INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 21, No. 5, p. 239-245, 2022

RESEARCH PAPER OPEN ACCESS

Enhancement of phosphate solubilization by *Bacillus subtilis* induced by UV mutagenesis

Chibani Hiba Rahman*, Hamoum Hakim, Chibani Abdelwaheb

[1]*Laboratory of Microbiology and Plant Biology, Faculty of Natural and Life Sciences, University of Mostaganem, Algeria*

Key words: Phosphate solubilizing bacteria, *Bacillus subtilis*, UV-mutagenesis, Enhancement

http://dx.doi.org/10.12692/ijb/21.5.239-245 Article published on November 20, 2022

Abstract

The ability of PSB to solubilize phosphate is considered to be one of the most important traits in plant nutrition. These bacteria play an important role in providing plants with phosphorus. The aim of the present study is the isolation of phosphate solubilizing bacteria as well as the enhancement of phosphate solubilization of *Bacillus subtilis* using UV mutagenesis. 24 bacteria were isolated from the rhizosphere of different regions in a western of Algeria on the basis of their ability to solubilize tricalcium phosphate in on Pikovskaya's agar plates. Using liquid medium significant amounts of phosphorus solubilized by the isolates were recorded, reaching a value of 346.34µg/ml of free phosphorus by the isolate GC9. This later was subjected to UV mutagenesis for its high phosphate solubilizing ability and it was identified as *Bacillus subtilis* using 16S rRNA gene sequence analysis. Induction of mutation in strain was carried out at different exposure times: 0, 4, 8, 12 and 16 min at a distance of 10 cm between UV source and treated bacteria. A high rate of improvement of phosphate solubilization in liquid medium was observed by the mutant GCM11 (109.78%).

* **Corresponding Author:** Chibani Hiba Rahman ✉ hibachibanidoc@gmail.com

Introduction

Phosphorus is an indispensable nutrient for plant growth and development. However, it is usually present at low concentrations in soil, due to numerous geochemical processes constraining its mobility and availability (Plassard *et al.,* 2015). In addition, the majority of soluble phosphorus added to the soil is either absorbed (immobilized) by calcium or precipitated by free forms of iron or aluminum, depending on the type of soil (Khan *et al.,* 2014). In agriculture, the deficiency of phosphate is generally compensated by the addition of chemical phosphate fertilizers to the soil. However, it is quickly immobilized and therefore becomes useless for the plants (Gyaneshwar *et al.,* 2002).

Among the proposed alternatives, the use of phosphate solubilizing bacteria (PSB) which is one of the ecological options to avoid or minimize the excessive use of chemicals in agriculture and could be a promising source as a bio-fertilizer in agriculture (Khan *et al.,* 2007). These bacteria are common in the rhizosphere and make phosphorus available to plants by solubilization of precipitated phosphates (Vessey *et al.,* 2003). In fact, phosphate-solubilizing bacteria exhibit multifunctional properties and can also exhibit characters that promote plant growth like biological nitrogen fixation, phytohormone synthesis and the decrease of environmental stress (Cattelan *et al.,* 1999). Soil bacteria belonging to genera *Bacillus*, *Pseudomonas*, *Rhizobium*, *Enterobacter*, and *Burkholderia* have been established to be powerful phosphate solubilizers (da Costa *et al.,* 2015; Shintu and Jayaram, 2015). Hence the genus *Bacillus* is one of the most extensively distributed in nature and is frequently used for commercial productions. The *Bacillus* species have a fast growth rate, extremely adaptable metabolism, and excellent physiological characteristics (Saxena *et al.,* 2020)

The genetic improvement of microbial isolates plays a key role in the commercial development of the microbial fermentation process. Generally wild strains solubilize limited amounts of the desired phosphate to be useful for agricultural applications (Glazer and Nikaido, 2007). The use of ultraviolet radiation-mutagenesis is one of the best known and most commonly used techniques in this context (Javed *et al.,* 2013). The main objective of this work consists in isolating phosphate-solubilizing bacteria from different plant rhizospheres and improving this solubilization capacity by using UV mutagenesis.

Materials and methods

Bacterial isolation

Soil samples were randomly collected from the rhizosphere regions of lentils, artichokes, chickpeas, beans and wheat plants growing at different sites at Relizane (western Algeria). Isolation of PSB was carried out by serial dilution method up to 10^{-6}. Subsequently 0.5mL of the suspension diluted to 10^{-6} were spread on nutrient agar (NA) medium. After incubation for 3 days at 30°C, detected bacteria were purified then maintained at -20°C in 50% glycerol as a stock.

