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Indigenous phosphate-solubilizing bacteria enhance germination in deteriorated rice seed

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Abstract

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Certain soil microorganisms have the potential to improve the growth of plants. The aim of this study was to isolate the indigenous bacteria from acid sulphate soil, and evaluate whether this could enhance the germination of deteriorating rice seeds. It was revealed that four bacteria isolates (4A, 6A, 8RH, 10RH) were able to solubilise phosphate and produce indole and siderophore. Molecular identification, using the 16S rRNA gene, showed that these four isolates are *Paenibacillus alvei* strain NBRC 3343, *P. alvei* strain DSM 29, *Bacillus cereus* strain ATCC 14579 and *B. cereus* strain ATCC 14579. Evaluation showed that the four strains were able to increase the percentage of germination by up to 46.5%, the uniformity of germination by up to 30% and the germination rate by 7.1%/d on seed with ≤ 50% viability. Germination of seeds with 80% viability increased by only 14%, with a slight increase in the uniformity of germination (16%) and germination rate (8.81%/d).

Keywords: indigenous; phosphate-solubilising bacteria; deteriorated rice seed; germination; 16S rRNA gene; indole; siderophore

Introduction

Seed germination is promoted through imbibition, which is stimulated by the availability of water. Seeds need enough water to activate certain components in them so that germination can proceed. The viability of germination also requires high seed vigour. Compared to normal germination, seeds with low viability (through deterioration) usually have a decreased percentage of germination power, require a longer time to germinate, show low growth speeds, and do not grow at similar rates. The strategy to improve growth power generally involves the use of seed hydro-priming and matriconditioning (Chithra et al., 2015; Singh et al., 2015; Lutts et al., 2016) and phytohormones.

Recent studies have found that certain soil microorganisms have a high potential to be plant growth promoters, such as the rhizobacteria, which can be used as biofertiliser due to their ability to solubilise phosphate, creating phytohormones and siderophore. Plant-growth-promoting rhizobacteria (PGPR) colonies are very active in roots systems, and can improve crop growth and yields (Wu et al., 2005).

Application of the bacterium *Bacillus* sp. has been shown to improve plant growth and mineral content in banana leaves (Jaizme-Vega et al., 2004) and, when inoculated into rice seed, has improved growth and productivity by up to 43%, but with *Pseudomonas fluorescens* has been shown to improve yield by up to 100% (Thakuria et al., 2004). Consequently, there is a basic need to conduct an evaluation of the

germination-boosting ability of PSB. If proved to be effective, indigenous PSB can be used to improve the viability of rice seeds that have already deteriorated.

The function of PGPR in improving plant growth and yield involves a hypothesis linked to the ability to synthesise growth hormone; *Bacillus* sp. has been reported as being able to synthesise gibberellin (Joo et al., 2004) and indole acetic acid (IAA) (Thakuria et al., 2004).

Evaluation of the ability of PSB to act as a germination booster was conducted on rice seeds that had low growth potential (i.e. were deteriorated), so that optimum plant growth in the field could be achieved. This study aimed to evaluate this ability in PSB that produce indole and siderophore, as well as the influence of inoculating deteriorated rice with such bacteria.

Methods

PSB sampling

The PSB were obtained from healthy rice plants, growing in acid sulphate soils with a pH of 3.9, at 0°0.2048′S, 109°14.7487′E in Sungai Rengas village, Sungai Kakap, West Kalimantan. The plants were taken, with soil attached, from the field, placed in sealed plastic container, and stored in refrigerator at 10°C.

Isolation of PSB, and chacterisation of their morphology

Bacteria were isolated from the rhizosphere and endophytic rice roots using a method involving tryptone soya agar (TSA; 15 g casein, 5 g soy extract, 5 g NaCl, 15 g agar agar and 1000 mL of distilled water). Bacteria from the rhizosphere were isolated by taking 1 g of soil, and mixing it with 9 mL of sterile distilled water. The endophyte bacteria were isolated by weighing 1 g of rice roots, and then sterilising their surfaces by soaking them in 3% NaOCl for 2 min, then 96% ethanol for 1 min, and rinsing with sterile distilled water up to 3 times. The sterilised tissue of the roots was added to 9 mL of distilled water, and crushed to the point of disintegration. Then, 0.1 mL of suspension from the soil and root mixtures were taken, and diluted by 10^{-3}, using a pouring method. The diluted samples were incubated at 26–28°C for 96 h. The grown bacterial colony was purified on new TSA at room temperature, and then characterised according to it macroscopic and microscopic (Olympus Optilab, at 400 x magnification) morphology, followed by Gram staining of a single colony.

