ISSN: 2347-4688, Vol. 10, No.(3) 2022, pg. 290-299

Current Agriculture Research Journal

www.agriculturejournal.org

A Correlational Analysis of the Phosphate Solubilising Bacteriathe Growth Rate of Lady Rosetta Potato

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Abstract
Phosphate-Solubilizing Bacteria (PSB) contribute to healthy plant growth, improved yield, and phosphorus utilization in several crops and are potentially useful as a bioinoculant for enhancing sustainable production. The objective of this study is to examine the response of Lady rosetta potatoes to *Acinetobacterrudis* inoculation as PSB under controlled and field conditions in Kotdachakar village, Ta. Bhuj, Dist. Kutchh, State- Gujarat, India. Plant growth and the productivity of Lady Rosetta potatoes, laboratory isolations, and culture experiments were conducted. The potato tubers were harvested 90 days post-inoculation. The results indicated that there is a significant ($p < 0.05$) increase in germination plant species compared to the control plots. *Acinetobacterrudis* has an equal impact on the weight and size of potato tubers, as well as the height and growth of Lady Rosetta potatoes. The control plotsshowed poor germination and stunted growth of the potato tubers and plants. An increase in P contentswas observed following *Acinetobacterrudis* inoculation, which resulted in an overall increase in plant height, size, and weight of potato tubers. The study concluded that *Acinetobacterrudis* has enormous potential to be applied as an alternative to PSB for environmental reasons.

Article History
Received: 07 September 2022
Accepted: 26 December 2022

Keywords
Acinetobacterrudis;
Lady Rosetta Potatoes;
Phosphate-Solubilizing Bacteria;
Potato Tubers.

Introduction

One of the most significant macronutrients for plants is phosphorus (P). Phosphorus is second to nitrogen in importance. The P plays a role in a bunch of plant metabolism processes, like photosynthesis, nutrient uptake, and cell division. As the plant grows, P nutrition helps it grow deeper and more numerous roots.[1-3] The biosynthesis of phosphates occurs in plants toa limited degree and resulted in the production of small amounts of phosphorus,

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Doi: http://dx.doi.org/10.12944/CARJ.10.3.11

while the remainder is broken down into insoluble compounds in the soil.[4,5] Reduced growth and leaf expansion, and less respiration and photosynthesis were observed in the P-deficient plants, and the plants become darker green (a higher concentration of chlorophyll), and reddish due to an increase in anthocyanin production. The initial symptoms of P deficiency are symptoms of slow, weak, and stunted growth since P is required in high amounts during the early phase of cell division.[6] Reported that reducing phosphorus nutrition affected plant growth. Greenhouse provided a soilless growth medium for poinsettias, petunias, and geraniums. The foliage or bracts of any poinsettia did not exhibit symptoms of P deficiency.Soil phosphorus (P) forms compounds with other elements, phosphates are one example. Plant remains, composts, and microorganisms contain organic phosphorus. Phosphorus in the soil is created by apatite, which is complexes with calcium, aluminum, and iron phosphates. Phosphorus is absorbed by clay particles as P. By mobilizing insoluble phosphorus from fertilizers and soils into soluble phosphorous, phosphate-solubilizing microbes (PSM) can efficiently increase fertilizer uptake in soils.[7] It was demonstrated[7] that phosphate-solubilizing bacteria and fungi produce a synergistic actionand increase rock phosphate mobilization and plant growth.[8]

Phosphorus is an element that occurs both organically and inorganically in soils. Due to its insoluble/fixed form, this mineral is difficult for plants to absorb, since it is insoluble or fixed, and cannot be absorbed directly by plants. As well as cycling phosphorus in the soil, soil microorganisms play an important role in solubilizing inorganic phosphorus and mobilizing organic phosphorus.[9] Also, 85% of the soluble phosphate added to soil gets immobilized due to a reaction between the soluble phosphate and soil ingredients. It has recently come to light that some studies have been carried out using microbes to increase the availability of P in crops. Microbes can transform insoluble P into soluble forms. According to research,[10] PSB, which are attached to roots by exudates from the root, are relatively dependent on roots. The carbon contents of the soil are up to 40% from rhizode position and root rotation, which provides nutrients for microorganisms.[11]

