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Arsenic-Resistant Plant Growth Promoting *Pseudoxanthomonas mexicana* S254 and *Stenotrophomonas maltophilia* S255 Isolated from Agriculture Soil Contaminated by Industrial Effluent

Noor ul Huda [1], Rabia Tanvir [2], Javaria Badar [1], Iftikhar Ali [3] and Yasir Rehman [3,*]

[1] Institute of Microbiology and Molecular Genetics (MMG), University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan
[2] Institute of Microbiology (IOM), University of Veterinary and Animal Sciences (UVAS), Lahore 54000, Pakistan
[3] Department of Life Sciences, University of Management and Technology (UMT), Lahore 54770, Pakistan
* Correspondence: yasir.rehman@umt.edu.pk; Tel.: +92-333-8636475

Abstract: In many areas of developing countries, agriculture soil is irrigated with water from drains contaminated with industrial wastewater that contains many toxic substances including arsenic. Such sites could be explored for arsenic-resistant plant growth-promoting microbes. Ten arsenic-resistant bacteria were isolated from such a site and were characterized. Their ability to resist and reduce/oxidize arsenic was determined. The bacteria were also analyzed for plant growth-promoting abilities such as auxin and hydrogen cyanide production, phosphate solubilization, and nitrogen fixation. The effect of these bacteria on plant growth was determined using *Vigna radiata* both in presence and absence of arsenic. Bacterial isolates S254 and S255 showed maximum resistance against arsenic; up to 225 mM of As(V) and 25 mM of As(III). The phylogenetic analysis revealed that strain S254 belonged to the species *Pseudoxanthomonas mexicana* and strain S255 belonged to the species *Stenotrophomonas maltophilia*. Both *P. mexicana* S254 and *S. maltophilia* S255 showed positive results for hydrogen cyanide production, auxin production, and nitrogen fixation. *P. mexicana* S254 produced auxin at a concentration of 14.15µg mL^{-1} and *S. maltophilia* S255 produced auxin as high as 68.75µg mL^{-1}. Both the bacteria-enhanced the growth of *V. radiata* and a statistically significant increase in shoot and root lengths was observed both in the presence and absence of arsenic. The application of such bacteria could be helpful for the growth of plants in arsenic-contaminated lands.

Keywords: arsenic resistance; arsenate reduction; bioremediation; plant growth promotion; auxin production

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1. Introduction

Arsenic (As) is a common element found in the earth crust. The prevalent forms of As are the reduced form i.e., arsenite (As(III)) and the oxidized form i.e., arsenate (As(V)). Both these forms of As are toxic and associated with serious health issues; however, the trivalent form is 100 times more toxic as well as more mobile [1]. Since it is a carcinogen, any exposure to it increases the risk of tumors in humans such as those of the liver, kidneys, bladder, and lungs [2, 3]. The majority of the exposure to As occurs through the consumption of As-contaminated water and through food produced from or irrigated with As-contaminated water [4]. One of the major sources of such contaminated water is the industrial wastewater that contains high concentrations of heavy metals. In developing countries such as Pakistan where an increasing population has overtaken the

infrastructure of proper wastewater management, it is reported that only 2% of wastewater is properly treated before its disposal [5]. Therefore, As contamination has become a serious health and environmental concern, especially in South Asia. Recent estimates indicate that more than 150 million people are at a risk for arsenicosis that is manifested later as cancer [6].

Like other under-developed countries, the sewage system in Pakistan is constructed as an open combined sewer system. In this type of system, a single drain is present for collectively transporting residential wastewater, rainwater, floodwater, and industrial wastewater to a chosen disposal location. Because of rapid industrialization in already populated cities of Pakistan, such drains contain a variety of contaminants and expose the land as well as its inhabitants to a concentrated source of pollution [5]. Rohi Nala is one such large drain that is located near the densely populated city of Lahore. Industries, residential and commercial areas, and agriculture fields surround it, and the wastewater of tanneries and other industries is discharged into it. The effluent from it is then often used for irrigating downstream areas [7]. Such areas were used as sampling sites in this study (Figure 1).