Bacterial phosphate-solubilisation ability

Inoculation was used to select for the ability to solubilize phosphate. 1 ml liquid culture ($\pm 10^9$) of pure bacterial inoculum was dropped onto Pikovskayas agar (10 g glucose, 0.5 g $(NH_4)_2SO_4$, 7.69 g Fe_2PO_3, 0.1 g $MgSO_4.7H_2O$, 0.002 g $MnSO_4.H_2O$, 0.002 g $FeSO_4.7H_2O$, 0.5 g yeast extract, 20 g agar and 1000 mL of aquadest) (Saraswati et al., 2012), which detects phosphate-solubilising microorganisms. This was incubated for 7 d at room temperature. The ability to solubilise phosphate was more characteristic of the clear zone around the colony.

Indole production

The indole test determines the ability of bacteria to produce indole by converting tryptophan. One dose of pure bacterial culture was inoculated into tryptone soya broth (TSB) with added L-tryptophan (15 g casein, 5 g soybean extract, 5 g NaCl, 0.1% L-tryptophan and 1000 mL of aquadest). Each culture was replicated up to 3 times, including the control medium that was not inoculated with bacteria. This was incubated on an incubator-shaker for 72 h at room temperature. Indole production was determined by dripping 2–3 drops of Kovacs reagent onto the samples and observing the indole ring. A positive reaction was reflected by the ring being pink to dark red, whereas a negative reaction was indicated by a green to yellow ring.

Siderophore production

To determine siderophore production, use like CAS medium consist of 20 g sucrose, 2 g L-asparagine, 1 g K_2HPO_4, 0.5 g $MgSO_4.7H_2O$ and 1 L aquadest at pH 7.0. The culture was incubated at 27˚C for 24 h, and then centrifuged at 1100 rpm for 30 min, and filtered through a 0.2 µm-pore nitrocellulose membrane. For the analysis, 3 mL of supernatant were mixed with 1 mL of 0.01 M $FeCl_3$, as a trace element. For comparison, a second sample was analysed without the use of $FeCl_3$. To detect the siderophores, the absorbance value was measured with a spectrophotometer at λ500 nm.

Molecular identification of bacteria using 16S rRNA

The first step in identifying the PSB is through genome extraction. A pure culture was grown on TSB for 24 h at room temperature. The genome isolation then followed the Genomic DNA Mini Kit (blood/cultured cell) protocol from Geneaid (Lerteanawanichakul, 2015). After that, several steps were conducted to obtain the DNA of the bacteria, amplified through a polymerase chain reaction (PCR) using a TI-thermocycler (Biometra, Goettingen, Germany). The bacteria genomes were amplified by PCR using two 16S rRNA primers (27F (AGAGTTTGATCMTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT)) (Lane, 1985) as much as 25 µL. To confirmation of PCR result only as much as 4 µL PCR product running and then 1 µL of loading dye was added. This was mixed, placed into a well containing

1% agarose gel (1 g agarose and 100 mL of TBE buffer 1 x), and then electrified at 220 volts and 75 mA for 45 min. The result of the gel electrophoresis was soaked in 10% ethidium bromide for 10 min, and then rinsed with double-distilled water. The DNA targets on the agarose were visualization on a G:Box gel documentation system (Syngene, Frederick, MD, USA) with the DNA target size are 1400 base pairs.

Bioinformatic analysis and contruction of phylogenetic tree of 16S rRNA

The DNA sequencing was conducted at the 1st Base DNA Sequencing Services Laboratory, Selangor (MY). The DNA sequence analysis and phylogenetic tree construction were performed using MEGA 6.0, and compared to the Genebank database using the Basic Local Alignment Search Tool for nucleotides (BLAST-N) from http://www.ncbi.nlm.nih.gov/. The phylogenetic tree was generated using the neighbour-joining method, with 1000 x bootstrap (Saitou & Nei, 1987).

Effects of bacterial inoculation on seed germination

Inoculated seeds were soaked in TSB (10^9 cfu mL^{-1}) for 24 h. Then, they were germinated on a sterilized sand medium, laid in a 30 x 20 x 10 cm (length x weight x height) plastic box. The treatment consisted of *Paenibacillus alvei* strain NBRC 3343*, P. alvei* strain DSM 29, *Bacillus cereus* strain ATCC 14579, *B. cereus* strain ATCC 14579 and an untreated control. The rice seeds used in the treatment had <50% and 80% germination viability. Three replications of 100 seeds were used. Before and after inoculating the seeds, the quality of the seed water was measured. The influence of inoculation on seed viability was measured by the normal percentage of sprouts, the speed of seed growth and unison of growth.

Statistical analysis

The data were statistically treated with ANOVA, and Duncan's Multiple Range test. A probability level of 0.05 was used to compare the means.