An inoculant, known as a biofertilizer, is capable of converting non-useful nutrients in the soil into a form that can be exploited by crops during all stages of the growing season. Due to their renewable, low-cost, and environmentally friendly properties, agricultural and food production have become increasingly dependent on biofertilizers over the past decade. Chemical fertilizers and pesticides have contributed to climatic changes. As a result of different types of fertilizers being used, there is a negative impact on the environment, including nitrogen leaching and runoff and phosphorus fixation in the soil.[12] To complement phosphorus and nitrogen fertilizers, researchers are studying the use of bacteria (*Phosphobacterium, Azotobacter, Rhizobium, Azospirillum,* and *Acinetobacter*) and VAM fungi as biofertilizers. Since this treatment was implemented, several crops have enjoyed noticeable improvements in growth. Environmentally friendly, sustainable farming practices include the application of microbiological fertilizers. *Azotobacter, Azospirillum*, and *Acinetobacter* are effective biofertilizers. They are also bacteria that dissolve P. A few studies have examined the effects of biofertilizers alone or in combination with other chemicals.

There are many species of *Acinetobacter* listed in the *Acinetobacter* genus that are known to flourish in natural and man-made environments alike[13,14] *Acinetobacter* species are commonly found in soil, water, insect guts, and plant-associated environments, such as floral nectar and tree bark.[15] In the present state, the genus consists of 58 species, some with validly published namesand some with genomic names. Raw milk and dairy wastewater were also found to contain *Acinetobacterrudis*. In the presence of *Acinetobacterrudis*, the soil's fixed phosphorus is dissolved, releasing citrate and soluble phosphorus. The microorganisms in these wastes also act as mineralization agents for organic phosphate compounds. The rate of composting can be accelerated during the thermophilic phase by adding these materials. By using bacteria in neutral to alkaline soils and fungi in acid soils, soil phosphorus application can be improved. In addition, the fixation of phosphorus can be avoided. There are many sources of phosphorus and sulfur found in nature, and therefore, this species will be able to use most of them.[16] Although Potato is a widely consumed fruit vegetable, not one study has been undertaken. Accordingly, the objective of the present study is to investigate the effects of *Acinetobacter* as a pesticide on a variety of potato properties. *Acinetobacter rudis* are currently subject to considerable interest since they are being exploited for crop production due to their rapid colonization, creation of growth-promoting substances, and mobilization of rock phosphorous.

Materials and Methods

Collection of Soil Sample

The soil of crops (such as paddy, maize, sorghum, redgram, blackgram, etc.)was taken to the laboratory immediately after harvesting to isolate bacteria that can fix minerals. After removing the loosely bound rhizosphere soil, the roots were gently shaken to remove it. Further analysis was carried out on these soil samples.

Isolation of Phosphate Solubilizing Bacteria

The oldest methods for isolating Phosphate Solubilizing Bacteria (PSB) use insoluble mineral phosphates as the P source (tricalcium phosphate) and the resulting medium is light and whitish. A phosphate solubilizing microbe forms a clear zone around its colony.[17] Using Pikovskaya medium, PSB were isolated and enumerated by dilution plate technique. Using 95 mL of sterile distilled water, 10 g of soil from each rhizosphere sample was suspended and shaken at 80 rpm for 30 min. Furthermore, soil suspensions were serially diluted.

Measurement of Solubilization Zone

After inoculating the PSB strains on a solid medium, they should be incubated for 7 days at 35*2°C. The incubation period was followed by a measurement of the diameter of the solubilization zone.

Culture Selection and Maintenance

The purity of various strains of mother cultures is preserved in Agricultural Universities, the Indian Agricultural Research Institute, some ICAR centers, and National/Regional Centers for Organic Farming in MOA. From the identified sources, the desired strain's mother culture can be purchased in test tubes. A trained microbiologist will have to subculture them and maintain them exclusively for mass production, according to standard techniques.

