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Proceeding Paper

Identification and Characterization of Potential Chalky Soil Plant Growth Promoting Bacteria (PGPR) Isolated from the Rhizosphere of *Chamaecytisus ruthenicus* (Russian Broom) †

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Abstract: Plant growth-promoting rhizospheric bacteria (PGPR) are well known for their significant roles in agriculture and the environment. In our previous study, 23 chalky soil bacterial isolates were obtained from the rhizosphere of *Chamaecytisus ruthenicus*. Seven out of them were generally reported for their potential effect on plant growth. However, the identification and further characterization of those chalky soil bacteria were not done yet. Therefore, the purpose of the present study was to identify and characterize chalky soil rhizospheric bacteria (seven previously investigated and one additional bacteria). A total of eight bacterial isolates were cultured in LB and other growth media to investigate their morphological behavior, antibitoic sensitivity or resistance status, and their effect on plant growth. Moreover, 16S rRNA gene sequencing was used to identify those potent bacterial isolates. The results of the present study demonstrated that all bacterial isolates obtained stable morphology in the three types of growth media. However, four bacterial isolates (Z11, Z12, Z15 and Z44) showed colour change. The antibiotic test result also revealed that all the tested bacterial isolates except Z11 and Z24 were resistant to both ampicillin (10 μg) and oxacillin (1 μg). where as all bacterial isolates were sensitive to polymyxin (300 units), amoxicillin (20 μg), vancomycin (30 μg), ceftazidime (30 μg), erythromycin (15 μg), ciprofloxacin (5 μg), bacitracin (10 units), and streptomycin (30 μg). The result of growth stimulation effect revealed that few bacterial isolates showed stimulation effect on the germination rate of an oats and lentils, on the shoot length of maize and oats, on the root length of wheats, maize and lentils, on the fresh weight of wheats and oats and on the dry weight of an oat seeds. Furthermore, the 16S rRNA gene sequence analysis result revealed that the bacterial isolates belonged to *Streptomycetes* spp. and *Jantinobacterium* sp. As a conclusion, those potential chalky soil rhizospheric bacteria would have a substantial impact on agriculture and the environment.

Keywords: PGPR; antibiotic sensitivity/resistance; morphological variability; 16S rRNA gene sequencing; plant growth stimulation

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1. Introduction

Plant grwoth-promoting rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth through a wide variety of mechanisms [1,2,7]. The mode and mechanism of PGPR activitity differ depending on the host plant species, soil

type and soil nutritional status [3]. Recently, the use of PGPR steadily increasing [1] and they have been used as bioremediation, biopesticides, biofertilizers, probiotics and antibiotics in modern agriculture [4,8,9]. Many studies have been reported that growth promoting activity has been shown in several PGPR species from the genera *Azotobacter, Azospirillum, Pseudomonas, Acetobacter, Burkholderia, Serratia*, *Bacillus*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Aeromonas*, *Herbaspirillum*, *Acinetobacter*, *Agrobacterium, Bradyrhizobium, Xanthomonas, Stenotrophomonas, Arthrobacter, Streptomyces* [3,5,6]. In our previous study, about 23 chalky soil bacteria were isolated from the rhizosphere of *Chamaecytisus ruthenicus*. Few of them showed antimicrobial activity against phytopathogenic microbes such as *Erwinia herbicola*, *Micrococcus roseus*, *Pectobacterium carotovorum*, *Fusarium avenaceum*, *Rhizoctonia solani*, *Alternaria brassicicola*, *Bipolaris sorokiniana* and *Pythium ultimum*. Moreover, these bacterial isolates also showed growth stimulation effect on the germinated seeds of wheat, maize, oats and lentils [3]. Eventhough few information was available, further characterization will be requried for the complete describption of those chalky soil bacteria. Therefore, the aim of the present study was to describe the morphological behavior on different growth medium, to evaluate the level of bacteria resistance or sensitivity to different antibiotics, to evaluate growth stimulation effect on germinated seeds, and to identify those selective chalky soil bacteria using 16S rRNA gene sequencing.