Results and Discussion

Bacterial ability to solubilise phosphate and produce indole and siderophores

Twenty-three bacterial isolates were obtained from healthy rice plants that were growing well in acid sulphate soil. This study used only four of these; based on their ability to solubilize phosphate, and produce indole and siderophores (4A and 6A were isolated from the roots, 8RH and 10RH from the rhizosphere). The ability of the bacterial isolates to solubilize phosphate was marked by a clear zone around the bacterial colony on Pikovskayas agar (Fig. 1A). Indole production was evidenced by a pink ring using Kovak's reagent (Fig. 1B). Siderophore production was marked by a change from blue to orange-yellow in the medium (Fig. 1C).

The ability to form indole indicates that the bacteria are able to promote growth (Aly et al., 2001; Ebrahim & Aly, 2004; Ardakani et al., 2010; Merzaeva & Shirokikh, 2010). In general, the indole usually formed is a natural auxin, which is a product of the metabolism of L-tryptophan by microorganisms (Aly et al., 2012).

Four of the isolated PSB have the ability to produce siderophores, which are metal-chelating agents with low molecular mass (Schwyn & Neilands, 1987) that are able to scavenge Fe and form complexes with other essential elements, such as Mo, Mn, Co and Ni (Bellenger et al., 2008). They can also bind metal Pb (Bhattacharya, 2010) and heavier metals, such as Cd, Cu, Zn and Al, allowing them to reduce poisonous materials (Chamongkolpradit et al., 2008). Fe is a source of nutrition for all organisms, including microorganisms. In bacteria that produce siderophores, Fe is unavailable for producing pathogens, so pathogens do not develop. *Pseudomonas* spp. are the one of genus bacteria that produce the largest amounts of siderophores (Meyer & Abdallah, 1980).

Quantitative measurement of siderophores on the selective medium gave varying results (Table 1). The 6A isolate

Fig. 1. The ability of bacteria to solubilise P (A), produce indole (B), and produce siderophore (C)

Table 1. Siderophore compound values from four isolates of PSB

Isolate code	Siderophore value (ppm)
4A	0.008
6A	0.037
8RH	0.011
10RH	0.009

had the highest values, compared to the other isolates, followed by 8RH, 10RH and 4A.

Identification and molecular characterisation of PSB isolates

Based on the results shown in Table 2, the 4A, 6A, 8RH and 10RH isolates that were tested for PGPR are from the Gram-positive group; most PGPR groups are Gram-negative (Wahyudhi, 2009).

The BLAST-N results (Table 3) show that isolate 8RH is closely related to *Bacillus cereus* strain ATCC 14579 and *B. cereus* strain ATCC 14579, with a 96% similarity. Isolate 10RH is closely related to *B. cereus* strain ATCC 14579 and *B. cereus* strain ATCC 14579, with a 93% similarity. Isolate 4A has a 92% similarity with *Paenibacillus alvei* strain 3343 and *P. alvei* strain DSM 29. Isolate 6A has a 91% similarity with *P. alvei* DSM 29 and a 90% similarity with *P. alvei* strain NBRC 3343.

Phylogenetic analysis of the 16S rDNA showed that isolates 8RH and 10RH are closely related. These two isolates were different from isolates 4A and 6A, with an extensive genetic distance (Fig. 2).

The results of the 16S rDNA amplification show that isolates 4A and 6A share the greatest similarity with the comparative strain of *P. alvei*, and isolates 8 RH and 10 RH share the greatest similarity with the comparative strain of *B. cereus*. *Bacillus* and *Paenibacillus* are ubiquitous Gram-positive bacteria. A closer look at the molecular identifications and phylogenetic tree of the four bacteria isolates shows that the bacteria tested belong to the genera *Paenibacillus* and *Bacillus* (Table 3). The 4A isolate is *P. alvei* strain NBRC 3343, the 6A isolate is *P. alvei* strain DSM 29, isolate 8RH is *B. cereus* strain ATCC 14579, and isolate 10RH is *B. cereus* strain ATCC 14579.

These species of bacteria can be found in most environments, due to their high survivability in extreme environments. *Bacillus* and *Paenibacillus* are known to be abundant in agricultural environments, playing a significant role in agricultural productivity. They are known to produce various metabolites that support cells in unfavourable environments. Some of the important metabolites are antimicrobial peptides, signal peptides and extracellular enzymes. The ability to produce such metabolites has been influenced by agroecosystem conditions affecting the bacterial habitat (Gardener, 2004). *Bacillus* and *Paenibacillus* have significant roles in increasing plant growth, resisting phytopathogens and inducing plant immunity (Govindasamy et al., 2010). *Bacillus* and *Paenibacillus* are also known as the main producers of a wide range of antibiotic peptides that can inhibit various microorganisms and pathogenic nematodes. These two genera are mostly known to reside in plant tissue as endophytic bacteria.