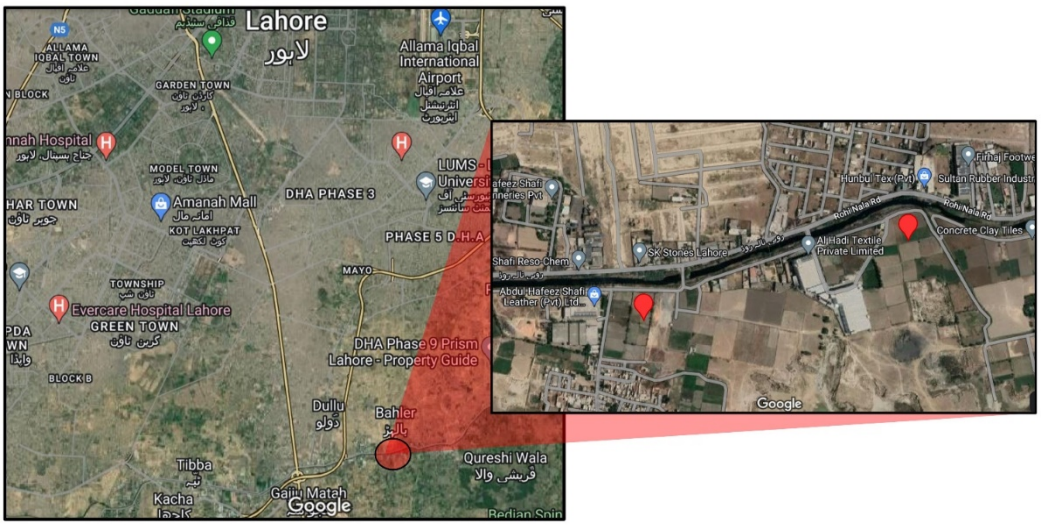

Figure 1. Map of the sampling sites. Soil samples were collected from agricultural land surrounding Rohi Nala drain (containing industrial wastes) being irrigated by its water, as shown by the red location markers.

In previous years, a significant number of studies have been carried out on the removal of As to increase its uptake from the environment. The currently employed methods include ion exchange, electrokinetic methods, chemical precipitation, and electrocoagulation. Each process is useful in removing As from soil and water to a certain extent but they have significant drawbacks, especially high costs and low efficiency [8]. Studies are now carried out on microorganisms with the capability to resist and detoxify As [9, 10]. These microorganisms employ a variety of mechanisms such as biosorption, exclusion, formation of complexes, compartmentalization, and enzymatic detoxification along with efflux systems [11, 12]. In addition, more studies are now focused on As-resistant plant growth-promoting microorganisms (PGPMs) since they not only resist As but at the same time promote plant growth [13-15]. They also provide a sustainable biological tool that is safe as well as low-cost for removing As toxicity and for regulating As accumulation in plants [16].

This study sought to ascertain whether the bacteria isolated from a field, irrigated by a drain containing industrial effluents (Rohi Nala), were tolerant to As and to determine whether they could promote plant growth. The identification of such microorganisms can

be further used for the bioremediation of As and for plant growth promotion in As-contaminated areas.

2. Materials and Methods

2.1. Soil Sampling

Two samples were taken from the rhizosphere of two different fields (F1 and F2) located in Kasur, Pakistan. These fields were being irrigated with the water of Rohi Nala drain. The samples were taken close to the plant rhizosphere at the depth of approximately 60–75 cm. The pH and temperature of the sampling site was recorded at the time of sampling.

2.2. Isolation of As-Resistant Bacteria

The samples were transported to the lab in labeled sterile bags and serial dilutions were prepared. The spread-plate technique was used, and the dilutions were plated on tryptic soy agar (TSA) plates with and without As(V) (Na_3AsO_4). Morphologically distinct colonies were selected and purified through quadrant streaking on TSA media plates supplemented with 1.0 mM As(V). Gram staining was carried out and biochemical testing was performed through catalase, oxidase, motility, citrate utilization, triple sugar iron, oxidation, fermentation, methyl red, and Voges Proskauer (MRVP) tests [17].

2.3. Determination of As Resistance by the Isolates

Tryptic soya broth (TSB) containing 75, 150, 175, and 200 mM of As(V) and 5, 10, 15, and 20 mM of As(III) was prepared and dispensed in 96 well microtiter plates. The wells were inoculated with 5.0 µL of fresh overnight TSB cultures. A negative control was also included in the microtiter plates. The microtiter plates were incubated at 37 °C for 48 hrs. After incubation, absorbance was measured at 600 nm using a spectrophotometer (CECIL CE 7200, Aquarius, UK) [18].

2.4. Determination of Cross Element Resistance of the Isolates

The bacterial isolates were cultured on TSA plates supplemented with 1.0 mM Ni ($NiCl_2.6H_2O$), Cd ($CdCl_2.5H_2O$), Zn ($ZnSO_4.7H_2O$), Se (Na_2O_3Se), Co ($Co(NO_3)_2.6H_2O$), and Cr ($KCrO_4$). The plates were incubated at 37 °C for 48 h. After incubation, plates were observed for the presence or absence of growth.

