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GAMMA IRRADIATION EFFECT ON PHOSPHATE SOLUBILIZING BACTERIA *ACINETOBACTER CALCOACETICUS*

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ABSTRACT

Phosphorus deficiency is a growing global issue on approximately 40% of cultivated land, primarily in tropical, acidic, and alkaline regions. This deficit, as a result of soil fixation, erosion, and phosphorus lag behind absorption, severely limits plant growth, crop yield, and soil fertility. While chemical fertilizers are used regularly to correct this situation, excessive use leads to environmental pollution. Alternatively, phosphate-solubilizing bacteria (PSB), such as Acinetobacter calcoaceticus, offer a biodegradable solution through the conversion of insoluble phosphorus to plant-accessible forms. This study explores the application of gamma irradiation, a mutagenesis technique that induces genetic alterations to improve the phosphate-solubilizing capability in the bacteria. The results showed from 931 bacteria colonies chosen after the irradiation treatment, 9.88% of bacteria strains showed a hypermorphic effect on the phosphate solubilization compared to the control, and 36.84% of bacteria strains showed hypomorphic effects. The distribution of hypermorphic bacteria strains across the phosphate solubilization index (SI) range was the higher SI range (3.1-3.5) was 8, the middle SI range (2.1-2.5) was 50, and the lower SI range (1.1-1.5) was 0. Among the 8 higher hypermorphic mutants, B346, B394, C92, C371, D20, D49, D96, and D100, only 3 mutant strains (C371, D20, and D100) showed statistical significance compared to control. This study has successfully produced several improved Phosphate-Solubilizing Bacteria (PSB) strains with strong potential for development as biofertilizers.

Keywords: Gamma irradiation, Phosphorus, Phosphate-solubilising bacteria, *Acinetobacter calcoaceticus*, Mutagenesis

INTRODUCTION

Phosphorus deficiency is one of the issues that is widely affecting agricultural lands across the globe. Phosphorus (P) is an essential macronutrient in plants. It is needed in plant photosynthesis, respiration, energy transport, and nucleic acid formation (Khan *et al*., 2023). According to Solangi *et al.* (2023), there is a possibility of around 40% of agricultural land being phosphorus deficient due to the immobilization or slow uptake of phosphorus ions from the soil into roots. About 10 million hectares of cultivated land, particularly in tropical, acid, and alkaline soils, are experiencing some degree of phosphorus deficiency in the form of low native phosphorus in soil, erosion and runoff, fixation, immobilization, and slowed phosphorus uptake, making the phosphorus unavailable for plant use (Sharmin *et al*., 2024). Phosphorus deficiency was very 0detrimental to agriculture by limiting plant growth and productivity, leading to poor yields of crops and ultimately poor soil fertility (Solangi *et al*., 2023). Farmers will, therefore, be strongly obligated to apply enormous amounts of chemical fertilizers to offset the deficiency. Meanwhile, overuse of chemical fertilizers can lead to pollution of the environment (Bisht *et al.,* 2021).

Besides the use of chemical fertilizers, plant growth-promoting rhizobacteria (PGPR), such as phosphate-solubilizing bacteria (PSB), can convert insoluble phosphorus into plant-available forms. PSB is a class of beneficial bacteria that infects the roots and converts insoluble phosphorus into soluble forms that are taken up by plants directly (Zhang *et al.,* 2024). PSB occurs in *Pseudomonas, Bacillus, Rhizobium, Azotobacter, Enterobacter, Serratia, Burkholderia*, and *Acinetobacter* (Pan *et al.,* 2023). PSB from *Acinetobacter* sp. will be investigated in this study. *Acinetobacter calcoaceticus* is a non-motile, aerobic, Gram-negative rod. They are usually found in isolation from water or soil and are known to have phosphate solubilization potential (Wakako *et al*., 2014).