Screening for phosphate solubilization

Isolates were tested by plate assay using Pikovskaya medium (Pikovskaya, 1948) supplemented with 0.5% $Ca3\ (PO4)_2$ to screen its efficiency to solubilize phosphate. Inoculated plates were incubated for 7 days at 30°C. Appearance of clear halo zone on Pikovskaya's agar plates indicates positive phosphate solubilization ability.

Phosphate solubilization in liquid Medium

Phosphate solubilizing capacity of each strain was determined on National Botanical Research Institute's Phosphate growth medium (NBRIP) (Nautiyal, 1999) supplemented with 5g of TCP as sole phosphorus source. The flasks containing 50mL of NBRIP medium were inoculated with 1mL of bacterial suspension (2×10^9CFU/mL) and incubated at 30°C on a rotary shaker for 6 days. The cultures were harvested by centrifugation at 6000 rpm for 30 min. The phosphorus in supernatant was estimated by Vanado-molybdate-yellow color method (Jackson, 1973). The values of soluble phosphate liberated were expressed asμg/ml over control.

16S rRNA gene sequence analysis

The PSB isolated from soil were identified on the base on morphological and biochemical tests as specified

in Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994) (data not shown). The bacterial isolate GC9 was selected for its high phosphate solubilizing ability to be used in further experiments. Molecular identification of bacterial isolate (GC9) was carried out using 16S rRNA gene amplification using a universal primer pair 1390 (5'-AACGGGCGGTGTGTRCAA-3') and PA2 (5'-AGTTTGATCMTGGCTCAG-3'). The sequences of 16S rRNA gene were analyzed using the BLAST searching program at the National Center for Biotechnology Information (NCBI) website: http://www.ncbi.gov.

Preparation of bacterial suspension

Bacterial suspension was prepared by transferring colonies from Luria-Bertani medium to a 250mL flask containing 50mL of LB broth. The flask was placed in a shaker incubator at 30° C with stirring at 160 rpm for 18 h. After incubation the optical density of the bacterial suspension was adjusted to reach 0.8 at 600 nm (corresponding to approximately 10^9 CFU/mL). This suspension was used as a cell source for irradiation.

UV mutagenesis

The UV mutagenesis of GC9 was carried out according to the method of Courcelle *et al.* (2001) using different exposure times. 5mL of previously prepared bacterial suspension were poured into 10-cm diameter-Petri dishes at a distance of 10 cm from the UV lamp (30-W germicidal lamp, 2540-2550Å) and exposed to UV rays for 0, 4 , 8, 12 and 16 mins. 0.1mL of suitable dilutions of each treatment was spread on nutrient agar plates and incubated at 30° C for 24 hours. After incubation the bacterial colonies were counted and transplanted onto NA dishes for further studies. Isolates from each treatment were selected on the basis of differential macroscopic characteristics. The ability of mutant isolates to solubilize phosphate was tested in NBRIP liquid medium.

Statistical analysis

The data obtained in this study was subjected to analysis of variance (ANOVA) and comparisons of means were performed by Newman and Keuls test at $p \leq 0.05$ using statbox.

Results and discussion

Screening for Phosphate Solubilization

Plant Growth Promoting Rhizobacteria (PGPR) are a group of beneficial rhizobacteria that can enhance yield and growth of agricultural crops. The ability of some rhizospheric bacteria to solubilize phosphate in soil and make it available for plants is an important activity in improving plant growth.

A total of 37 phosphate solubilizing bacteria were obtained from different rhizosphere soil samples at Relizane region (western Algeria). The ability of the studied isolates to solubilize the inorganic phosphate was tested using solid Pikovskaya medium supplemented with tricalcium phosphate by the formation of transparent halo around bacterial colony (Fig. 1). Out of the 37 isolates 24 (64.86%) were marked as phosphate solubilizing bacteria with reference to their ability of transparent halo production with different diameters around their colonies. Phosphate solubilizing capacity of each isolate was also determined on NBRIP liquid medium. The results showed that the phosphate solubilizing ability of tested bacteria varied from 31.76 to 346.34µg/mL using TCP as a source of insoluble P. The isolate GC9 that showed the best capability in phosphate solubilization (346.34µg/mL) was selected to be used in further tests (Table 1).

Molecular identification of GC9 using 16S rRNA gene showed that it belonged to the genus *Bacillus* identified as *Bacillus subtilis*. The result was expressed as a percentage of similarity of the bacteria to be identified with the closest strains.