2. Methods

2.1. Morphological Behavior of Bacterial Isolates

To evaluate the morphological behavior of those selected bacterial isolates, eight bacterial isolates were cultured on three different kinds of growth media: LB with concentration 5% reduced (yeast extract = 0.2 g/L, Peptone = 0.4 g/L, NaCl = 0.2 g/L and Agar = 4 g/L), LB with normal concentration (yeast extract = 1 g/L, Peptone = 2 g/L, NaCl = 1g/L, and Agar = 4 g/L) and sugar growth medium (Tripton = 0.6 g/L, Peptone = 1 g/L, sugar = 2 g/L, NaCl = 1 g/L, and Agar = 4 g/L). The plates were incubated for 48 h at 29 °C. Colony morphology including shape, margin, elevation, surface, colour and pigmentation of each isolate were examined.

2.2. Evaluation of Bacterial Sensitivity or Resistance to Antibiotics

The disc diffusion method was utilized to evaluate the level of sensitivity or resistance of those selected bacterial isolates. Eighten types of antibiotics including Cefotaxime (30 μg), Streptomycin (300 units), Oxacillin (1 μg), Trimethroprim (75 μg), Polymyxin (300 units), Ceftazidime (30 μg), Gentamicin (10 μg), Tetracycline (30 μg), Erythromycin (15 μg), Ofloxacin (5 μg), Vancomycin (30 μg), Bacitracin (10 units), Ampicillin (10 μg), Lincomycin (15 μg), Meropenem (10 μg), Amoxicillin (20 μg), Rifampin (5μg), and Ciprofloxacin (5 μg) were used. The cultured bacterial strains (72 h at 29 °C) were inoculated and evenly distributed on plate containing LB growth medium (composition: yeast extract 1 g/L, peptone 2 g/L, sodium chloride 5 g/L, and agar 20 g/L). Then antibiotic disks were applied on the surface of the inoculated LB growth medium. Thus, the zones of growth inhibition surrounding each antibiotic disk were measured to the nearest millimeter after incubation period (48 h at 29 °C).

2.3. Growth Stimulation Effect of Bacterial Isolates

Seed germination was performed to evaluate growth stimulation effects of bacterial isolates on four seeds (wheat, maize, oats, and lentils). In 10 mL of LB liquid medium, eight bacterial isolates (Z10, Z11, Z12, Z15, Z24, Z26, Z44, and Z82) were cultured and incubated at 29 °C for 72 h. On a total of 36 plates, 25 surface sterilized seeds from each variety were placed. Thereafter, except for the control group, 15 mL of bacterial solution that had beed diluted to an OD 600 of 0.1 was applied to each plate. The control group, however, received merely water as an addition. For a week, 15 mL of water was added to the plates each day. The growth stimulation experiment was performed in triplicate. The

germination rate, shoot length, root length, fresh and dry weight of the seedlings were measured after the seeds had germinated. The data were statistically evaluated using a *t*-test at $p = 0.05$.

2.4. Identification of Bacterial Isolates Using 16S rRNA Gene Sequencing

Genomic DNA was isolated from cells using the Fungal / Bacterial DNA Kit (ZymoResearch, 160 USA) according to the manufacturer's recommendation. The 16S rRNA gene was amplified by PCR using primers universal for 16S rRNA prokaryotes: 27f (5′-AGAGTTTGATCCTGGCTCAG3′) and 1492r (5′-TACGGYTACCTTGTTACGACTT3′). The amplified DNA was purified using the Zymoclean Gel DNA Recovery Kit (ZymoResearch, Irvine, CA, USA). Sequencing of PCR DNA fragments was performed on an Applied Biosystems Genetic Analyzer automatic sequencer. Primary phylogenetic screening of the obtained sequences was performed using the BLAST program [http://www.ncbi.nlm.nih.gov/blast] in the EzBioCloud database (www.ezbiocloud.net). The nucleotide sequences of the 16S rRNA gene obtained for the strain 82 were manually aligned with the sequences of reference strains of the nearest microorganisms. Phylogenetic tree constructed using partial 16S rRNA gene sequences by the neighbor-joining method with a bootstrap test of 1000 replicates was performed using MEGA 11.0.

3. Results

3.1. Morphological Behavior of Bacterial Isolates

Colony morphology of the bacterial isolates were examined. The morphological characteristics of the bacterial isolates were presented in Table 1 and Figure 1. Except colour, all bacterial isolates showed stable morphological characteristics. In general, four bacterial isolates (Z11, Z12, Z15 and Z44) showed colour change in the three types of growth medium.