Table 2. Morphological characteristics of selected isolates in TSA medium after seven days incubation at ambient room temperature

Isolate code	Colour		Colony shape	Elevation	Colony edge	Surface texture	Cell shape	Gram stain
	Surface	Base						
4A	White	White	Circular	Raised	Erose	Smooth	Rod	Gram-positive
6A	White	White	Circular	Convex	Entire	Smooth	Rod	Gram-positive
8RH	Clear white	White	Irregular	Flat	Lobate	Smooth	Rod	Gram-positive
10RH	White	White	Circular	Umbonate	Undulate	Rugose	Rod	Gram-positive

Table 3. BLAST-N result of 16S rDNA of PSB from acid sulphate soils in Sungai Rengas village, West Kalimantan

Isolate code	Comparative strain	Total base (Isolate/Genebank)	Query Cover	% Identity	Accession number
4A	*Paenibacillus alvei* strain NBRC 3343	1190/1476	100%	92%	NR_113577.1
	Paenibacillus alvei strain DSM 29	1190/1527	100%	92%	NR_042091.1
6A	*Paenibacillus alvei* strain DSM 29	770/1527	100%	91%	NR_042091.1
	Paenibacillus alvei strain NBRC 3343	770/1476	100%	90%	NR_113577.1
8RH	*Bacillus cereus* ATCC 14579	1073/1512	99%	96%	NR_074540.1
	Bacillus cereus strain ATCC 14579	1073/1482	99%	96%	NR_114582.1
10RH	*Bacillus cereus* ATCC 14579	928/ 1512	98%	93%	NR_074540.1
	Bacillus cereus strain ATCC 14579	928/1482	98%	93%	NR_114582.1

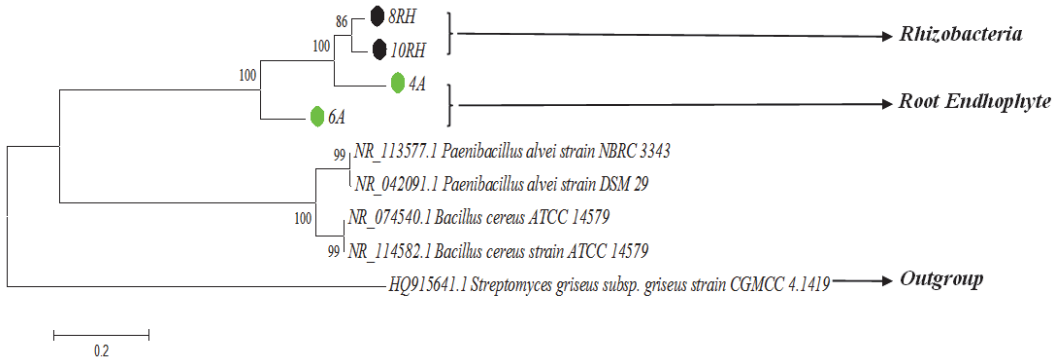

Fig. 2. Neighbour-joining tree of 16S rDNA of PSB from acid sulphate soil. The tree was constructed using the Kimura two-parameter model in Mega 6.0 with 1000 x bootstrap

Effect of bacterial inoculation on seed germination

Firstly, the seeds were germinated, and then they were measured before undergoing the inoculation process. The measurements show that the water content of the rice seeds, before and after inoculation, on both seeds viability types, was slightly increased after inoculation (Table 4, Fig. 3).

The increase in water content in the untreated/control seeds with a ≤50% viability was 10.82%, which was greater than in seeds with an 80% viability, which only increased by 4.32%. Inoculated seeds showed a slight increase in water content.

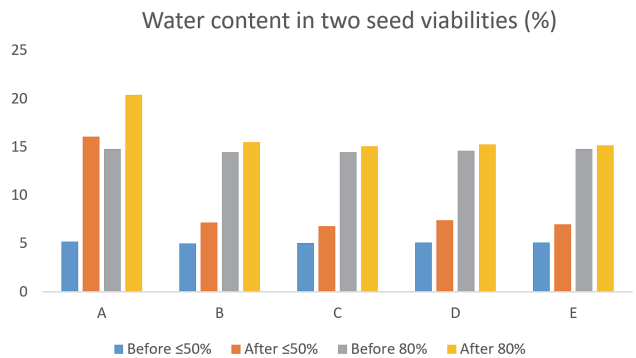

Fig. 3. Water content before and after inoculation of seeds (≤50% and 80%)

Note: *A* – untreated, *B* – *P. alvei* strain NBRC3343, *C* – *P. alvei* strain DSM29, *D* – *B. cereus* strain ATCC14579, *E* – *B. cereus* strain ATCC1457

Inoculation with PSB increased seed viability (≤50% and 80%), showed a significant effect (Table 5, Fig. 4); in seeds with ≤50% viability, inoculation increased the germination percentage by 46.5%, whereas in seeds with 80% viability, the germination percentage increased by only 14%.