A. calcoaceticus in this study has been enhanced in the ability for phosphate solubilization through gamma irradiation technology. The method, known as mutagenesis, involves the exposure of PSB to gamma rays that are capable of making alterations to DNA or chromosomes and initiating genetic mutations artificially (Durland & Ahmadian-Moghadam, 2022). These mutations can lead to new characteristics that increase their ability to solubilize phosphate in the soil. Previous research proved that ionizing radiation is promising for improving the effectiveness of PSB. *Pseudomonas fluorescens* were gamma-ray irradiated (100 – 1000 Gy) and assayed for phosphate-solubilizing activity (Kuheli *et al*., 2003). The mutant strains showed 2-3 folds higher phosphate-solubilizing activity than that of the wild-type strains. Whilst Chaudhary *et al.* (2022) has examined the enhancement in phosphate solubilization of *Bacillus subtilis* by UV mutagenesis and a superior PSB mutant strain GCM11 was obtained. Recent study on the gamma irradiation of *Aspergillus niger*, a phosphate-solubilizing fungus has shown an increased citric acid yield and improved phosphate solubilization (Peng *et al*., 2022). Yang *et al.* (2022) found that ion beam irradiation of *Penicillium oxalicum* resulted in mutant strains exhibiting higher organic acid secretion and consequently, greater phosphate-solubilizing activity. These findings collectively suggest that induced mutation using ionizing radiation can effectively breed superior fungal strains with enhanced bio-fertilizing potential.

Gamma irradiation facility at Malaysian Nuclear Agency (Gamma Cell Biobeam GM8000, Germany), has been used for mutation induction on agriculture samples as well as on biological samples including bacteria and fungus. Gamma irradiation was chosen in the study because gamma rays can cause changes in the DNA or chromosome level, which may increase the production or activity of some enzymes and enhance the phosphate-solubilizing capability (Abozahra *et al.,* 2025). Besides, gamma irradiation can induce mutations that increase stress tolerance in PSB and make it more tolerant to environmental stresses (Mohamed *et al.,* 2021). Moreover, the mutated PSB may have improved root colonization in the rhizosphere and led to more effective phosphate solubilization (Elhaissoufi *et al.,* 2020). Ultimately, this proven method of inducing beneficial mutations in PSB allows for the development of enhanced biofertilizer strains, which can significantly boost soil fertility and phosphorus availability for optimal crop growth.

METHODOLOGY

Bacteria Strain

A. calcoaceticus bacteria used in the study was isolated from compost and soil at the Malaysian Nuclear Agency by Phua *et al.* (2019). The species identification was done using the 16S rRNA method by Chong *et al*. (2024).

Gamma Irradiation

Irradiation was conducted at Malaysian Nuclear Agency using gamma cell (Biobeam GM8000, Germany). Dose mapping was done before gamma irradiation was performed because the dose distribution inside any irradiation chamber is inherently non-uniform. Therefore, we used calibrated dosimeters placed at the sample position provides empirical confirmation that the bacteria actually receive the intended dose (Sharma *et al*., 2021). A radiosensitivity test previously conducted by Phua *et al.* (2024) established the lethal dose (LD50) of *Acinetobacter calcoaceticus* at 445.5 Gy. Accordingly, this dose was employed for gamma irradiation-mediated mutagenesis in the present study. The bacterial strains were cultured on nutrient agar (NA) plates in 90 mm diameter Petri dishes and were incubated at 28 ± 2 °C for 24 hours. The plate had roughly 50-100 single colonies per plate selected for further irradiation treatment. Ten plates were needed for each irradiation treatment, and the experiment was repeated 4 times. Culture plates were wrapped with aluminium foil and irradiated in the gamma cell. Fricke dosimetry was used to measure the doses absorbed during the treatment. The irradiated plates were placed at room temperature for 24 hours.

Colonies isolation

Approximately about 20-30 colonies were picked randomly from the plate. The single colony was picked using a sterile inoculating loop and then inoculated into a sterile test tube with 15 mL nutrient broth. The cultures were incubated in an incubator shaker at 28 ± 2 °C for 24 hours.

Screening for Phosphate-Solubilizing Strains

The qualitative screening method used and Potato Dextrose Agar (PDYA) media according to Silva *et al.* (2014). The media contains 39 g of potato dextrose agar, 1 g of yeast extract, and a calcium phosphate mixture of 50 mL of K_2HPO_4 (10% w/v) and 100 mL of $CaCl_2$ (10% w/v), which were separately autoclaved and added to the media before the resulting liquid medium was poured onto plates. Approximately 20 µL of irradiated bacterial suspension (>10^8 cfu/mL) was spotted on the PDYA agar plate. The non-irradiated *A. calcoaceticus* suspension was also spotted side-by-side with the sample as a control. The plates were incubated at 28 ± 2 °C for 6 days or until the expansion of the clear zone became stagnant. The expansion of the clear zone was quantified using the Solubilization Index (SI) (Mengesha & Legesse, 2024).

$$SI = \frac{\text{Colony Diameter} + \text{Halo Zone Diameter}}{\text{Colony Diameter}}$$

Colony Diameter: The bacterial or fungal colony diameter.
Halo Zone Diameter: The diameter of the clear zone around the colony where phosphate has been solubilized.