Table 1. Morphological characteristics of bacterial isolates on the three types of growth media.

Bacterial isolates			Parameters			
	Shape	Margin	Elevation	Surface	Colour	Pigmentation
Z10	Circular	Entire	Flat	Rough	White	Yellow
Z11	Circular	Entire	Flat	Rough	Orange ⁜, Yellow Δ, (Orange and yellow)#	None
Z12	Circular	Undulate	Flat	Rough	White ⁜, Green Δ, White#	Brown
Z15	Circular	Entire	Flat	Rough	Orange ⁜, YellowΔ, (Orange and yellow)#	None
Z24	Circular	Entire	Flat	Rough	White ⁜ Δ #	None
Z26	Circular	Entire	Flat	Rough	Grayish white ⁜ Δ #	None
Z44	Circular	Entire	Raised	Smooth	Purple⁜ Δ, Dark Purple#	None
Z82	Circular	Entire	Raised	Smooth	Purple⁜ Δ, Dark Purple#	None

* = LB growth medium with concentration 5% reduced, Δ = LB growth medium with normal concentration. # = sugar medium.

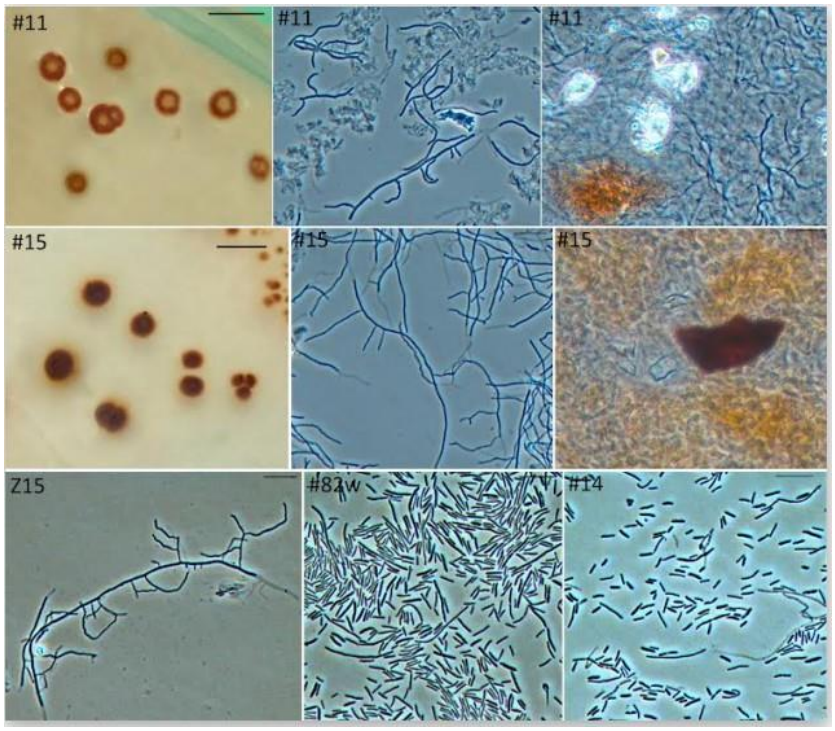

Figure 1. Photo of colonies bar—5 mm. Other photos: bar—10 mkm. For culture No. 11 and No. 15, photographs of colonies + cells after subculture + cells from colonies with crystals are presented. Photos of bacteria in the bottom row are cells from freshly inoculated cultures.

3.2. Evaluation of Bacterial Sensitivity or Resistance to Antibiotics.

Antibiotic test was examined to evaluate the level of bacterial sensitivity or resistance. The antibiotic test result (Table 2) was revealed that, all the tested bacterial isolates except Z11 and Z24 were resistant to both Ampicillin (10 μg) and Oxacillin (1 μg). Where as all bacterial isolates were sensitive to Polymyxin (300 units), Amoxicillin (20 μg), Vancomycin (30 μg), Gentamicin (10 μg), Erythromycin (15 μg), Ofloxacin (5 μg), Ciprofloxacin (5 μg), Bacitracin (10 units), and Streptomycin (30 μg).