It has been reported that higher concentrations of phosphate-solubilizing bacteria are commonly found in rhizospheric soil compared to non-rhizospheric soil (Reyes *et al.*, 2007).

It has also been reported that *Bacillus, Pseudomonas, Rhizobium and Enterobacter* are the most efficient P solubilizers (Whitelaw, 1999). In another study, *Bacillus subtilis* has emerged as the best phosphate solubilizer compared to *Pseudomonas fluorescens* (Sivasakthi *et al.*, 2013)

Table 1. Phosphate solubilization by bacterial isolates.

Isolate	Concentration of phosphate (µg/ml)
GA5	130.04± 5.19 ^{n}
PA8	283.73±7.78^{c}
GA9	179.44± 5.61^{k}
LA11	326.1± 8.92^{b}
LB2	324.62± 10.67^{b}
GB3	295.22± 4.48^{d}
GB12	84.65± 2.81^{o}
PB16	31.76 ± 1.14^{s}
LB17	44.32± 1.30^{r}
GC1	284.82± 9.92^{e}
PC4	47.27± 2.08^{r}
PC8	296.08± 7.91^{d}
GC9	346.34 ± 8.82^{a}
LD3	268.58± 7.37^{f}
GD5	138.35± 6.48^{m}
GD10	146.33± 6.31^{l}
PD11	197.76± 7.04^{j}
GD13	244.88± 8.52^{h}
LE6	259.52± 8.73^{g}
GE7	76.63± 3.54^{p}
PE9	57.41± 3.78^{q}
PE11	148.83± 5.92^{l}
LE13	233.69± 7.18^{i}
GE15	305.47± 8.53^{c}

Values: Mean ± standard deviation, abc: homogeneous groups indicating statistically different values according to the Newman-Keuls test at p ≤0.05.

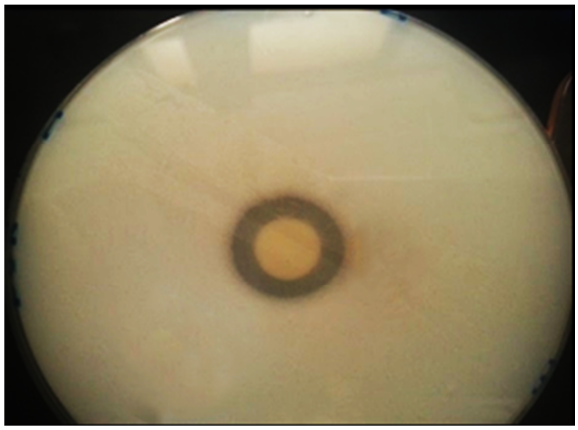

Fig. 1. Phosphate solubilization by GC9.

The mechanism of phosphate solubilization by *Bacillus* species is through the release of organic acids, chelation, and ion exchange while the action of organic acids is recognized as a major mechanism responsible for phosphorus release (Cheng *et al.*, 2017). Several studies showed almost similar amount of phosphate solubilized by *Bacillus* (Mahdi *et al.*, 2020; Bhatt and Maheshwari, 2020). Patil (2014), has stated that *B. subtilis* is a powerful phosphate solubilizer that tolerates soil salinity.

It has been known that inoculation of different plant with *Bacillus* species can increase the concentration of phosphorus in the host plant (Abdelmoteleb and Gonzalez-Mendoza, 2020; Mahdi *et al.*, 2020; Zhao *et al.*, 2022).

UV mutagenesis

UV radiation is a standard mutagen, and its mutagenicity has been studied extensively due to its ubiquity in nature and convenience of handling. In addition, UV is also applied in many fields such as UV disinfection technology in clinical microbiology and evolution engineering in the biotechnology. The bacterial suspension of GC9 was exposed to UV rays for different time intervals (4, 8, 12, 16 and 20 min). After incubation, a bacterial count was performed and the CFU for each time interval was calculated. The CFU decreased sharply over the exposure time as well as the survival rate of the bacterial isolates, it showed a reduction ranging from 100% to 22.39% after 4 min of exposure.

It also became almost zero after an exposure time of 16 and 20 minutes (Table 2). Mutant isolates were selected according to their differentiation in macroscopic appearance to test their ability to solubilize phosphate in NBRIP liquid medium. The amount of phosphate solubilized by the mutants varies from 159.41to 726.76µg/ml. In parallel, the percentage of improvement was calculated and it varies from -53.98% to 109.78%. Among all the mutant isolates 44.44% showed a positive improvement compared to the wild type isolate. A maximum improvement was observed in the GCM11 mutant with a percentage of 109.78% (Table 3).

