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Efficiency of polysaccharide and *Nostoc* sp. up2 cells on growth of rice seedling (san-pah-twang 1) and soil quality improvement

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Abstract

This research aimed to determine the effects of polysaccharide (PS) and *Nostoc* sp. UP2 cells on the growth of rice and soil quality improvement. The analysis of polysaccharide component showed the total sugar and reducing sugar contents of 17.61% and 6.04%, respectively. Even though PS and *Nostoc* cells did not affect the germination percentages, they tended to increase the speed of germination, seedling vigor index, germination energy, length and weight of shoot and root. Considering the 21 days old rice seedlings, the PS concentration of 240 $mg.kg^{-1}$ promoted the length of shoot and root of 25.15 and 15.15 cm, respectively. The biochemical contents of rice seedling treated with PS and *Nostoc* cells showed the higher contents of total sugar, total free amino acid and total chlorophyll compared with the controls. Additionally, the study of soil quality improvement found that the pH, organic matter, total nitrogen and available phosphorus in soil were not affected by all concentrations of PS and *Nostoc* cells. In contrast, cation exchange capacity, available Fe^{2+}, exchangeable K^{2+}, Ca^{2+}, Mg^{2+} and Na^{2+} were increased. Furthermore, the moisture content and porosity of the soil were also significantly influenced by PS and *Nostoc* cells.

Introduction

Thailand is an agricultural country that has a long history of exporting agricultural products. The major emphasis in agriculture was on rice, followed by rubber, which together accounted for over half the value of all agricultural commodity exports. Other crops regularly grown included maize, cassava, fruit, cotton etc.[1] Thai farmers use 5-6 million tons of agrochemicals per year, and the chemical fertilizer use rate in 2010 - 2014 was increased by 7.4 percent per year. This high rate of agrochemical usage might probably demonstrate an inappropriate use of agrochemicals, particularly for the local farmers, who are highly motivated to use these strategies by most agricultural development policies. Consequently, this situation can lead to soil and water pollution, contamination in agricultural products and effect on the human health.[2]

Experience across the world shows that agrochemicals not only reduce the yields but also increase the costs of production, leading to the need of new alternatives such as the use of animal manure, microbial biofertilizers and the integrated farming system. Cyanobacteria are considered one of the most interesting implications for reducing agrochemical environmental impacts. *Nostoc* sp. is cyanobacterium or blue-green algae, which can photosynthesize and fix the atmospheric nitrogen (N_2) and release hormones to stimulate the growth of plants. In addition, it secretes polysaccharides (PS) containing fixed carbon and nitrogen. Many soil

microorganisms utilize these polysaccharides as a source of nourishment, contributing to the soil fertility.[3,4,5] *Nostoc* spp. are able to release some exopolysaccharides that play a specific role in the stabilization of soil particles and regulation of water penetration and retention in soils. Besides, the inoculation of *Nostoc* sp. and its polysaccharides promoted seed germination, growth, and metabolic process of seedling.[6,7] Cyanobacteria polysaccharides are complex anionic heteropolymers containing many different monosaccharides, uronic acids, and non-saccharide components.[8] When added to the soil, they promote the plant growth and increase the gas exchange ability, which provides energy to the rice root that is trapped in water. Therefore, this research aimed to determine the effects of PS and *Nostoc* sp. UP2 cells on the growth of rice and soil quality improvement.

Methodology

Cyanobacteria and culture conditions

Nitrogen fixing cyanobacteria *Nostoc* sp. UP2 were isolated from soil in University of Phayao area. Cyanobacteria were cultured in BG-11 liquid medium at room temperature and illuminated by white fluorescent tubes with the light intensity of 3,000-5,000 lux. Cultures were harvested by filtration.

Extraction and determination of carbohydrate

PS were prepared according to the method of Huang *et al.*[9] Cyanobacterial cells were extracted with 100°C distilled water. The pellets were collected by centrifugation at 10,000 rpm for 15 min at 4 °C. The pellets were resuspended in 100 °C distilled water for the second extraction. The supernatants were combined and precipitated with 80% ethanol for 24 h. Then, ethanol was evaporated with rotary evaporator and dried with a freeze-dryer.

PS powder was weighed and dissolved in sterile distilled water. Total sugar content was quantified according to the phenol-sulfuric acid method[10] using glucose as a standard. Reducing sugars were quantified according to the dinitrosalicylic acid method, using glucose as a standard.[11]

Effect of the cells and PS from Nostoc sp. UP2 on rice seed germination at 7 days

The seed of *Oryza sativa* L. cv. San-pah-twang 1 rice were obtained from Phayao rice seed center, Phayao province. Healthy rice seeds of were soaked in water for 1 h and then disinfected for 10 min with 10 % hypochlorite solution, then rinsed 3 times with deionized water. The PS powder was dissolved in deionized water to generate PS concentrations of 15, 30, 60, 120, and 240 $mg.L^{-1}$. Fresh cells of *Nostoc* sp. UP2 were weighed for preparing for the concentration of 4.5, 9.1, 18.2, 36.4, 72.2, 144.4 and 288.8 $g.L^{-1}$. These concentrations of fresh cells were calculated based on their amount of PS extracted from *Nostoc* sp. UP2. Meanwhile, a filter paper was placed on a Petri dish and moistened with 5 mL of those concentrations of PS and fresh cell solutions. Seed germination tests were conducted according to Chon *et al.*[12] Petri dishes containing deionized water and zeolite were used as a negative and positive controls, respectively. The germination parameters i.e. germination percentage (GP)[13] germination energy (GE),[14] speed of germination (SG)[15] and the seedling vigor index (SVI)[16] were determined. The shoot and root lengths were measured at 7 days after the application. Fresh weight of each sample was determined, and the samples were then oven-dried at 80 °C for 24 h before the dry weight was obtained.