PSB inoculation increased the germination percentage. This is similar to the findings of (Chookietwattana & Maneewan, 2012), where the use of PSB increased the percentage of tomato seed germinations. The improvement in sprouting is triggered by the ability of the bacteria to produce indole, which can take the form of auxin, gibberellin or cytokinin. *Bacillus* sp. has been reported to synthesise IAA (Thakuria et al., 2004) and gibber-

Table 5. Effect of inoculation of PSB on the percentage of germination

Treatments	Percentage of germination (%)	
	Viability ≤50%	Viability 80%
Untreated/control	50.5 b	86 b
P. alvei strain NBRC 3343	81.0 a	100 a
P. alvei strain DSM 29	97.0 a	100 a
Bacillus cereus ATCC 14579	94.0 a	100 a
B. cereus strain ATCC 14579	96.0 a	100 a

Numbers followed by the same letter in the same column are not significant different in DMRT 0.05

Table 4. Water content before and after the inoculation of PSB on two viabilities of rice seed

Treatment	Water content before inoculation		Water content after inoculation	
	Viability ≤50%	Viability 80%	Viability 50%	Viability 80%
Untreated/control	5.2	14.8	16.08	20.4
P. alvei strain NBRC 3343	5	14.5	7.2	15.5
P. alvei strain DSM 29	5.05	14.5	6.8	15.1
B. cereus strain ATCC 14579	5.12	14.6	7.4	15.3
B. cereus strain ATCC 14579	5.12	14.8	7.01	15.2

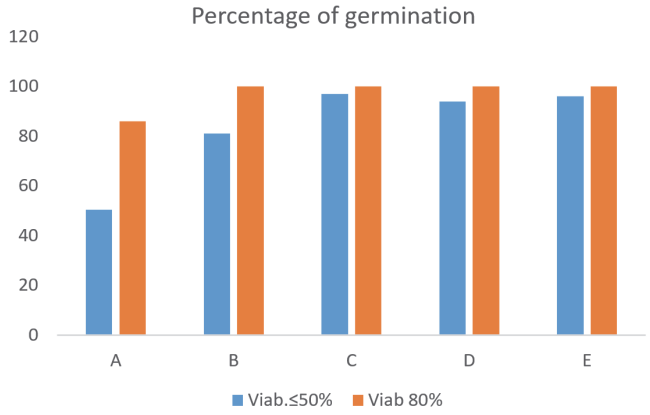

Fig. 4. Effect of inoculation of PSB on percentage of germination

Note: *A* – untreated, *B* – *P. alvei* strain NBRC3343, *C* – *P. alvei* strain DSM29, *D* – *B. cereus* strain ATCC14579, *E* – *B. cereus* strain ATCC1457

ellin (Joo et al., 2004). (Sutariati & Ilyas, 2006) showed that inoculation using *Bacillus* sp. increased the germination power, the uniformity of germination and the growth rate in chili seeds.

Inoculation significantly increased the uniformity of germination and the growth rate of the two viabilities of seed (Table 6, Fig. 5).

The uniformity of germination and germination rate of rice seeds, as shown in Table 6, appear to be significantly increased for both seed viabilities. Seeds with ≤50% viability show an increase in the uniformity of germination of up to 30.67%, compared to the control sample, while there was only a 16% increase in the uniformity of germination in seeds with 80% viability. In seeds with ≤50% viability, inoculation increased the seed germination rate by 7.08%/d, while, in seeds with 80% viability, this increased by 8.81%/d

The effects of PGPR vary among plant types (Bashan et al., 1989). Bacterial inoculation can increase the speed of germination, improve seedling growth, enable responses to stress due to environmental factors, and protect plants from disease (Lugtenberg et al., 2002).

The influence of PSB inoculation on rice seed germination was affected by indole production from the excretion of the bacteria. The mechanism of PGPR in promoting growth is not well understood, but is believed to result from phytohormone production (Shaharoona et al., 2006; Egamberdi-

Table 6. Effect of inoculation of PSB on germination rate and uniformity of germination

Treatment	Germination rate (%/d)		Uniformity of germination (%)	
	Viab. ≤50%	Viab. 80%	Viab.≤50%	Viab. 80%
Untreated/control	9.17 c	14.09 c	9.33 c	77 b
P. alvei strain NBRC3343	13.74 b	20.53 b	17.33 c	89 a
P.alvei strain DSM29	15.89 a	21.10 b	30.67 b	91 a
Bacillus cereus ATCC14579	16.26 a	21.27 b	40.00 a	92 a
B. cereus strain ATCC14579	16.27 a	22.90 a	34.70 a	93 a