2.5. Optimization of Culture Conditions

For the optimization of culture conditions, the bacterial isolates were grown in TSB media over a range of pH and temperatures. For pH optimization, the pH of the media was set at 3, 5, 7, and 9 and the tubes were inoculated with overnight fresh cultures of the isolates followed by incubation at 37 °C at 120 rpm. For optimal temperature, TSB tubes were incubated at a range of temperatures, i.e., 25, 30, 37, and 45 °C, at 120 rpm after inoculating with overnight isolates. After incubation, absorbance was measured at 600 nm using a spectrophotometer (CECIL CE 7200, Aquarius, UK).

2.6. Qualitative Analysis of Bacterial Cultures for As Oxidation/Reduction

The assay for As oxidation/reduction was performed as described by Salmassi, et al. [19]. According to the method, Brunner mineral medium [14] was supplemented with 10 mM glucose and 5 mM As(III) or 10 mM As(V) and inoculated with overnight cultures of the isolates. After 48 h incubation at 37 °C, the media were centrifuged at 4000 rpm for 5 min and 200 µL of supernatants was mixed with 10 µL of 0.01 M $KMnO_4$. A standard of known concentration of arsenate was used as a positive control and a standard not containing any arsenate was used as a negative control. A change of color from purple to yellowish brown indicated a positive result, i.e., the reduction of As from As(V) into As(III).

2.7. Estimation of Plant Growth-Promoting Activities

2.7.1. Phosphate Solubilization Activity

The isolates were checked for their ability to solubilize phosphate by culturing them on Pikovskaya agar plates supplemented with 5 g L^{-1} tricalcium phosphate [20, 21]. The plates were incubated at 28 °C for 8–10 days and observed daily for the appearance of clear halos around bacterial growth indicating the solubilization of the phosphate.

2.7.2. Hydrogen Cyanide (HCN) Production

The isolates were checked for their ability to produce hydrogen cyanide (HCN) [22]. For this purpose, the isolates were inoculated on nutrient agar (Difco, Detroit, MI, USA) plates supplemented with glycine (4 g L^{-1}). Whatmann filter paper No.1 soaked in 2% sodium carbonate (in 0.5% Picric acid) and was placed at the agar surface. The plates were incubated at 28 °C for 7 days and observed daily for the development of orange to red color indicating the production of hydrogen cyanide.

2.7.3. Nitrogen Fixation

The isolates were checked for their ability to fix nitrogen. For this purpose, the strains were inoculated on plates containing nitrogen free mannitol agar [17]. The plates were incubated at 28 °C for 2–3 days and observed daily for the appearance of growth indicating a positive result.

2.7.4. Auxin Estimation

The isolates were checked for their ability to produce auxin. For this purpose, nutrient broth supplemented with tryptophan (500 μg mL^{-1}) was inoculated with overnight cultures of the isolates. The tubes were incubated at 37 °C for 4 days. After incubation, auxin estimation was carried out. The supernatants were taken and Salkowski reagent was added to determine the concentration of IAA [23]. The intensity of the color was measured by taking the absorbance at 535 nm by a microtiter plate reader (Epoch, Santa Monica, CA, USA). The standard curve was prepared in order to estimate the concentration of auxin produced.

2.8. 16S rRNA Gene Sequencing and Phylogenetic Analysis

For the amplification of 16S rRNA gene, genomic DNA was isolated using GeneJet genomic DNA extraction kit (ThermoFisher, Waltham, MA, USA). 16S rRNA gene was amplified using the primers 518F (CCAGCAGCCGCGGTAATACG) and 800R (TACCAGGGTATCTAATCC) [24, 25]. The PCR products were sent to a commercial sequencing facility (Macrogen, Seoul, Korea). After checking the quality of the base calling of the sequences, sequence alignment was done by using ClustalW in MEGA 5.0 (Geospiza). The neighbor-joining (NJ) method was applied for phylogenetic tree construction [26] in MEGA 5.0 [27]. A total of 100 replicates of boot-strap test [28] were taken as the measure of reliability of the constructed phylogenetic trees.

2.9. Evaluation of Plant Growth in the Presence of the Bacterial Isolates

The effect of bacterial strains on the growth of plants (*Vigna radiata*) was also analyzed. The pots of plants were divided into four sets: Set 1: plants only, Set 2: plants with As(V), Set 3: plants with individual bacterial inoculation, Set 3B: plants with combination of all selected strains, Set 4A: plants with As(V) and individual bacterial inoculation, and Set 4B: plants with As(V) and with combination of all selected strains. The pots where As(V) was added, 1.0 mM concentration of Na_3AsO_4 was used.

V. radiata seeds were acquired from Punjab seed corporation, Lahore. Uniformly sized healthy seeds were selected, and surface sterilization was done with 0.1% $HgCl_2$ for 1.0 min, followed by thorough washing with autoclaved distilled water. The seeds were then soaked in suspension of selected bacterial strains (0.5 OD_{600}) for 15 min and were

sown in pots containing thoroughly sifted soil. Per pot, eight seeds were sown and later thinned to 5 plants per pot after germination. Pots were kept under conditions of 12 h of photoperiod at 25 ± 2 °C and were watered regularly. After two weeks of growth, root length, shoot length, and fresh and dry weight were recorded.