Table 2. Survival data for GC9 after UV treatment at different exposure times.

Exposure time (min)	CFU/ml	Survival %
0	1.67x 10^{9}	100
4	3.74 x 10^{8}	22.39
8	8.35 x 10^{7}	5
12	1.4 x 10^{7}	0.84
16	1.82 x 10^{6}	0.01
20	5.46 x 10^{4}	0.003

Table 3. Phosphate solubilization of mutants after UV treatment.

Isolate	Concentration of phosphate (µg/ml)	Improvement %
GC9	346.44 ± 9.05^{g}	0
GCM1	254.61 ± 8.34^{l}	-26.50
GCM2	334.39 ± 9.67^{i}	-3.48
GCM3	256.55 ± 8.27^{l}	-25.94
GCM4	343.55 ± 8.93^{h}	-0.83
GCM5	580.44 ± 14.73^{e}	67.54
GCM6	658.63 ± 18.43^{c}	90.11
GCM7	321.42 ± 9.27^{j}	-7.22
GCM8	268.65 ± 7.75^{k}	-22.45
GCM9	239.78 ± 6.97^{m}	-30.78
GCM10	181.64 ± 5.32^{p}	-47.57
GCM11	689.00 ± 19.01^{b}	98.88
GCM12	726.76 ± 19.80^{a}	109.78
GCM13	159.41 ± 3.91^{r}	-53.98
GCM14	192.46 ± 4.61^{o}	-44.44
GCM15	234.64 ± 6.12^{n}	32.27
GCM16	578.31 ± 18.38^{e}	66.92
GCM17	486.13 ± 15.49^{f}	40.32
GCM18	176.38 ± 6.32^{q}	-49.08
GCM19	649.29 ± 19.14^{d}	87.41

Values: Mean ± standard deviation, abc: homogeneous groups indicating statistically different values according to the Newman-Keuls test at p ≤0.05.

The results of several studies illustrated that the mutant *Bacillus subtilis* species obtained by ultraviolet irradiation is a promising biosorbent for the remediation of many heavy metals (Wang, *et al.,* 2014, 2018). It has been also reported that a UV mutant strain of *Bacillus subtilis* enhanced acetoin production compared to the wild strain (Xu *et al*., 2011).

The salt tolerance of *Bacillus subtilis* isolates was improved from 10 to 14% using UV mutagenesis and PGP traits were also improved by mutant strains (Hingole and Pathak, 2016). Moreover, treatment of the mung bean seeds with UV mutant strain of *Bacillus subtilis* M-1 had a significant stimulation effect on plant growth compared to parent strain (Olga *et al*., 2019).

In our study the survival rate decreased from 100% to 20% after 4 min of exposure. Those results are similar with the work reported by El-Hamshary *et al.* (2018). The decrease in survivability of *bacillus* strain with an increase in exposure time has been reported by Nadeem *et al.* (2010). UV Mutant strains of *E. cloacae* isolated from Purslane rhizosphere improved the solubilization of soil phosphorus (El-Hamshary *et al*., 2018). It has been reported by Sivasakthi *et al.* (2015) that the UV mutated *Bacillus subtilis* that were efficient in phosphate solubilization improved in antagonistic activity against *Pyricularia oryzae* compared to the wild type.

Conclusion

The results of the present investigation revealed that among different rhizospheric isolates GC9 was the best phosphate solubilizer. UV mutagenesis treatment of *Bacillus subtilis* (GCM11) was able to enhance phosphate solubilization in liquid medium with an improvement rate of 109.78%. This UV mutant bacterial isolates can be used as inoculants to enhance the phosphorus uptake by plants and reduce the utilization of phosphorus fertilizers and increase yield of crop production.

Acknowledgements

The authors are thankful to the Laboratory of Microbiology and Plant Biology, Faculty of Natural and Life Sciences, University of Mostaganem, Algeria for providing the facilities for the work.

References

Abdelmoteleb A, Gonzalez-Mendoza D. 2020. Isolation and identification of phosphate solubilizing Bacillus spp. from *Tamarix ramosissima* rhizosphere and their effect on growth of Phaseolus vulgaris under salinity stress. Geomicrobiology Journal **37(10),** 901-908. https://doi.org/10.1080/01490451.2020.95321.