Culture Augmentation

The culture progressively scaled up from a small mother culture to a broth in the required quantities in the following step. The requisite mother culture is inoculated by preparing a defined synthetic medium (specific to the organism). Bacteria are cultivated in this broth for up to 7 days. A variety of fermentation vessels are required depending on the quantity to be harvested. The long-term storage of isolated bacterial strains was conducted using Pikovskaya broth for overnight growth with moderate shaking (120 rpm). The cells were harvested by centrifugation at 6000 rpm for 10 minand resuspended in Pikovskaya broth. After mixing 500 µlof bacterial suspensions with 500 µlof 30% glycerol in 1.8 mL screw cap cryovials, the mixtures were stored at -20°C.

Field Experiment

The experiment took place atKotdachakar village, Ta. Bhuj,Dist. Kutchh, State- Gujarat, India.(Lat-Long) from 20/10/2021 to 18/01/2022Several parameters of the soil were determined before the experiments and it found a silt loam containing 5.5% oxidizable organic matter (Walkley-Black Method), 213 Kg/Ha total nitrogen (KjeldahlTechnique), 37 ppm of available phosphorus (Bray-Kurtz I Method), 0.7% available potassium (Morgan Method), 10.167 dS m-1 of electrical conductivity and a pH of 7.4. The *Acinetobacter rudis*(MW582308) culture was blended with 90% FYM and 10% Rock phosphate and maintained at 30% moisture. To evaluate the effectiveness of PSB, seeds of Lady Rosetta potatoes were used as hosts. Mix well and incubate it for 30 days at room temperature, then apply to the field Lady rosetta potato experimental plot. Following the contract, the control plot was fertilized with 90% FYM and 10% Rock Phosphate, mixed well, and applied in the potato field.After four days interval germination of both the plots was analyzedand recorded. Both the farms were irrigated as per the requirement specified for the species of the potato crop. Harvest of Lady rosetta potato was taken on 90th days after harvesting, where the height of all the plants was measured in cm, the size of potato tuber diameter was measured in cm, and the weight of potato tuber was measured in gms.

Statistical Analysis

In order to analyse the collected data, Pearson Correlation Coefficient, Mean, and Standard Deviation tests were conducted using SPSS software (Version 22.0) and ADANCO software. *A.rudis* were examined for their association with the growth of Leady rosetta potatoes. Through Pearson correlation, an attempt was made to identify the degree to which bio fertilizer is related to various properties of Lady Rosetta potatoes. In this study, we chose statistical methods that are significant at a level of 0.05. The impact analysis model of Cohen's test was aptly suited due the multiple levels of tangible variables like growth, size, height and weight.

Results and Discussion

A) Leady Rosetta Potato Germination Stimulated by *Acinetobacterrudis* (Mw582308)