Numbers followed by the same letter in the same column are not significant different in DMRT 0.05

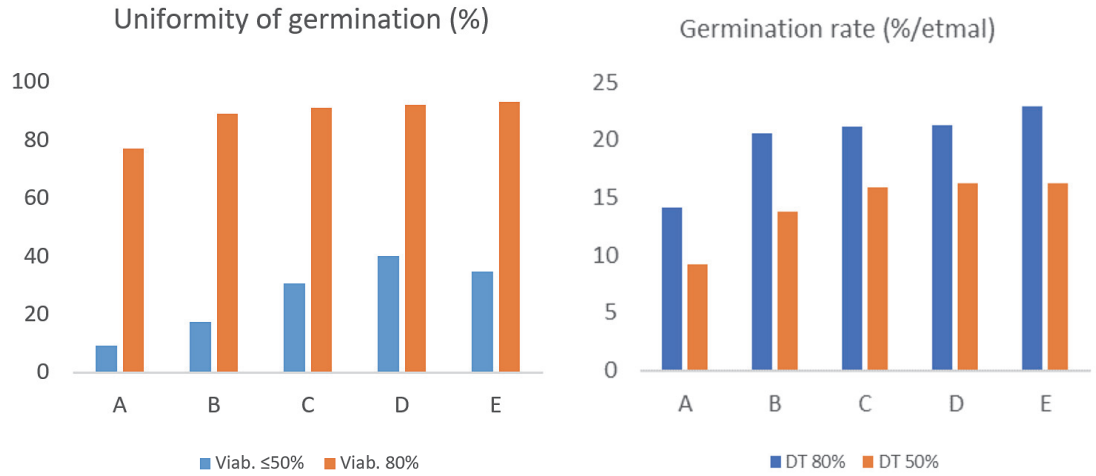

Fig. 5. Effect of inoculation of PSB on germination rate (%/etmal) and uniformity of germination (%)

Note: *A* – untreated, *B* – *P. alvei* strain NBRC3343, *C* – *P. alvei* strain DSM29, *D* – *B. cereus* strain ATCC14579, *E* – *B. cereus* strain ATCC1457

yeva, 2007), nonsymbiotic N-fixation (Mrkovacki & Milic, 2001) against phytopathogenic microorganisms through the production of siderophores, the synthesis of antibiotics (Bharathi et al., 2004; Nuruddin et al., 2006), and the ability to solubilise phosphates (Cattelan et al., 1999).

Bacillus derived from the plant rhizosphere is known to promote growth through the production of certain potentially bioactive compounds, such as 3-hydroxy-2 butane (acetoin), 2,3-butanediol, IAA, phytase, lipopeptide, siderophores, and the induction of systemic resistance (Koumoutsi et al., 2004; Idris et al., 2007; Zhao et al., 2011). These beneficial effects are important because they enable seeds to grow well in the field under suboptimal conditions, as suggested by (Callan et al., 1997), with some bacteria acting as biocontrolling agents, increasing the effectiveness of colonic formation at the roots, supporting plant growth directly or indirectly after germinating in the field. With bacteria able to produce siderophores, seeds can be protected from pathogens present in the soil, or from seed-borne pathogens.

Efforts to increase the viability of seeds that have already deteriorated are usually conducted by applying supplemental growth regulators, such as priming (osmopriming, matriconditioning, hydropriming), or hormonising (with gibberellin or auxin). Priming can increase the uniformity of seed germination (Singh et al., 2015). The discovery of PGPR, which can produce phytohormones, such as those produced by PSB, provides an opportunity to replace commonly-used growth regulators. The use of PSB inoculation can be regarded as biopriming, in accordance with the opinion of (Callan et al., 1997).

The ability for bacteria to solubilize phosphate is currently very much needed. Although herein, only germination was addressed, this initial work will benefit future studies because these bacteria can provide P for the plants, thus supporting their growth in the field. Furthermore, synthetic P fertilizer is an expense for farmers, and so these bacteria can be an alternative source. These bacteria are also able to make P nutrients that are not available in the soil become available to plants; while P is generally abundant (Khan et al., 2009), the availability of P in soil is low.

The effects of PGPR include the production of plant growth regulators, such as auxin, gibberellic acid and cytokinin. They directly or indirectly provide favourable conditions for growth (Nadeem et al., 2014). In this study, it was shown that PSB are able to increase the percentage of germination, the uniformity of germination and the growth rate of seeds, not solely because of the production of phytohormones, but also through the positive influence between bacteria and seeds.