3. Statistical Analysis

Each experiment was performed in triplicate and their results were taken as mean values. Statistical analysis was carried out using Microsoft Excel 2013 (Microsoft, Washington, DC, USA). Standard deviation (SD) is displayed as error bars. A two-tailed *t*-test was applied to determine the statistical significance of bacterial effects on plants' growth ($p = 0.05$).

4. Results

4.1. Isolation and Characterization of As-Resistant Bacteria

The temperature of the sample taken from the first agriculture field (F1) was 32 °C and its pH was 7.9. The temperature of the sample taken from the second agriculture field (F2) was 29 °C; its pH was 7.5. Ten isolates were selected based on their As resistance and As reduction potential. Seven of the isolates (S252, S253, S254, S255, S257, S258, and S4E1) belonged to the field F1 whereas the remaining three isolates (S43, S46, and S48) were from field F2. The isolates were observed to be both Gram positive as well as Gram negative rods. Several biochemical tests such as catalase, oxidase, citrate utilization, motility test, triple sugar iron test, oxidative fermentative test, and MR-VP tests were also performed (data not shown).

4.2. Phylogenetic Analysis of the Selected Isolates

Based on the results of As-resistance, As-reduction, and plant growth-promoting activities, bacterial isolates S254 and S255 were selected for phylogenetic analysis. 16S rRNA gene sequences of the bacterial isolates S254 and S255 were checked for sequence homology by using NCBI BLAST (https://blast.ncbi.nlm.nih.gov/). The isolate S254 was showing 99% homology with *Pseudoxanthomonas mexicana* (GenBank accession no. KY651247) and S255 was showing 99% homology with *Stenotrophomonas maltophilia* (GenBank accession no. KY651248). The phylogenetic tree is shown in Figure 2.