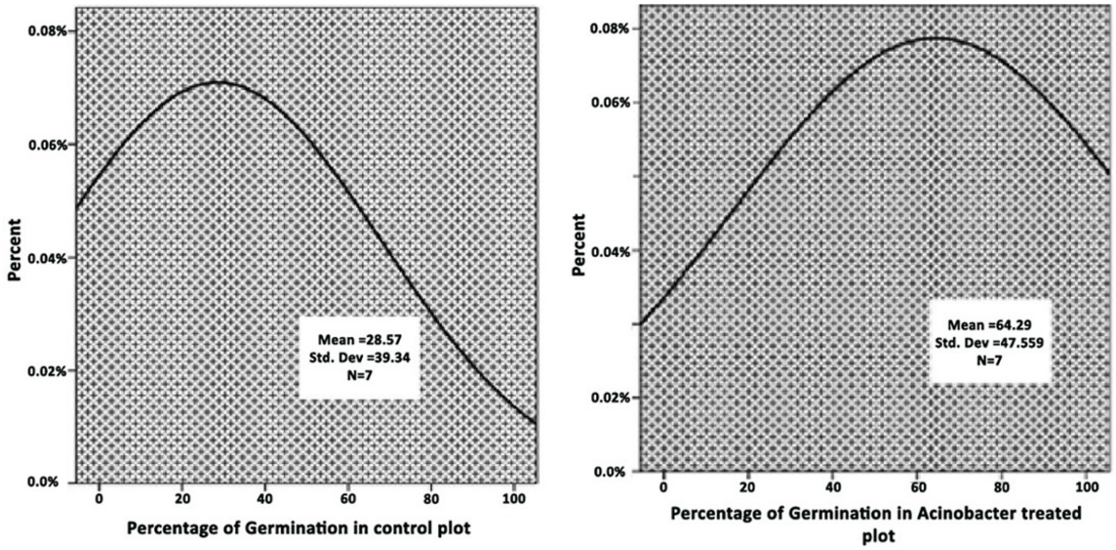

Fig. 1: Germination difference of *Lady rosetta* sp. potatoes in Control and Acinobaterrudistertiated plot.

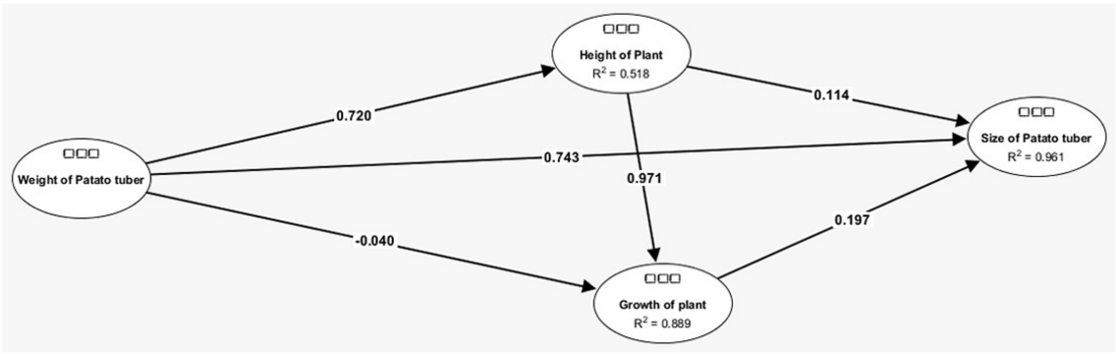

Fig. 2: Relation of various features of Lady Rosetta potatoes in A.*rudis* plot

As compared to the Control plot, the germination rate of Lady rosetta potatoes treated with *A. rudis* biofertilizer was remarkably high among *A. rudis* biofertilizer treated plots (Figure 1). There is a significant ($p < 0.05$) difference between the *A. rudis*-treated plot and the control plot in that the *A. rudis*-treated plot has a mean value of 64.29 and a standard deviation of 47.553. In this context, it is well-known that germination of Lady Rosetta sp. due to the presence of biofertilizers, phosphorus-solubilizing bacteria are present, for example, *A. rudis*. Specifically, the "P" solubilizing properties of *A. rudis* (MW582308) are attributable to its simple crystal structure and simple amorphous structure, which makes it a suitable solubilizerof rock phosphates.[10] Therefore, this solubilizes phosphorous helping to promote good germination in Lady rosetta species of potatoes.

B) Experimental and Relational Analysis of *Acinetobacter rudis* (Mw582308) Over Leady Rosetta Potato

As represented in Figure 2 the correlation coefficient of various features of Lady Rosetta potatoes in the *A. rudis*-treated plot. From this, it is clear that the growth of plants and height has a very strong association. Successively between size and weight, there is the second most strong association and which is also seen amount the weight of the plant and height of the plant. However, there is no association between the weight of the plant and the growth of the plant. Furthermore, it is also found that the descending order of those aspects is Weight of Plant potato tuber >Size of potato tuber >Height of plant >Growth of plant (Figure-2).