Table 2. Measurement of the inhibition zone around the antibiotic discs (mm).

	Antibiotics																	
Bacterial Isolates	Cef	Str	Oxa	Tri	Pol	Ceft	Gen	Lin	Ery	Ofl	Van	Bac	Amp	Tet	Mer	Amo	Rif	Cip
Z10	0	35	0	30	20	10	10	11	14	12	12	10	0	14	10	10	10	37
Z11	18	20	15	14	15	15	20	12	10	16	12	19	20	14	18	22	17	18
Z12	12	8	0	28	10	0	13	12	15	10	35	17	0	10	0	12	0	18
Z15	18	26	0	15	20	20	22	0	25	27	17	17	0	0	15	16	16	18
Z24	18	24	0	19	17	13	16	22	15	10	29	12	11	12	11	20	25	23
Z26	30	32	0	0	19	18	20	25	20	16	30	17	0	11	12	40	20	28
Z44	15	20	0	18	14	16	18	0	17	25	18	20	0	11	18	15	15	24
Z82	15	19	0	21	15	18	22	10	13	25	15	12	0	0	15	15	10	23

The measurement of Inhibition zone: 0 = Bacterial isolates resistance to antibiotics, >0 = Bacterial isolates sensitive to antibiotics.

3.3. Growth Stimulation Effect of Bacterial Isolates

The growth stimulation effect of bacterial isolates on the germinated seeds (germination rate, shoot and root length, and plant fresh and dry weight) was presented in Figure 2. In Figure 2a, it is shown that the bacterial isolates Z12, Z24, Z44, and Z82 showed a significant increase in the germination rate of oat. Where as bacterial isolates Z82 significantly increased the germination rate of lentil ($p = 0.05$). Figure 2b, presented the effect of

bacterial isolates on the shoot length of the germinated seeds. The shoot length of maize was significantly increased by Z44. However, bacterial isolates Z12, Z24, Z26, Z44, and Z82 were significantly increased the shoot length of oat ($p = 0.05$). As Figure 2c, indicated that bacterial isolates Z24, Z26, and Z44 were significantly increased the root length of wheat. Where as the root length of maize and lentil was significantly increased by Z10, Z24, and Z44 (($p = 0.05$). In Figure 2d, it was shown that the fresh weight of wheat plants was significantly increased by Z10, Z26, Z44 ($p = 0.05$). Where as bacterial isolates Z44 and Z82 were singificantly increased the fresh weight of oat plants. However, Figure 2e, indicated that the dry weight of wheat plants significantly increased by Z10, Z15, and Z24. Moreover, the dry weight of oat plants was significantly increased by Z44 and Z82 ($p = 0.05$).