A higher SI indicates greater phosphate-solubilizing activity. Each bacterial culture had five replicates. ANOVA analysis with the means separated by Duncan's Multiple Range tests ($P \leq 0.05$) using One-Way ANOVA, SPSS (IBM Corp., 2021).

RESULTS AND DISCUSSION

The number of bacterial strains irradiated in the experiment was 931, which an adequate data to work with (Table 1). Approximately 92 strains (9.88%) showed a higher phosphate solubilization index (hypermorphic effect) than that in the control. This indicated that irradiation enhanced the capacity of the bacteria to solubilize the phosphate. However, 343 strains (36.84%) showed a hypomorphic effect on the phosphate solubilization index compared to the control, which means that irradiation reduced the capacity of the bacteria to solubilize the phosphate. This result show that irradiation would rather suppress or impair the activity of phosphate solubilization of *A. calcoaceticus* than enhance it where only 9.88% of the strains showed a positive effect. The low percentage indicated that only a few strains had their activity increased due to some genetic or physiological features that make them more susceptible to irradiation. This finding supports by a 2025 review on radiation mutagenesis states that most newly induced mutations are thought to be deleterious or neutral with no effects. Accordingly, mutations with beneficial effects on traits of interest are very rare (Geras'kin *et al.,* 2025)**.** The remaining strains (53.28%) showed no significant difference in solubilization activity compared to the control. These strains were not affected by irradiation, and the effects on their solubilization activity were not statistically detectable.

Table 1. The number of bacteria irradiated with gamma irradiation and the effect of the gamma-ray on the phosphate solubilization index (SI) of the sample.

Number of strains	Number of hypermophic strains	Percentage of hypermorphic strains (%)	Number of hypomorphic strains	Percentage of hypomorphic strains (%)
931	92	9.88	343	36.84

The results in Figure 1 showed that most hypermorphic mutant strains, 50 out of 92, fell in the SI range of 2.1–2.5, suggesting that irradiation typically induced a moderate enhancement in solubilization activity rather than extreme improvements. The second-highest number of hypermorphic mutant strains was 26 out of 92, falling in the SI range of 2.6–3.0. Approximately 8 strains were found in the higher SI range (3.1–3.5) and lower SI range (1.6–2.0). Only 8 strains fell in the 3.1–3.5 SI range, indicating that very high solubilization activity after irradiation was rare. These strains could be of interest for further study and potential biofertilizer applications as they represent the best-performing subset. The 8 strains in the 1.6–2.0 SI range showed only a slight improvement over the control, suggesting their response to irradiation was minimal. The absence of strains in the 1.1–1.5 SI range indicated that irradiation does not produce strains with very low solubilization activity among the hypermorphic group.

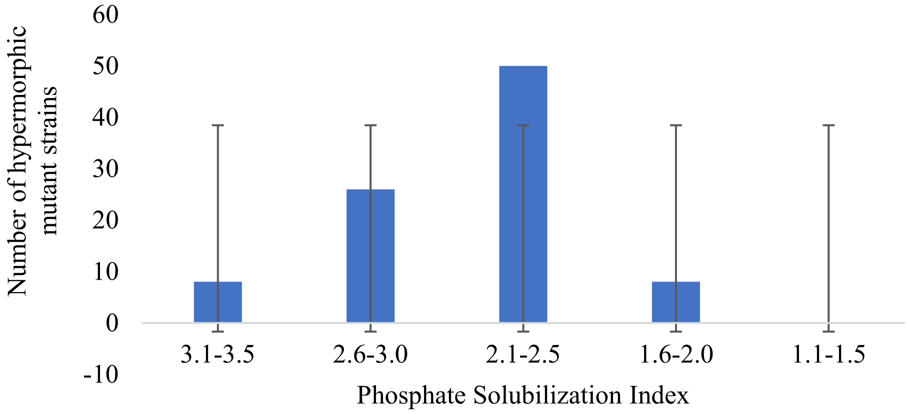

Figure 1. The number of hypermorphic *A. calcoaceticus* strains based on their phosphate solubilization index (SI) ranges (Premono *et al.,* 1996). SI>1: Indicates some level of phosphate solubilization. The higher SI reflects the greater phosphate-solubilizing activity. SI=1: No solubilization.