The effects on various features for potato species Lady Rosetta by *A. rudis* species (Table 1). The degree of weightage of Weight of Potato tuber -> Size of Potato tuber (f^2=6.7317), Height of Plant -> Growth of plant (f^2=4.0860), and Weight of Potato tuber -> Height of Plant (f^2=1.0759) have a large effect among all the other pairs while the growth of plant ->the size of Potato tuber (f^2=0.1098) is small effect whereas the height of Plant ->the size of Potato tuber (f^2=0.0314) poor effects. In the case of the weight of Potato tuber ->the growth of the plant (f^2=0.0070) has don't have any effects. From this, it is discovered that *A. rudis* have impacting equally on the weight and size of potato tuber, as well as the height and growth of plant species of Lady Rosetta.[12] Showed that PSB species caused potato plants to grow at a faster rate to increase their root/shoot weight than control plants.The effect of *A. rudis* led to an increase in the height and weight of potatoes, to prove that this bacterium works as a growth promoter.[17] This is because the nutrient phosphorus had been significantly ($p < 0.05$) enhanced.

Table 1: Relation of various features of Lady Rosetta potatoes

Effect	Beta	Indirect effects	Total effect	Cohen's f^2
Growth of plant -> Size of Potato tuber	0.1968	-	0.1968	0.1098
Height of Plant -> Growth of plant	0.9713	-	0.9713	4.0860
Height of Plant -> Size of Potato tuber	0.1141	0.1911	0.3052	0.0314
Weight of Potato tuber -> Growth of plant	-0.0402	0.6993	0.6590	0.0070
Weight of Potato tuber -> Height of Plant	0.7199		0.7199	1.0759
Weight of Potato tuber -> Size of Potato tuber	0.7430	0.2118	0.9548	6.7317

There are symbiotic relations with the *A. rudis* with the germination, plant growth, size, and weight of Lady Rosetta potatoes confirmed from the above hypothetical explorations. There are many considerable pieces of evidence approved that *A. rudis* species mobilize rock phosphate to solubilize for the uptake of plant roots and transport system.[19] However, the obtained results are corresponding to the study,[20] where the transformation of plant physiology like growth, size, and weight has been significantly ($p < 0.05$) associated with *A. rudis* as a commercial biofertilizer. The application of rock phosphate to potatoes inoculation could provide beneficial results in the potatoes cropping cycle, as this practice produced high crop yields and added more phosphate to the soil for high crop production.

C) Hypothetical Correlational Impact of *Acinetobacter rudis* over Leady Rosetta Potato

Null Hypothesis (H_0)–There is no significant impact of *Acinetobacter rudis* (MW582308) biofertilizer on the growth, size, and weight of Lady Rosetta potatoes.

H_0:µX≠µY

Alternate Hypothesis (H_1)

There is a definite significant impact of *Acinetobacter rudis*(MW582308) biofertilizer on the growth, size, and weight of Lady Rosetta potatoes.

H_1:µX=µY

Germination rates in *A. rudis*-treated plot and the average height of *A. rudis* treated plot crop was found to be correlated very strongly (r=0.942 f=0.01 at 0.05); as well as having distinct positive correlations (r=0.794 f=0.033 at 0.01) with the average size of *A. rudis*-treated plot potato tubers in cm, as well as strong positive correlations (r=0.659 f=0.107 at 0.00) with the average weight of *A. rudis*-treated potatoes in gms.Thus it was established that there is a strong to very strong correlation between *A. rudis* plots as a measure of growth, size, or weight of plants (Table 2).

Table 2: Pearson Correlations Coefficient of growth, size, and weight of Control and Acinebacterrudis biofertilizer Treated plot of Lady Rosetta potatoes

