate-mobilizing rhizobacteria not only for their plant growth-promoting traits but also for their antibiotic resistance profiles. The observed differences among strains emphasize their genetic diversity and the potential risk of resistance gene transfer within soil microbial communities. Strains demonstrating strong phosphate-mobilizing capacity alongside antibiotic sensitivity are promising candidates for the development of safer biofertilizers, whereas moderately resistant isolates, such as *Pantoea agglomerans* 19, require further molecular genetic studies to verify the absence of ARGs. Moreover, the sequencing of *Rahnella aquatilis* strain UT4 contributes additional insights into the diversity of phosphate-mobilizing bacteria and will serve as a reference point in forthcoming comparative analyses. Future work will expand the screening to a broader set of strains and apply molecular approaches, including PCR and sequencing, to characterize resistance determinants more precisely and evaluate their biosafety for agricultural applications.

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Authors' contributions

SIZ designed and carried out experiments, cultivated phosphate-mobilising bacteria and prepared the manuscript. KMK, HKK and NAE participated in the experiments and drafted the manuscript. ZSS coordinated the study. OUJ collected the literature and participated in drafting the manuscript. BKR performed the processing of data and revised the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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