Conclusions

Four bacterial isolates, taken from healthy rice plants grown in acidic sulphate soils, have the ability to solubilise phosphates and produce indole and siderophores. These four bacteria are *P. alvei* strain NBRC 3343, *P. alvei* strain DSM 29, *B. cereus* strain ATCC 14579 and *B. cereus* strain ATCC 1457. These PSB have the ability to improve seed viability, as shown in an increased percentage of germination, germination rates and uniformity of seed growth. They are most effective on seeds with ≤50% viability.

References

Aly, M. M., El-Sabbagh, S., El-Shouny, W., & Ebrahim, M. K. (2001). Salt tolerance of maize under the effect of Azotobacter chroococcum and Streptomyces niveus. *J Union Arab Biol*, *10*, 62-84.

Aly, M. M., El Sayed, H. E. A., & Jastaniah, S. D. (2012). Synergistic effect between Azotobacter vinelandii and Streptomyces sp. isolated from saline soil on seed germination and growth of wheat plant. *Journal of American Science, 8*(5), 667-676.

Ardakani, S. S., Heydari, A., Tayebi, L., & Mohammadi, M. (2010). Promotion of cotton seedlings growth characteristics by development and use of new bioformulations. *International Journal of Botany, 6*(2), 95-100.

Bashan, Y., Ream, Y., Levanony, H., & Sade, A. (1989). Nonspecific responses in plant growth, yield, and root colonization of noncereal crop plants to inoculation with Azospirillum brasilense Cd. *Canadian Journal of Botany*, *67*(5), 1317-1324.

Bellenger, J. P., Wichard, T., Kustka, A. B., & Kraepiel, A. M. L. (2008). Uptake of molybdenum and vanadium by a nitrogen-fixing soil bacterium using siderophores. *Nature Geoscience*, *1*(4), 243-246.

Bharathi, R., Vivekananthan, R., Harish, S., Ramanathan, A., & Samiyappan, R. (2004). Rhizobacteria-based bio-formulations for the management of fruit rot infection in chillies. *Crop Protection, 23*(9), 835-843. https://doi.org/10.1016/j.cropro.2004.01.007

Bhattacharya, A. (2011). Siderophore mediated metal uptake by Pseudomonas fluorescens and its comparison to iron (iii) chelation. *Ceylon Journal of Science (Biological Sciences)*, *39*(2), 147-155.

Callan, N. W., Mathre, D. E., Miller, J. B., & Vavrina, C. S. (1997). Biological seed treatments: factors involved in efficacy. *HortScience, 32*(2), 179-183.

Cattelan, A. J., Hartel, P. G., & Fuhrmann, J. J. (1999). Screening for plant growth–promoting rhizobacteria to promote early soybean growth. *Soil Science Society of America Journal*, *63*(6), 1670-1680.

Chamongkolpradit, W., Budzikiewicz, H., Chanthai, S., & Ruangviriyachai, C. (2008). Immobilized and characteristics of pyoverdin I onto modified micelle-templated silica (MTS) surface as a chelating agent. *Asia-Pacific Journal of Science and Technology*, *13*(2), 182-196.

Chithra, K., Siji, K., & Thomas, B. (2015). Phosphate solubilizing bacteria (PSB) and its priming effect on the germination and growth of Abelmoschus esculentus Linn – A case study. *Journal of Science, 5*(2), 71-76.

Chookietwattana, K., & Maneewan, K. (2012). Screening of efficient halotolerant phosphate solubilizing bacterium and its effect on promoting plant growth under saline conditions. *World Applied Sciences Journal, 16*(8), 1110-1117.

del Carmen Jaizme-Vega, M., Rodríguez-Romero, A. S., & Guerra, M. S. P. (2004). Potential use of rhizobacteria from the Bacillus genus to stimulate the plant growth of micropropagated bananas. *Fruits*, *59*(2), 83-90.

Ebrahim, M. K., & Aly, M. M. (2005). Physiological response of wheat to foliar application of zinc and inoculation with some bacterial fertilizers. *Journal of plant nutrition*, *27*(10), 1859-1874.

Egamberdiyeva, D. (2007). The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. *Applied Soil Ecology, 36*(2-3), 184-189.

Govindasamy, V., Senthilkumar, M., Magheshwaran, V., Kumar, U., Bose, P., Sharma, V., & Annapurna, K. (2010). Bacillus and Paenibacillus spp.: potential PGPR for sustainable agriculture. In: *Plant growth and health promoting bacteria,* Springer, Berlin, Heidelberg, pp. 333-364.

Idris, E. E., Iglesias, D. J., Talon, M., & Borriss, R. (2007). Tryptophan-dependent production of indole-3-acetic acid (IAA) affects level of plant growth promotion by Bacillus amyloliquefaciens FZB42. *Molecular Plant-Microbe Interactions*, *20*(6), 619-626.