		Percentage of Germi nation in A. Treated plot	**Average Height of *A.rudis* treated plot crop in cm**	**Average Size of *A.rudis* Treated plots Potato tuber in cm**	**Average Weight of Potatoes tuber treated by A. rudis in gms**
Percentage of Germination in Acinetobacter Treated plot	Pearson Correlation	1	0.942**	0.794*	0.659
	Sig. (2-tailed)		.001	.033	.107
	N	7	7	7	7
Percentage of Germination in Control Plot	Pearson Correlation	0.136	0.287	0.099**	0.050**
	Sig. (2-tailed)	.124	.088	.006	.001
	N	7	7	7	7
Average Height of Control plot crop in Cm	Pearson Correlation	0.164**	0.197**	0.258*	0.139
	Sig. (2-tailed)	.000	.000	.013	.058
	N	7	9	7	7
Average Height of Acineto bacterrudis treated plot crop in Cm	Pearson Correlation	0.942**	1	0.834*	0.720
	Sig. (2-tailed)	.001		.020	.068
	N	7	9	7	7
Average Size of control plots Potatoes tuber in Cm	Pearson Correlation	0.157*	0.090*	0.196**	0.062**
	Sig. (2-tailed)	.049	.034	.000	.001
	N	7	7	7	7
Average Size of Acinetobact errudis Treated plots Potato tuber in Cm	Pearson Correlation	0.794*	0.834*	1	0.955**
	Sig. (2-tailed)	.033	.020		.001
	N	7	7	7	7

Average Weight of control plot of Potatoes tuber in Grm	Pearson Correlation	0.106	0.157*	0.171**	0.198**
	Sig. (2-tailed)	.076	.049	.000	.000
	N	7	7	7	7
Average Weight of Potatoes tuber treated by Acinetobacter in Gms	Pearson Correlation	0.659	0.720	0.955**	1
	Sig. (2-tailed)	.107	.068	.001	
	N	7	7	7	7

*Significance level 0.01 **Significance level 0.05

A. rudis-treated plot and the percentage of germination in the control plot have an extremely weak/poor positive correlation (r=0.136 f=0.124 at 0.00); *A. rudis*-treated plot crops have a poor positive correlation (r=0.287 f=0.088 at 0.00) with average height in cm, and potatoes tubers treated by *A. rudis* have poor to negligible positive correlation (r=0.099 f=0.006 at 0.05) with an average size in cm, there is also a poor to a negligible correlation between average weight and *A. rudis* treatment (r=0.050 f=0.001 at 0.050). The result from the analysis suggests that there is no significant correlation between the percentage of germination in the control plot, the growth of the plant, the size, or the weight of the plot treated with *A. rudis*. Inoculated strains benefit from rich Acinobacter species solubilization in soil because it buffers the pH of the soil, and it also plays a key role in promoting solubilization of phosphorus (P).[21]

There is a very weak positive correlation (r=0.164 f=0.000 at 0.05) between the average height of the control plot crop in cm and the percentage of seedlings that germinate in the *A. rudis*-treated plots, *A. rudis*-treated plot crops have a poor positive correlation (R=0.197 f=0.000 at 0.05) with average height, and potato tubers treated with *A. rudis* have a poor positive correlation (R=0.258 f=0.013 at 0.05) with average size. Moreover, there is a low positive correlation (r=0.139, f=0.058 at 0.000) between the average weight of Potato Tubers treated by *Acinetobacter rudis*(MW582308) in Gms. Because of the foregoing, it appears that there is no significant correlation between the height of control plots and germination rates, growth of plants, size, and weight of *A. rudis*-treated plots.

The correlations found between the average height of *A. rudis* treated plot crop in Cm and the average size of *A. rudis* treated plots Potato tuber in cm are extremely strong (r=0.834 f=0.020 at 0.01), on the other hand, the correlation with an average weight of *A. rudis* treated plots Potato tuber in cm is extremely strong (r=0.720 f=0.068 at 0.00).*Acinetobacter rudis*(MW582308) plots showed a strong to a very strong positive correlation between plant size with germination percentage, and plant weight with the Lady Rosetta potato species.