Bhatt K, Maheshwari DK. 2020. Zinc solubilizing bacteria (*Bacillus megaterium*) with multifarious plant growth promoting activities alleviates growth in *Capsicum annuum* L. 3 Biotech **10(2),** 1-10. https://doi.org/10.1007/s13205-019-2033-9.

Cattelan AJ, Hartel PG, Fuhrmann JJ. 1999. Screening of plant-growth promoting rhizobacteria to promote early soybean growth. Soil Science Society of America Journal **63(6),** 1670-1680. https://doi.org /10.2136/sssaj1999.6361670x.

Cheng J, Zhuang W, Li NN, Tang CL, Ying HJ. 2017. Efficient biosynthesis of d-ribose using a novel co-feeding strategy in *Bacillus subtilis* without acid formation. Letters in Applied Microbiology **64(1),** 73-78. https://doi.org/10.1111/lam.12685 .

Courcelle J, Khodursky A, Peter B, Brown PO, Hanawalt PC. 2001. Comparative gene expression profiles following UV exposure in wild-type and SOS-deficient *Escherichia coli*. Genetics **158(1),** 41-64. https://doi.org/10.1093/genetics/158.1.41.

da Costa EM, de Lima W, Oliveira-Longatti SM, de Souza FM. 2015. Phosphate-solubilising bacteria enhance *Oryza sativa* growth and nutrient accumulation in an oxisol fertilized with rock phosphate. Ecological Engineering **83,** 380-385. https://doi.org/10.1016/j.ecoleng.2015.06.045.

El-Hamshary OIM, Soliman EA, AL-Musalam HJ, Alhebshi A. 2018. Genetic comparison among high phosphate-solubilizing mutants of *Enterobacter clocae* via rapd-pcr analysis. Asian Journal of Microbiology, Biotechnology & Environmental Sciences **20(2),** 643-654.

Glazer AN, Nikaido H. 2007. Microbial biotechnology: fundamentals of applied microbiology. Cambridge University Press.

Gyaneshwar P, Kumar GN, Parekh LJ, Poole PS. 2002. Role of soil microorganisms in improving P nutrition of plants. In: Adu-Gyamfi JJ (Ed.), Food Security in Nutrient Stressed Environments: Exploiting Plants' Genetic Capabilities. Springer, Netherlands pp. 133-143.

Hingole SS, Pathak AP. 2016. Saline soil microbiome: A rich source of halotolerant PGPR. Journal of Crop Science and Biotechnology **19(3),** 231-239.

Holt JG, Krieg NR, Sneath PHA, Stately JT, Williams ST. 1994. Bergey's manual of determinative bacteriology, 9th ed. Williams and Wilkins, Baltimore pp 787.

Jacksonm L. 1973. Soil chemical Analysis. Prentice Hall of Englewood cliffs. New Jersey, USA pp. 378-398.

Javed S, Meraj M, Bukhari SA, Irfan R, Mahmood S. 2013. Hyperproduction of alkaline protease by mutagenic treatment of *Bacillus subtilis* M-9 using agroindustrial wastes in submerged fermentation. Journal of Microbial & Biochemical Technology **5,** 074- 080. https://doi.org/ 10.4172/ 1948-5948.1000103.

Khan MD, Zaidi A, Ahmad E. 2014. Mechanism of phosphate solubilization and physiological functions of phosphate-solubilizing microorganisms. Springer, Cham.

Khan MS, Zaidi A, Wani PA. 2007. Role of phosphate-solubilizing microorganisms in sustainable agriculture-a review. Agronomy for Sustainable Development **27(1),** 29-43 https://doi.org/10.1051 /agro:2006011

Mahdi I, Fahsi N, Hafidi M, Allaoui A, Biskri L. 2020. Plant growth enhancement using rhizospheric halotolerant phosphate solubilizing bacterium *Bacillus licheniformis* QA1 and *Enterobacter asburiae* QF11 isolated from *Chenopodium quinoa* wild. Microorganisms **8(6),** 948. https://doi.org/10.3390/microorganisms8060.

Nadeem M, Qazi JI, Baig S. 2010. Enhanced production of alkaline protease by a mutant of *Bacillus licheniformis* N-2 for dehairing. Brazilian Archives of Biology and Technology **53,** 1015-1025. https://doi.org/10.1590/S1516-891320100500003.

Nautiyal CS. 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiology Letters **170,** 65-270. http://dx.doi.org/10.1111/j.1574-6968.1999.tb13.