The eight strains with a higher SI range (3.1–3.5) from Figure 1 were repeated in the phosphate solubilization test with at least five replicates per strain on the PDYA medium. The clear zone around bacteria colonies on the PDYA medium was a key indicator of the phosphate-solubilizing activity of *A. calcoaceticus* (Fig. 2). The size of the clear zone reflects the efficiency of the bacteria in solubilizing insoluble phosphate compounds into soluble forms that plants can absorb. The expansion of the clear zone was quantified using the Solubilization Index (SI) (Mengesha & Legesse, 2024). Based on the results shown in Figure 3, the average SI values ranged from 2.59 (D49) to 2.79 (D20), indicating relatively close performance among the strains with no significant differential among them. Moreover, the variability in the SI ranged from 0.0927 to 0.2272 was generally low, indicating the data were robust and reproducible (Table 2).

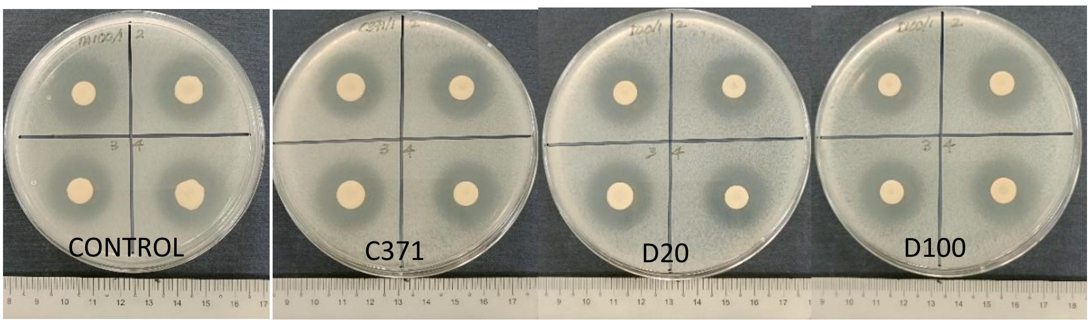

Figure 2. A phosphate solubilization clear zone appeared around the hypermorphic *A. calcoaceticus* strains (C371, D20, and D100) and the control strain on agar media.

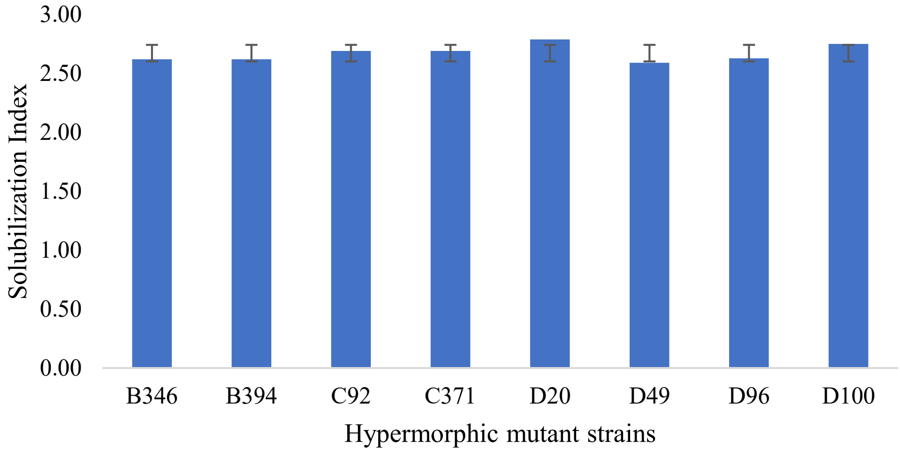

Figure 3. The hypermorphic mutant strains based on their phosphate solubilization index (SI) ranges (Premono *et al.,* 1996). The f-ratio value is 1.98446. The p-value is .118403. The result is not significant at $p < 0.05$.

The statistical significance (p-values) in Table 2 showed significant strains ($p<0.05$) C371, D20, and D100 with p-values 0.019953, 0.004327, and 0.010642, respectively. These strains exhibited statistically significant differences in SI compared to the control, indicating that they have higher phosphate-solubilizing activity. The remaining strains B346, B394, C92, D49, and D96 have p-values higher than 0.05, indicating their SI values were not significantly different. Among the significant strains, D20 was the most significant, standing out with the highest SI and the lowest p-value. However, C371, despite having a mid-range SI, shows very low variability and significance, making it a reliable candidate. Based on the results, D20, C371, and D100 were the most promising strains due to their statistical significance. These mutated phosphate-solubilizing bacteria strains have shown an enhancement in their ability to produce organic acids, enzymes, or metabolites that solubilize phosphate caused by gamma irradiation. Further investigation into their gene sequences at the molecular level is needed.