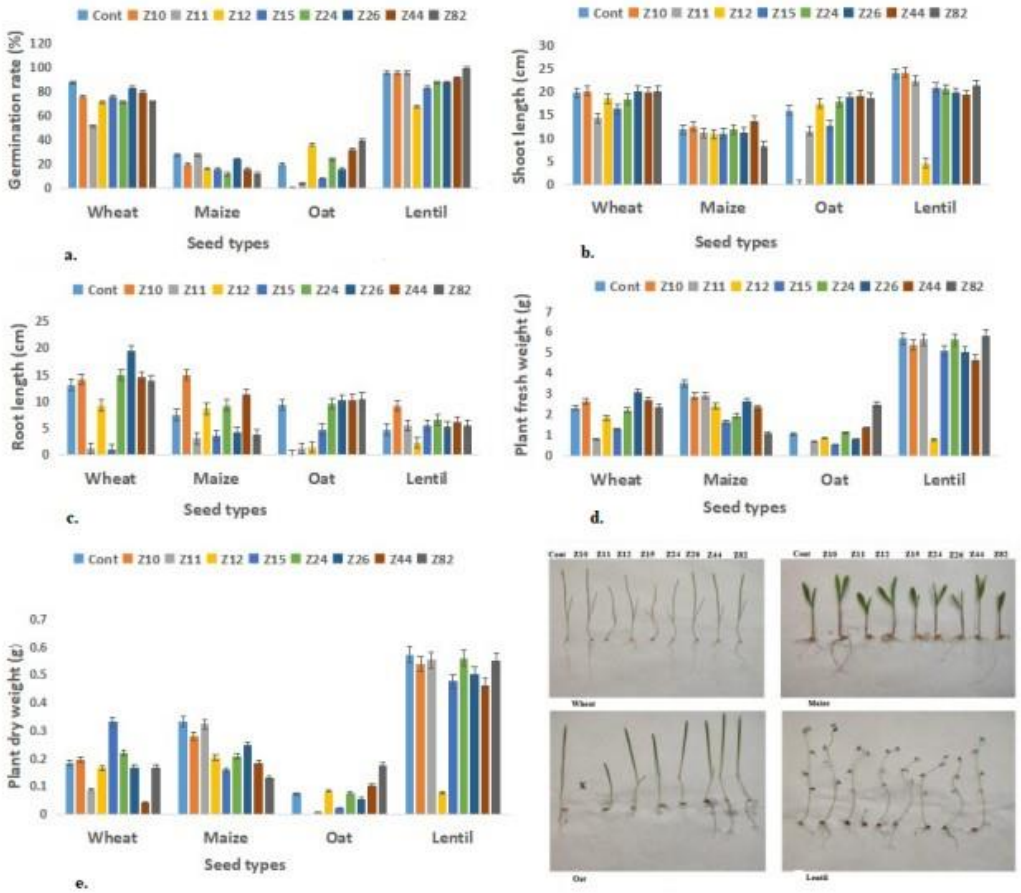

Figure 2. The growth stimulation effect of bacterial isolates on the germinated seeds of wheat, maize, oat, and lentil: (**a**). Germination rate (%), (**b**). Shoot length (cm), (**c**). Root length (cm), (**d**). Plant fresh weight (g) and (**e**). Plant dry weight (g).

3.4. Identification of Bacterial Isolates Using 16S rRNA Gene Sequencing

Molecular genetic identification of the strain 82 by 16S rRNA showed that it belongs to the species *Janthinobacterium rivuli* with 99% certainty. The accuracy of identifying representatives of the *Streptomycetes* family did not allow us to identify them to individual species at this stage. However, according to preliminary data, strain 11 can be tentatively attributed to the species *Streptomyces lasiicapitis*, 15—*Streptomyces griseoaurantiacus*, Z15—*Streptomyces aureoverticillatus/Streptomyces lasiicapitis/Streptomyces labedae/Streptomyces longissimus/Streptomyces rubrogriseus/Streptomyces thinghirensis*. Work to identify these strains will continue.

4. Discussion

In the current study, eight bacterial isolates were characterized for their morphological characteristics, antibiotic sensitivity or resistance, growth stimulation effect and molecular identification using 16S rRNA gene sequencing. Eventhough all bacterial isolates had stable morphological characteristics in the three types of growth media, four bacterial isolates (Z11, Z12, Z15 and Z44) showed colour change. The antibiotic test result revealed that almost all bacterial isolates were sensitive to all of the tested antibiotics. However, all bacterial isolates were resistant to oxacillin (1 μg) excluding Z11 and ampicillin (10 μg) excluding Z24. Moreover, antibiotic cefotaxime (30 μg) was resisted by Z10, ceftazidime (30 μg), Meropenem (10 μg) and Rifampin (5μg) were resisted by Z12, Lincomycin (15 μg) was resisted by Z15 and Z44, Tetracycline (30 μg) was resisted by Z15 and Z82, and Trimethroprim (75 μg) was resisted by Z26. Some published articles were reported that antibiotics like streptomycin and oxytetracycline were used in agriculture as a tool for the management of phytopathogens. Such kinds of antibiotics not only had impact on phytopathogen but also numerous beneficial bacteria such as plant growth promoting rhizobacteria [10,11]. Therefore, application of such kinds of antibiotics might be harmful for those chalky soil bacterial isolates which were examined in the present study. On the other hand, those chalky soil bacterial isolates which were resistant to antibiotics may be require further investigation especially on environmental concern. The growth stimulation effect of bacterial isolates was also examined in the present study. The result of growth stimulation effect revealed that few bacterial isolates showed stimulation effect on the germination rate of an oats and lentils, on the shoot length of maize and oats, on the root length of wheats, maize and lentils, on the fresh weight of wheats and oats and on the dry weight of an oat seeds. The 16S rRNA gene sequencing result revealed that those potent chalky soil bacterial isolates were belongs to *Streptomyces* spp. bacterial strains.