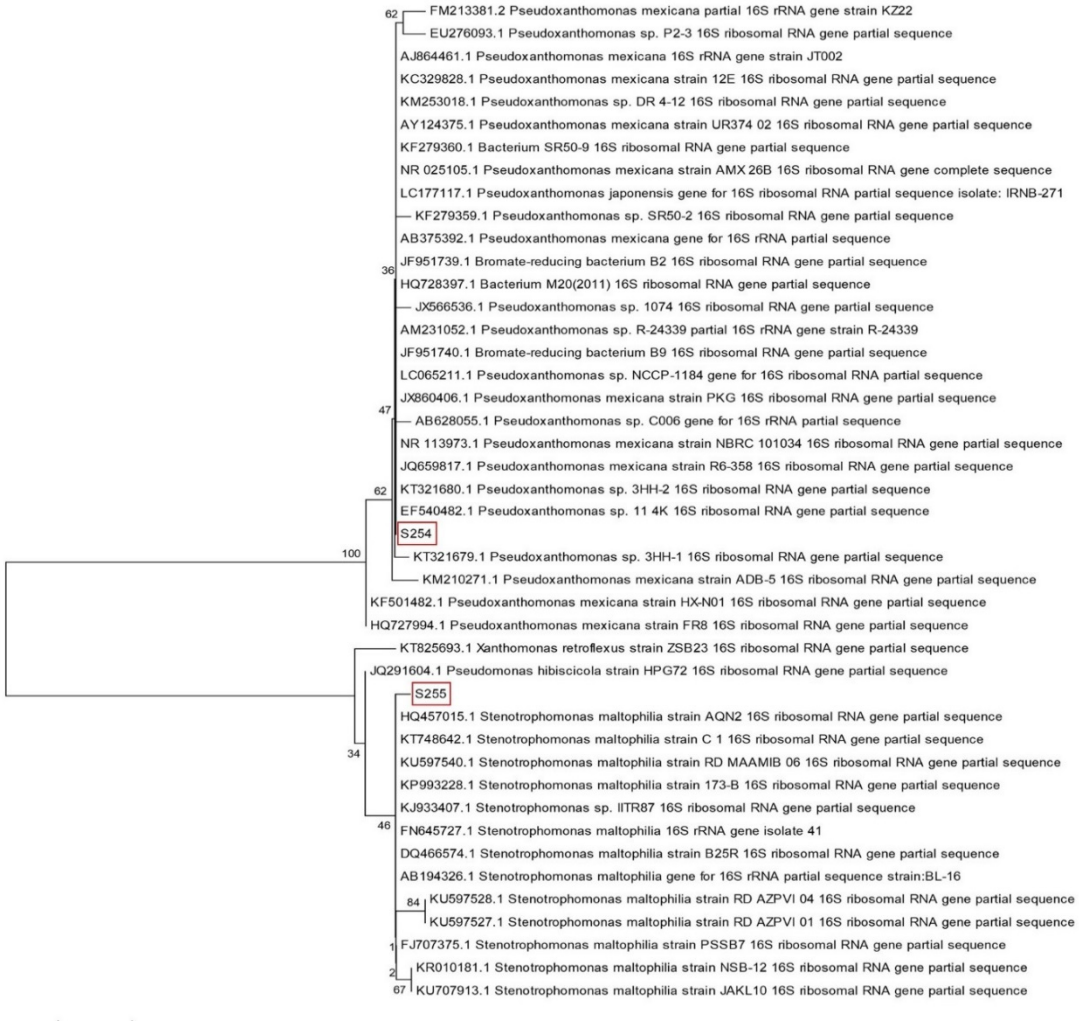

Figure 2. Phylogenetic tree of *Pseudoxanthomonas mexicana* strain S254 and *Stenotrophomonas maltophilia* strain 255 (marked Red in the figure).

4.3. Minimal Inhibitory Concentration (MIC) of the Isolates

All ten isolates were evaluated for the minimal inhibitory concentrations (MIC) of As(V) and As(III). The highest value for MIC of As(V) was shown by *S. maltophilia* S255 (225 mM), followed by *P. mexicana* S254 (175 mM). While checking for the MIC for As(III), the highest MIC was shown by *S. maltophilia* S255 (25 mM) (Figure 3).

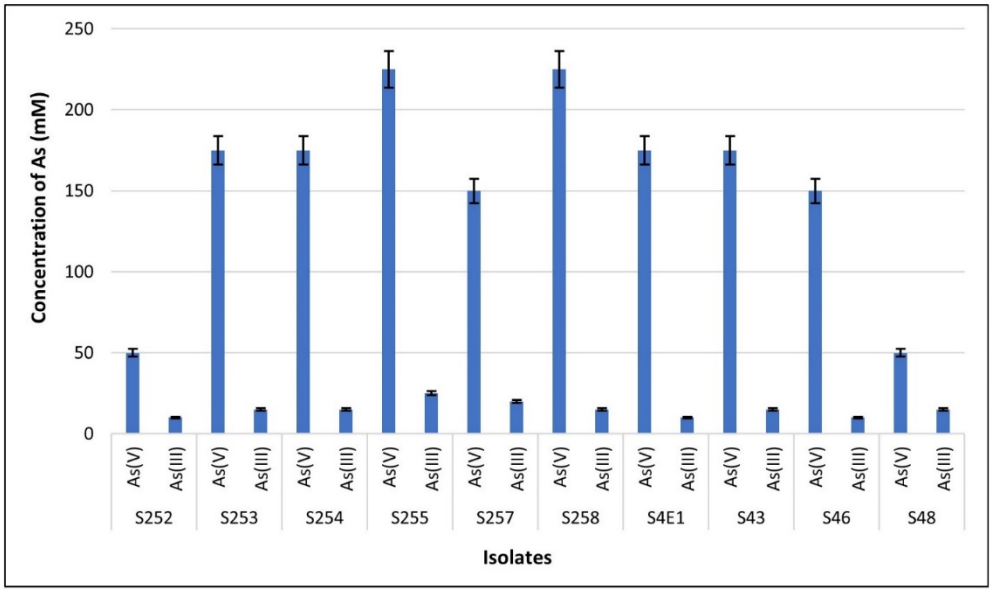

Figure 3. Minimal inhibitory concentration (MIC) of the isolates against As(V) and As(III).

4.4. Cross Element Resistance of the Isolates

Among the ten isolates, *S. maltophilia* S255 showed resistance towards all metals at 1.0 mM concentration, whereas the *P. mexicana* S254 showed resistance against all the metals except Cd. The intensity of resistance of all the isolates is shown in Table 1.

Table 1. Cross-metal resistance by the As-resistant isolates.

Strains	Metals					
	Co (1 mM)	Se (1 mM)	Zn (1 mM)	Ni (1 mM)	Cd (1 mM)	Cr (1 mM)
S252	+	+	+	+	−	+
S253	+	+	+	+	+	+
S254	++	+++	+++	+++	−	+
S255	+++	+++	+++	+++	+++	+++
S257	+	+	+	+	−	+
S258	+	+	+	+	−	+
S4E1	+	+	+	+	−	+
S43	+	+	+	+	−	+
S46	+	+	+	+	−	+
S48	+	+	+	+	−	+

Co: cobalt; Se: selenium; Zn: zinc; Ni: nickel; Cd: cadmium; Cr: chromium. +++: maximum growth; ++: intermediate growth; +: slight growth; −: no growth.