Table 2. The comparison between each potential mutated strain with the control SI (2.48) based on their average Selection Index (SI) (Premono *et al.,* 1996). Standard deviations and p-values were calculated using One-Way ANOVA with significance at the $p < 0.05$ level.

Potential mutated strains	Average SI	Standard Deviation	p-value	Significant at $p < 0.05$
B346	2.62	0.1409	0.154868	Not Significant
B394	2.62	0.1874	0.203302	Not Significant
C92	2.69	0.2272	0.087023	Not Significant
C371	2.69	0.0927	0.019953	Significant
D20	2.79	0.1383	0.004327	Significant
D49	2.59	0.1715	0.271801	Not Significant
D96	2.63	0.1498	0.120386	Not Significant
D100	2.75	0.1429	0.010642	Significant

CONCLUSION

Infertility of agricultural soils due to phosphate deficiency is a worldwide issue and causes a reduction in crop production. One solution to overcome this issue is to use phosphate-solubilizing bacteria as it can increase the phosphorus content in the soil and improve the soil condition in the long term. Mutagenesis using gamma irradiation was used in this study to develop new bacterial strains with enhanced characteristics, such as phosphate solubilization. These mutants exhibit greater phosphate solubilization activity than the original strain and can be further developed as biofertilizer products.

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REFERENCES

Abozahra, M. S., Amin, M. A., Sarker, T. C., Abd-ElGawad, A. M., and Aboelezz, E. (2025). Molecular, biophysical, and biochemical studies on irradiated *Zea mays* seeds using various sources of gamma rays for dosimetrical applications. *Scientific Reports* Vol. 15, 9340. doi.org/10.1038/s41598-025-87531-5

Bisht, N. and Chauhan, P. S. (2021). Excessive and disproportionate use of chemicals cause soil contamination and nutritional stress. Soil contamination - threats and sustainable solutions. *IntechOpen*. doi:10.5772/intechopen.94593

Chaudhary, R., Nawaz, A., Fouillaud, M., Dufossé, L., Haq, Iu., and Mukhtar, H. (2022). Enhanced production of phytase through UV mutagenesis of *Bacillus subtilis*. *Food Science & Nutrition* 10, 3700–3709. DOI: 10.1002/fsn3.2967

Chong, S. P., Phua, C. K. H., Mohd-Dzomir, A. Z., Bahari, N., and Deraman, M. (2024). Pyrroloquinoline quinone (pqq) genes isolation in *Acinetobacter calcoaceticus.* In *Seminar R&D 2024, 27-29 August 2024, Bangi, Selangor, Malaysia*

Durland, J. and Ahmadian-Moghadam, H. (2022). Genetics, Mutagenesis. In: *StatPearls [Internet]*. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK560519/

Elhaissoufi, Wissal., Khourchi, S., Ibnyasser, A., Ghoulam, C., Rchiad, Z., Zeroual, Y., Lyamlouli, K., and Bargaz, A. (2020). Phosphate solubilizing rhizobacteria could have a stronger influence on wheat root traits and above ground physiology than rhizosphere P solubilization. *Frontiers in Plant Science* Vol. 11. doi:10.3389/fpls.2020.00979

Geras'kin, S., Churyukin, R., and Kazakov, E. (2025). Application of ionizing radiation for crop improvement. *Planta* 262(3), 76. DOI: 10.1007/s00425-025-04796-w

IBM Corp. (2021). IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY, IBM Corp.

Khan, F., Siddique, A. B., Shabala, S., Zhou, M., and Zhao, C. (2023). Phosphorus plays key roles in regulating plants' physiological responses to abiotic stresses. *Plants (Basel)* 12(15), 2861. doi: 10.3390/plants12152861

Kuheli, D., Vandana, K., and Reeta, G. (2003). 'P' solubilization potential of plant growth promoting *Pseudomonas* mutants at low temperature. *Microbiological Research* Vol. 158(4), 359-362. doi.org/10.1078/0944-5013-00217

Mengesha, A. S. and Legesse, N. H. (2024). Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of lentil (*Lens culinaris* M.) collected from Hagere Mariam district, Central Ethiopia. *PLoS ONE* Vol. 19(11), e0308915. doi.org/10.1371/journal. pone.0308915

Mohamed, E. A., Osama, E., Manal, E., Samah, A., Salah, G., Hazem, K. M., Jacek, W., and Nabil, E. (2021). Impact of gamma irradiation pretreatment on biochemical and molecular responses of potato growing under salt stress. *Chemical and Biological Technologies in Agriculture* Vol. 8, 35. doi.org/10.1186/s40538-021-00233-8

Pan, L. and Cai, B. (2023). Phosphate-solubilizing bacteria: advances in their physiology, molecular mechanisms and microbial community effects. *Microorganisms* 11(12), 2904. doi: 10.3390/microorganisms11122904.