Joo, G. J., Kim, Y. M., Lee, I. J., Song, K. S., & Rhee, I. K. (2004). Growth promotion of red pepper plug seedlings and the production of gibberellins by *Bacillus cereus, Bacillus macroides* and *Bacillus pumilus*. *Biotechnology Letters, 26*(6), 487-491.

Khan, M. S., Zaidi, A., Wani, P. A., & Oves, M. (2009). Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. *Environmental Chemistry Letters*, *7*(1), 1-19.

Koumoutsi, A., Chen, X. H., Henne, A., Liesegang, H., Hitzeroth, G., Franke, P., Joachim Vater, J., & Borriss, R. (2004). Structural and functional characterization of gene clusters directing nonribosomal synthesis of bioactive cyclic lipopeptides in Bacillus amyloliquefaciens strain FZB42. *Journal of Bacteriology*, *186*(4), 1084-1096.

Lugtenberg, B. J. J., Chin-A-Woeng, T. F. C., & Bloemberg, G. V. (2002). Microbe-plant interactions: principles and mechanisms. Antonie van Leeuwenhoek, *International Journal of General and Molecular Microbiology, 81*(1-4), 373-383.

Lutts, S., Benincasa, P., Wojtyla, L., Kubala, S., Pace, R., Lechowska, K., Quinet, M. & Garnczarska, M. (2016). Seed priming: new comprehensive approaches for an old empirical technique. In: *New Challenges in Seed Biology-Basic and Translational Research Driving Seed Technology*. IntechOpen.

McSpadden Gardener, B. B. (2004). Ecology of Bacillus and Paenibacillus spp. in agricultural systems. *Phytopathology*, *94*(11), 1252-1258.

Merzaeva, O. V., & Shirokikh, I. G. (2010). The production of auxins by the endophytic bacteria of winter rye. *Applied Biochemistry and Microbiology, 46*(1), 44-50.

Meyer, J. M., & Abdallah, M. A. (1980). The siderochromes of non-fluorescent pseudomonads: production of nocardamine by Pseudomonas stutzeri. *Microbiology*, *118*(1), 125-129.

Mrkovacki, N., & Milic, V. (2001). Use of Azotobacter chroococcum as potentially useful in agricultural application. *Annals of Microbiology*, *51*(2), 145-158.

Nadeem, S. M., Ahmad, M., Zahir, Z. A., Javaid, A., & Ashraf, M. (2014). The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. *Biotechnology Advances*, *32*(2), 429-448.

Nuruddin, A. A., Leng, H. M., & Basaruddin, F. (2006). Peat moisture and water level relationship in a tropical peat swamp forest. *Journal of Applied Sciences, 6*(11), 2517-2519.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, *4*(4), 406-425.

Saraswati, R., Husen, E., & Simanungkalit, R. D. M. (2012). Metode analisis biologi tanah. Balai Besar Penelitian dan Pengembangan Sumber Daya Lahan Pertanian. Bogor. Indonesia (ID)

Shaharoona, B., Arshad, M., Zahir, Z. A., & Khalid, A. (2006). Performance of Pseudomonas spp. containing ACC-deaminase for improving growth and yield of maize (*Zea mays* L.) in the presence of nitrogenous fertilizer. *Soil Biology and Biochemistry*, *38*(9), 2971-2975.

Singh, H., Jassal, R. K., Kang, J. S., Sandhu, S. S., Kang, H., & Grewal, K. (2015). Seed priming techniques in field crops – A review. *Agricultural Reviews, 36*(4), 251-264.

Sutariati, Gu. A. K., & Ilyas, S. (2006). Effects of plant growth promoting Rhizobacteria on seed germination and seedling growth of hot pepper. *Indonesian Journal of Agronomy, 34*(1), 46-54.

Thakuria, D., Talukdar, N. C., Goswami, C., Hazarika, S., Boro, R. C., & Khan, M. R. (2004). Characterization and screening of bacteria from rhizosphere of rice grown in acidic soils of Assam. *Current Science, 86*(7), 978-985.

Wahyudi, A. T. (2009). Rhizobacteria Pemacu Pertumbuhan Tanaman: Prospeknya sebagai Agen Biostimulator & Biokontrol. *Nano Indonesia*.

Wu, S. C., Cao, Z. H., Li, Z. G., Cheung, K. C., & Wong, M. H. (2005). Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. *Geoderma*, *125*(1-2), 155-166.

Zhao, Q., Shen, Q., Ran, W., Xiao, T., Xu, D., & Xu, Y. (2011). Inoculation of soil by Bacillus subtilis Y-IVI improves plant growth and colonization of the rhizosphere and interior tissues of muskmelon (*Cucumis melo* L.). *Biology and Fertility of Soils*, *47*(5), 507-514.

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