4.5. Optimization for pH and Temperature

The growth of the strains was checked at different temperatures and 37 °C was recorded as the ideal temperature for the growth of the isolates. When the temperature was raised to 45 °C, the growth of all the strains decreased. All the isolates showed best growth at pH 7.

4.6. Qualitative Analysis of Bacterial Cultures for As(V) Reduction

In the assay for qualitative analysis of bacterial cultures for the reduction of As(V) to As(III), four among the ten isolates i.e., S252, *P. mexicana* S254, *S. maltophilia* S255, and S46 showed positive results. A color change from purple to yellowish brown indicated As-reducing capability (Figure S1).

4.7. Phosphate Solubilization, Hydrogen Cyanide (HCN) Production, Nitrogen Fixation, and Auxin Estimation

Among the ten isolates, no isolates gave positive results for phosphate solubilization as no zone of clearing was observed after incubation. For HCN production, strong intensity of the brown coloration was observed by *P. mexicana* S254 and *S. maltophilia* S255. Both the bacteria showed positive result for nitrogen fixation as well. For auxin estimation, although nine among the ten isolates gave positive results (Figure S2), *P. mexicana* S254 produced the highest concentration of auxin, i.e., 68.75 µg mL^{-1}, while *S. maltophilia* S255 produced 14.15 µg mL^{-1} of auxin (Figure 4). The results of the plant growth-promoting activities are summarized in Table 2.

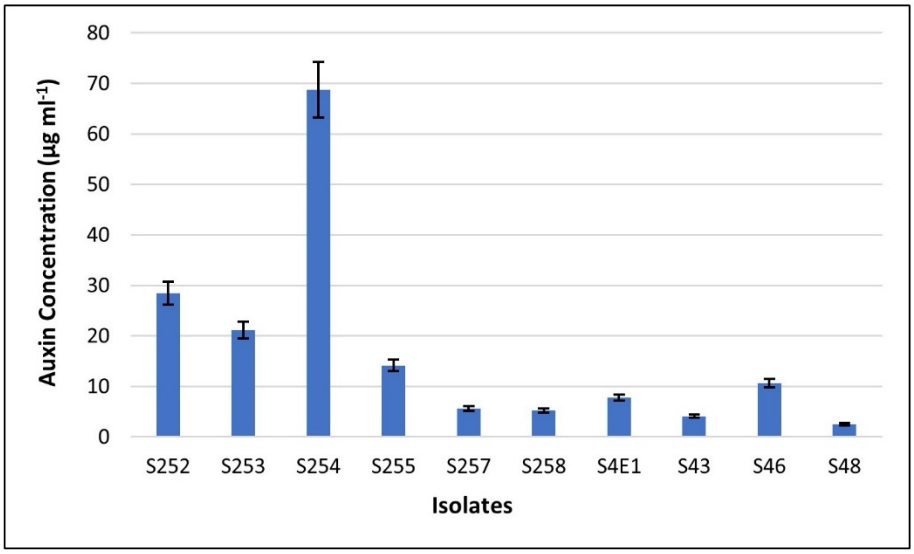

Figure 4. Concentration of auxin produced by the As-resistant bacterial isolates.

Table 2. Plant growth promoting activities by the As-resistant bacterial isolates.

Strains	Plant Growth-Promoting Activities			
	HCN Production	Phosphate Solubilization	Auxin Production	Nitrogen Fixation
S252	+	−	+++	−
S253	−	−	+++	−
S254	+++	−	+	+
S255	++	−	+++	+
S257	−	−	++	−
S258	−	−	+	−
S4E1	+	−	+	−
S43	+	−	+	−
S46	+	−	+	+
S48	−	−	−	−

HCN: hydrogen cyanide. +++: maximum growth; ++: intermediate growth; +: slight growth; −: no growth.

4.8. Effect of the Bacterial Isolates on Plant Growth

The shoot length of the plants devoid of As was 21.23 + 0.96 cm. In the presence of *P. mexicana* S254 and *S. maltophilia* S255, the shoot length of the plants increased to 24.15 + 0.18 and 26.5 + 0.5 cm, respectively. The presence of As had a severe negative effect on the plant growth and shoot length decreased to 4.1 + 0.1 cm. The presence of *P. mexicana* S254 and *S. maltophilia* S255 seemed to reduce the harmful effects of As on plants as the shoot

length was found to be 26.3 + 0.5 and 19.96 + 0.12 cm, respectively. The root length of the plants was measured to be 7.65 + 0.61 cm in the absence of As. In the presence of *P. mexicana* S254 and *S. maltophilia* S255, the root length of the plants was found to be 7.66 + 0.88 and 8.46 + 0.87 cm, respectively. As with the shoot, the presence of As also had negative effects on the length of the roots, and the length of the roots in the presence of As was measured at 1.36 + 0.5 cm. The presence of *P. mexicana* S254 and *S. maltophilia* S255 apparently alleviated the harmful effects of As on plant roots as the root length was measured to be 6.8 + 0.17 and 3.94 + 0.15 cm, respectively (Figure 5). These positive effects of the bacterial on the plant growth were found to be statistically significant by two tailed *t*-test, $p < 0.05$.