Peng, Q., Xiao, Y., Zhang, S., Zhou, C., Xie, A., Li, Z., Tan, A., Zhou, L., Xie, Y., Zhao, J., Wu, C., Luo, L., Huang, J., He, T., and Sun, R. (2022). Mutation breeding of *Aspergillus niger* by atmospheric room temperature plasma to enhance phosphorus solubilization ability. *PeerJ* Vol. 10, e13076. doi: 10.7717/peerj.13076

Phua, C. K. H., Ali, T. K. Z., Chong, S. P., Mohd-Dzomir, A. Z., and Abdullah, N. H. L. (2024). Guideline on mutagenesis of biofertiliser bacteria using gamma irradiation. Agensi Nuklear Malaysia, e ISBN 978-967-2706-19-9

Phua, C. K. H., Saud, H. M., Abdul-Rahim, K., Ishak, C. F., and Wahab, P. E. M. (2019). Effects of gamma-irradiated *Acinetobacter calcoaceticus* on nitrogen and phosphorus uptake of green mustard (*Brassica chinensis*). *Malaysian Journal of Soil Science* Vol. 23, 149-166

Premono, M. E., Moawad, A. M., and Vlek, P. L. G. (1996). Effect of phosphate-solubilizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere. *Indonesian Journal of Crop Science* 11(1), 13–23.

Sharma, D., Sharma, A., Singh, B. (2021). Effect of geometry and position of dosimeters on dose mapping of Gamma Chamber-900 using ceric-cerous dosimeter. *Journal of Radioanalytical and Nuclear Chemistry* 330, 1447–1454. DOI: 10.1007/s10967-021-07983-0

Sharmin, H., Tahsina, Deepranjan, S., Rahul, D., Mohammad, G. K., Rafi, U., Nazeer, A., Mohammad, A. H., Asim, M., and Naser A. A. (2024). Sustainable Management of Phosphorus in Agriculture for Environmental Conservation. Phosphorus in Soils and Plants. *IntechOpen* doi:10.5772/intechopen.113086

Silva, M. L. R. B., Figueirôa, C. dos S., Mergulhão, A. C. do E. S., and Lyra, M. do C. C. P. de. (2014). Diversidade e potencial de solubilização de fosfato in vitro por bactérias endofíticas associadas à cultura da palma forrageira (Opuntia e Nopalea) em Pernambuco. *Pesquisa Agropecuária Pernambucana* 43(1), 1-12. DOI: 10.12661/pap.2014.013

Solangi, F., Zhu, X., Khan, S., Rais, N., Majeed, A., Sabir, M. A., Iqbal, R., Ali, S., Hafeez, A., Ali, B., Ercisli, S., and Kayabasi, E. T. (2023). The global dilemma of soil legacy phosphorus and its improvement strategies under recent changes in agro-ecosystem sustainability. *ACS Omega* Vol. 8(26), 23271-23282. doi: 10.1021/acsomega.3c00823

Wakako, S., Masayuki, S., Kyoko, M., and Masaaki, M. (2014). Plant growth-promoting bacterium *Acinetobacter calcoaceticus* P23 increases the chlorophyll content of the monocot Lemna minor (duckweed) and the dicot *Lactuca sativa* (lettuce). *Journal of Bioscience and Bioengineering* Vol. 118 (1), 41-44. doi.org/10.1016/j.jbiosc.2013.12.007

Yang, T., Li, L., Wang, B., Tian, J., Shi, F., Zhang, S., and Wu, Z. (2022). Isolation, mutagenesis, and organic acid secretion of a highly efficient phosphate-solubilizing fungus. *Frontiers in Microbiology* Vol. 13, 793122. doi: 10.3389/fmicb.2022.793122

Zhang, T., Jian, Q., Yao, X., Guan, L., Li, L., Liu, F., Zhang, C., Li, D., Tang, H., and Lu, L. (2024). Plant growth-promoting rhizobacteria (PGPR) improve the growth and quality of several crops. *Heliyon* Vol. 10(10). doi.org/10.1016/j.heliyon.2024.e31553