Olga S, Zhanara S, Gulnar U, Gulna M, Zhanar R. 2019. Effect of UV irradiation of PGPR bacteria on seeds germination and plant growth of mung bean (*Vigna radiata*). European science review **2(1-2),** 60-62.

Patil VS. 2014. *Bacillus subtilis*: A potential salt tolerant phosphate solubilizing bacterial agent. International Journal of Life Sciences Biotechnology and Pharma Research **3(2),** 141.

Pikovskaya RI. 1948. Mobilization of P in soil in connection with vital activity by some microbial species. Microbiologica **17,** 362-37.

Plassard C, Robin A, Le Cadre E, Marsden C, Trap J, Herrmann L, Hinsinger P. 2015. Améliorer la biodisponibilité du phosphore: comment valoriser les compétences des plantes et les mécanismes biologiques du sol. Innovations Agronomiques **43,** 115-138. https://agritrop.cirad.fr /579393/1/Plassard%20et%20al_2015.pdf.

Reyes I, Valery A, Valduz Z. 2007. Phosphate-solubilizing microorganisms isolated from rhizospheric and bulk soils of colonizer plants at an abandoned rock phosphate mine. In: First international meeting on microbial phosphate solubilisation, 16-19 July, 2002, Spain. Springer, Netherlands pp. 69-75.

Saxena AK, Kumar M, Chakdar H, Anuroopa N, Bagyaraj DJ. 2020. *Bacillus* species in soil as a natural resource for plant health and nutrition. Journal of Applied Microbiology **128(6),** 1583-1594. https://doi.org/10.1111/jam.14506.

Shintu PV, Jayaram KM. 2015. Phosphate solubilising bacteria (*Bacillus polymyxa*)-An effective approach to mitigate drought in tomato (*Lycopersicon esculentum* Mill.). Tropical. Plant Research **2,** 17-22.

Sivasakthi S, Kanchana D, Usharani G, Saranraj P. 2013. Production of plant growth promoting substance by *Pseudomonas fluorescens* and *Bacillus subtilis* isolates from paddy rhizosphere soil of Cuddalore District, Tamil Nadu, India. International Journal of Microbiological Research **4(3),** 227-233. https://doi.org/10.5829 /idosi.ijmr.2013.4.3.75171.

Sivasakthi S, Saranraj P, Sujitha D. 2015. Mutation Based Strain Improvement of Pgpr Isolates (*Pseudomonas fluorescens* & *Bacillus subtilis*) for the Improvement of Growth and Yield of Paddy (*Oryza sativa* L.). American-Eurasian Journal of Agricultural & Environmental Sciences **15(8),** 1591-1601.

Vessey JK. 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant and soil **255(2),** 571-586. https://doi.org/10.1023/A:1026037216893.

Voisard C. 2010. Suppression of black rot of tobacco by a *Pseudomonas* strain: Potential applications and mechanisms. In: Hornby D, Cook RJ, Henis Y(Eds), Biological Control of Soil Borne. CAB International pp. 93-108.

Wang T, Sun H, Mao H, Zhang Y, Wang C, Zhang Z, Sun L. 2014. The immobilization of heavy metals in soil by bioaugmentation of a UV-mutant Bacillus subtilis 38 assisted by NovoGro biostimulation and changes of soil microbial community. Journal of Hazardous Materials **278,** 483-490.

Wang T, Sun H, Ren X, Li B, Mao H. 2018. Adsorption of heavy metals from aqueous solution by UV-mutant *Bacillus subtilis* loaded on biochars derived from different stock materials. Ecotoxicology and Environmental Safety **148,** 285-292. https://doi.org/10.1016/j.ecoenv.2017.10.039.

Whitelaw MA. 1999. Growth promotion of plants inoculated with phosphate solubilizing fungi. Advances in Agronomy **69,** 99-151.

Xu H, Jia S, Liu J. 2011. Development of a mutant strain of *Bacillus subtilis* showing enhanced production of acetoin. African Journal of Biotechnology **10(5),** 779-799.

Zhao D, Ding Y, Cui Y, Zhang Y, Liu K, Yao L, Wang C. 2022. Isolation and Genome Sequence of a Novel Phosphate-Solubilizing Rhizobacterium *Bacillus altitudinis* GQYP101 and Its Effects on Rhizosphere Microbial Community Structure and Functional Traits of Corn Seedling. Curr. Microbiol **79(9),** 1-12.