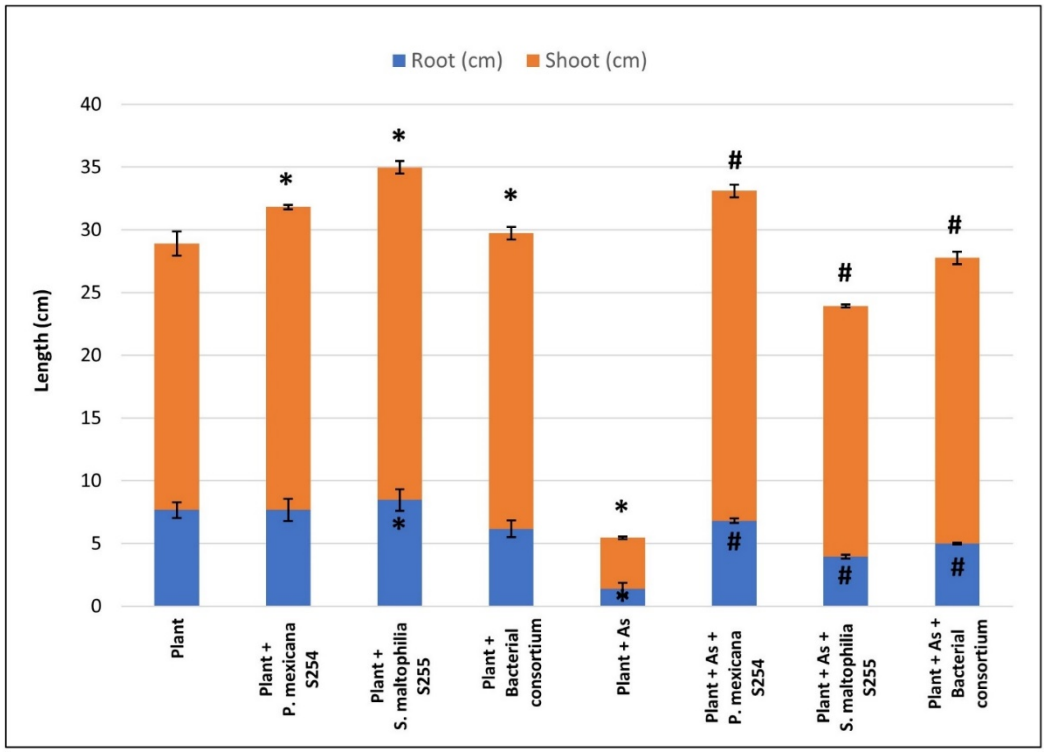

Figure 5. Effects of the bacteria on plant growth *Vigna radiata*. Both *Pseudoxanthomonas mexicana* strain S254 and *Stenotrophomonas maltophilia* strain 255 showed positive effects on the plant growth especially in the presence of arsenic. * Statistically ($p < 0.05$) significant difference as compared to control plants (in the absence of As), # statistically ($p < 0.05$) significant difference as compared to control plants (in the presence of As)

5. Discussion

The leather industry plays a main part in Pakistan's economy; however, the tanneries that produce them also produce many toxic wastes. This waste includes As along with chromium that contaminate the agriculture soil in the area [29]. There is also a high concentration of other heavy metals, metalloids, trihalomethanes (THMs), and pathogens in it [5].
Studies have reported a drop in the yield of crops such as rice, wheat, and berseem, and vegetables that are grown in such areas due to effects on the soil fertility [7].

Human exposure to As through plant-derived food intake is a major health hazard [30, 31]. When croplands are irrigated with industrially contaminated water, the rhizospheric microbes may also acquire resistance to As as well as mechanisms to detoxify it. Because these bacteria are resident of rhizosphere, they may also possess the plant growth promoting potential. We can increase the crop yield of As-contaminated agriculture areas by providing As-resistant PGPMs, which can detoxify As as well as enhance plant growth.