A poor positive correlation was found (r=0.157, f=0.049, P=0.01) between the average size of control plots Potatoes tubers in cm, and Percentage of Germination in *Acinetobacter rudis* treated plots; incredibly poor positive correlation (r=0.196 f=0.000 at 0.05) with Average size of Potatoes tubers inoculated with *Acinetobacterrudis*(MW582308); whereas it has a weak positive correlation (r=0.067 f=0.000 at 0.05) with Average height of plot crops treated with *Acinetobacter rudis*(MW582308) and possesses a weak to negligible positive correlation (r=0.062 at 0.05 f=0.001) with Average Weight of Potatoes tubers inoculated with *Acinetobacter rudis*(MW582308). There wasn't a significant correlation between the average size of control plots Potatoes tubers in cm and germination rates, growth of plants, size, and weight of *A. rudis* treated plots. Similarly, the Average Size of *A. rudis*-treated plot crops in cm and the average weight of *A. rudis*-treated plots of Potato tubers in cm were found to be highly correlated (r=945 f=0.001 at 0.05). It has been found in these studies that there was a strong to a very strong positive correlation between seedling size with germination percentage growth or potato weight with *Acinetobacter rudis* plots.

In addition, a poor negative correlation (r=0.157 f=0.049 at 0.00) was found between the average weight of control plots of potatoes tubers and germination rates for *A. rudis*-treated plots; whereas a weak positive correlation (r=0.106 f=0.076 at 0.00) was observed for the average weight of *A. rudis* treated plots and germination rates. Moreover, it has a poor positive correlation (r=0.171 f=0.000 at 0.05) with the average height of *A. rudis*-treated plot crop; in addition, it has a poor correlation (r=0.198 f=0.000 at 0.05) with an average weight of *A. rudis* treated potatoes tubers in gms. The results indicate there is no significant correlation between the average weight of control plots of potato tubers in grams and germination percentage, growth of plants, or size of *A. rudis*-treated plots.

Acinetobacter rudis maximal colonization and maximal enhanced effect on plant growth parameters and nutrient uptake.[22,23] There is a direct effect of root exudates on the colonization behaviours of specific bacterial strains, as well as on the type of plant.[24] The presence of antigenic lipopolysaccharide (LPS) molecules in phosphate attachment to roots mediated the interaction with root cells to enhance root growth.[25] So, P solubilizing bacteria can reduce the phosphorous leaching loss and it can effectively enhance the plant growth.[26]

The results showed several extremely strong, very strong, and strong positive correlations between germination percentages, plant growth, and size of Lady Rosetta potatoes treated with *A. rudis* biofertilizer. It was found that there was no significant correlation between controls and treated plots of Lady Rosetta potatoes. According to our findings, it was found that biofertilizers such as *A. rudis* were significantly supportive of germination and plant growth stimulation, as well as increasing the size and weight of Lady Rosetta potatoes. Biofertilizers that solubilize phosphorus can also be important as they can store the nutrients in the soil. These organisms possess high-affinity transporters that can move sparingly soluble P, and as the bacteria die, this soluble P becomes available to plants through mineralization.[27-30]

Conclusion

Chemical fertilizers and animal manures must be eliminated to prevent a drastic decline in food production. To reduce the adverse impacts of chemical fertilizers on the environment, an integrated management approach must be implemented for nutrient inputs that are incorporated into the soil as agricultural inputs. This study determined that bacterial strains canpromote the germination of Lady Rosetta potatoes. *A. rudis* (MW582308) bacterial inoculation has also been found to lead to improved growth of Lady rosetta potatoes as measured by the increase in tuber size and weight following inoculation. As a bioinoculant, *A. rudis*, a P-solubilizing strain, proved very promising. Potato plants grew, yielded, and received P nutrition more effectively following the application of this bacterial strain. As a bioinoculant for enhancing sustainable agricultural production, the bacterial strain tested may well be used to reduce environmental pollution due to excessive use of chemical fertilizers and the high cost of P fertilizer production. In addition, this study emphasizes the importance of evaluating possible growth-promoting bacteria under field experimental conditions. The weight and size of potato tubers showed a close correlation with plant height in experimental plots but were not observed in control plots. The study concluded that *A. rudis* has a higher potential to solubilize rock phosphate into mobilizable mineral forms of phosphorous that can be used for growth and yield improvement without compromising the environment.

Acknowledgments

The authors are extremely grateful to the Ami group of companies for providing laboratory facilities.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors declare no conflict of interest.

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