5. Conclusions

The present study was done on the identification and characterization of chalky soil bacteria found in the rhizosphere of Chamaecytisus ruthenicus. Morphologically, all the bacterial colonies had stable morphological characteristics except colour change. Regarding the level of chalky soil bacterial isolates to antibiotics, the majority of the studied bacterial isolates were sensitive to most antibiotics. However, few bacterial isolates were also resistant to some antibiotics. Most of the bacterial isolates had growth stimulation effects on the germination rate, shoot and root length, fresh and dry weight of the germinated seeds (wheat, maize, oat and lentil). The 16S rRNA gene sequencing result revealed that those selected bacterial isolates were belongs to Streptomaces spp. In the future, an investigation on biochemical test and environmental significance of those potent chalky soil bacterial isolates will be required. On the basis of the present and past studies on chalky soil bacterial isolates, we can concluded that those bacterial isolates had plant growth promoting activities and they will play a great role both in agriculture and environment.

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References

1. Ashrafuzzaman, M.; Mossen, F.A.; Ismail, M.R.; Hoque, A.; Islam, M.Z.; Shahidullah, S.M.; Meon, S. Efficiency fo plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. *Afr. J. Biotechnol.* **2009**, *8*.
2. Hardiansyah, M.Y.; Musa, Y.; Jaya, A.M. Identification of plant growth promoting rhizobacteria from thorny bamboo rhizosphere with 3% KOH gram test and gram staining test. *Int. J. Appl. Biol.* **2020**, *4*, 7–17.
3. Asfha, Z.A.; Suzina, N.E.; Kocharovskaya, Y.; Delegan, Y.; Solyanikova, I.P. Isolation and Characterization of Plant Growth-Promoting Bacteria from the Rhizosphere of *Chamaecytisus ruthenicus* (Russian Broom) Growing in Chalky Soil. *Eng. Proc.* **2023**, *37*, 121.
4. Karnwal, A. Isolation and identification of plant growth promoting rhizobacteria from maize (*Zea mays L.*) rhizosphere and their plant growth promoting effect on rice (*Oryza sativa* L.). *J. Plant Prot. Res.* **2017**, *57*, 144–151.
5. Singh, R.; Pandey, K.D.; Kumar, A.; Singh, M. PGPR isolates from the rhizosphere of vegetable crop *Momordica charantia*: Characterization and application as biofertilizer. *Int. J. Curr. Microbiol. Appl. Sci.* **2017**, *6*, 1789–1802.
6. Kumar, A.; Paswan, D.; Vishwkarma, S.P.; Kumari, M.; Kumar, B. Screening and characterizations of suiable plant growth promoting rhizobacteria in maize based intercropping system. *Pharma Innov. J.* **2023**, *12*, 839–848.
7. Rathaur, P.; Raja, W.; Ramteke, P.W.; John, S.A. Effect of UV-B tolerant plant grwoth promoting rhizobacteria (PGPR) on seed germination and growth of Withania somnifera. *Adv. Appl. Sci. Res.* **2012**, *3*, 1399–1404.
8. Naveed, M.; Mubeen, S.; Khan, S.; Ahmed, I.; Khalid, N.; Suleria, H.A.R.; Bano, A.; Mumtaz, A. S. Identification and Characterization of rhizospheric microbial diversity by 16S rRNA gene sequencing. *Braz. J. Microbiol.* **2014**, *45*, 985–993.
9. Dong, W.; Liu, H.; Ning, Z.; Bian, Z.; Zeng, L.; Xie, D. Inoculation with *Bacillus cereus* DWO19 modulates growth, yield and rhizospheric microbial community of cherry tomato. *Agronomy* **2023**, *13*, 1458.
10. Osbiston, K.; Oxbrough, A.; Martinez, L.T. Antibiotic resistance levels in soils from urban and rural land uses in Great Britain. *Access Microbiol*. **2021**, *3*, acmi000181.
11. Mitchell, M.; Thornton, L.; Riley, M.A. Identifying mmore targeted antimicrobials active against selected bacterial phytopathogen. *J. Appl. Microbiol.* **2022**, *132*, 4388–4399.