In the present study, the soil samples were taken from the plant rhizosphere of two adjacent fields irrigated by industrial effluents. Of the ten isolated strains, the highest MIC

of As was observed with the isolates S254 and S255, i.e., 225 and 175 mM, respectively. 16S rRNA gene sequence of the bacterial isolate S254 displayed 99% homology with *P. mexicana*. An earlier study reported this species for its As reduction potential [32] and this was confirmed in a later study by Sarkar, et al. [33], in which As(V) reductase gene *arsC* was detected in this species. A recent study by Selvaraj, et al. [34] reported the isolation of *P. mexicana* from wastewater. The phylogenetic analysis of the isolate S255 revealed 99% homology to *S. maltophilia.* This species is ubiquitous in a wide range of habitats, even extreme environments [35]. In terms of its resistance to heavy metals, previous study reported that *S. maltophilia* was observed to be resistant to 10 mM As(III) and 20 mM As(V) [36].

Both the strains displayed resistance to a variety of toxic elements in the study, i.e., Co, Cr, Ni, Se, Zn, and Cd. *S. maltophilia* S255 resisted all the metals very efficiently which coincides with the results of Xiong, et al. [37], in which a *S. maltophilia* strain displayed heavy-metal adaptability especially against high concentration of Cd and Cu. Another study by Baldiris, et al. [38] showed that *S. maltophilia* was able to resist Cr as well as Zn and Cu. This species in our study displayed resistance to metals up to 1.0 mM concentration which is confirmed by another study by Liaquat, et al. [39], where it displayed a maximum metal tolerance concentration of 1.0 mM. Other studies such as by Gopi, et al. [40], Kumar, et al. [41], Aslam, et al. [42] and Raman, et al. [43] further confirm the presence of cross-element resistance in this species.

The strain *P. mexicana* S254 showed growth inhibition in the presence of Cd and Cr. A previous study showed this species was isolated from a canal contaminated with industrial effluent. It was observed to be effectively involved in phenanthrene degradation and the authors suggested its use in bioremediation [44]. A later study by Wang, et al. [45] showed the species was capable of growing in high concentration of Cd. Interestingly, *P. mexicana* is shown to harbor transposon containing genes for multi-drug resistance. It has already been proven that a strong relationship exists between heavy metal and antibiotic resistances [42]; therefore, it can be speculated that the *P. mexicana* may also be capable of multi-metal resistance as well. A study by Muehe, et al. [46] showed this strain growing in the rhizosphere of a metal-hyperaccumulating plant, *Arabidopsis halleri*. The plant has been previously shown to be capable of accumulating a high concentration of Cd and Zn.

In our study, in addition to evaluating the resistance of strains S254 and S255 to metals, they were also evaluated for their plant growth promoting potential. In previous studies, *P. mexicana* has been attributed as a plant growth-promoting bacteria positive for auxin and hydrogen cyanide (HCN) production [47]. This was consistent with the results obtained in our study, where *P. mexicana* S254 gave a distinct positive result for HCN production as well as giving the highest concentration of auxin production. A study by Lampis, et al. [48] showed that *Pseudoxanthomonas* sp. was capable of producing siderophores and indoleacetic acid (IAA). In another study by Liaqat and Eltem [49], *P. mexicana* was identified as an endophyte from pear plant. The strain isolated from it was observed to be positive for the nitrogen fixation, IAA production, and solubilization of phosphate.

In our study, *S. maltophilia* S255 also gave positive results for plant growth promotion with HCN production, nitrogen fixation, and high auxin production. Previous studies have established *S. maltophilia* as mainly associated with plant environment with a role in nitrogen and sulfur cycles and promotion of plant growth [35]. *Stenotrophomonas* spp. have also been reported as an endophyte capable of producing auxin. Another *Stenotrophomonas* sp. from common horsetail produced 19.2 μg mL^{-1} of auxin that is similar to the results in our study where auxin production was observed to be 14.15 μg mL^{-1} [50]. In one study, positive results were observed in terms of roots elongation when seeds of *Capsicum annuum* L. were treated with *S. maltophilia* strains [39].

6. Conclusions

From the study, it could be concluded that the bacteria isolated from the cropland irrigated with industrial wastewater not only have phytostimulatory activities, but they were also capable of resisting As and other heavy metals. The results showed that such bacteria can help the plants grow in soil contaminated with As. Such PGPMs could be used as bio-fertilizers to enhance the crop yield and survival rate of plants in As-contaminated areas as a future prospective.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su141710697/s1, Figure S1, Microtiter plate showing As-reduction by the bacterial isolates using $KNnO_4$. Purple colour indicates presence of As(V), whereas yellowish colour indicates presence of As(III). Figure S2, Microtiter plate showing production of auxin by the bacterial isolates. Development of pink colour is an indication of auxin production.

Author Contributions: N.u.H. performed the experiments. R.T. and J.B. did the analysis and prepared the draft of the paper. R.T., I.A. and Y.R. reviewed and edited the manuscript. R.T. and Y.R. supervised the research. All authors have read and agreed to the published version of the manuscript.

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