Effect of cells and PS from Nostoc sp. UP2 on rice seedling growth at 21 days

The experimental soil was excavated from the surface of a paddy field in Phayao province (0-20 cm depth) and was then air-dried, ground and sieved through a 6.00 mm sieve to remove rock and plant fragments. Plastic pots containing 500 g of sieved air-dried topsoil were prepared. Sieved air-dried topsoil was mixed with selected various concentrations of PS and fresh cells of *Nostoc* sp. UP2 (PS: 60, 120 and 240 $mg.kg^{-1}$; fresh cells: 36.4, 72.2 and 144.4 $g.kg^{-1}$). Deionized water and zeolite were used as controls. Healthy rice seeds were surface sterilized as described above. The rice seeds were soaked in deionized water overnight, then wrapped in a wet sheet cloth and incubated in the dark until germination. Consequently, ten germinated seeds were sown in the plastic pots containing the prepared soil and watered every 7 days.

Root and shoot lengths, fresh and dry weights were measured at the 21 days after the application. The total sugar content was quantified as described above. Total free amino acid was determined according to the ninhydrin carbon dioxide method, using leucine as a standard.[17] Chlorophyll content in seedlings was analysis by the method described previously.[18] Number of total bacteria and *Nostoc* sp. were determined according to the standard total plate count.[8]

Effect of cells and PS from Nostoc sp. UP2 on soil properties

Soil samples were collected and prepared as described above. Plastic pots (20 cm diameter × 18 cm height) containing 2 kg of sieved air-dried topsoil were prepared. The maximum concentration of PS (120 $mg.kg^{-1}$) and *Nostoc* sp. UP2 fresh cells (144.4 $g.kg^{-1}$) were mixed with prepared soil. The soil was immersed in tap water for 15 days in the greenhouse before being analyzed for their properties. Soil physical properties i.e. soil moisture,[19] total porosity[20] and bulk density[21] were measured. The chemical properties of soil were also investigated. The soil pH was quantified by pH meter and electrical conductivity (EC) was quantified according to EC meter. Cation exchange capacity (CEC) was quantified according to ammonium acetate (NH_4OAc) extraction method.[22] Total nitrogen was analyzed according to Kjeldahl method.[23] The organic matter (OM) was determined according to Walkley and Black titration method.[24] Available phosphorus was analyzed according to Bray II extraction method.[25] Available iron was determined by diethylene triaminepentaacetic acid (DTPA) method.[26] The exchangeable potassium, calcium, magnesium and sodium were quantified according to ammonium acetate (NH_4OAc) extraction method and analyzed by Atomic Absorption Spectrophotometer.[27,28]

Statistical analysis

Data were recorded as the mean ± standard deviation of the result in triplicate. Statistical analysis was performed using one-way analysis of variance (ANOVA). Difference between means were considering using Duncan's New Multiple Range Test (DMRT) at the significant level of 0.05.

Results and Discussion

Carbohydrate production from Nostoc spp.

Among three cyanobacterial strains, *Nostoc* sp. UP2 demonstrated the highest total sugar percentage of 17.61, followed by *Nostoc* sp. UP3 and *Nostoc* sp. UP1 (Table 1). In contrast, the *Nostoc* sp. UP2 showed the lowest reducing sugar percentage of 6.04 while the highest percentage was obtained from *Nostoc* sp. UP3. Considering the degree of polymerization (DP), it was found that *Nostoc* sp. UP2 gave the significantly highest DP value of 3.03. In general, the higher DP indicates the higher number of monosaccharide units in a PS structure and a higher molecular weight, resulting in more water holding capacity. In addition to those characteristics, *Nostoc* sp. UP2 and its PS were selected for determination of their properties in soil quality improvement and rice growth promoting efficiency.

Table 1. Total sugar and reducing sugar contents and degree of polymerization (DP) of *Nostoc* spp.

Treatment	Carbohydrate composition (Mean±SD[a])		
	Total sugar (%)	Reducing sugar (%)	DP
Nostoc sp. UP1	9.99±0.55[b]	7.99±1.50[ab]	1.28[b]
Nostoc sp. UP2	17.61±2.91[a]	6.04±1.09[b]	3.03[a]
Nostoc sp. UP3	15.61±0.19[a]	10.90±1.79[a]	1.46[b]

[a]Mean ± standard deviation followed by the same letter are not significantly different at $p<0.05$, according to Duncan's Multiple Range Test (DMRT).

Effect of the cells and PS from Nostoc sp. UP2 on germination of rice seed

Nostoc sp. UP2 cells and its PS did not pose a significant difference on the germination percentage and germination energy compared with the positive and negative controls. While, both of them demonstrated the significant higher speed of germination than that control. Moreover, considering the SVI, a high *Nostoc* sp. UP2 cells concentration of 72.2, 144.4 and 288.8 $g.L^{-1}$ strongly showed a significant growth promoting effect on the seedling length, resulting in the significant highest SVI. Whereas, the PS demonstrated a significant higher SVI than the negative control, but no significant difference was found compared with the zeolite (Table 2). These results are consistent with those obtained by Wang *et al*. (1991), Adam (1999) and Xu, *et.al* (2013) who found that the presoaking of several plants seeds with cyanobacteria cells and polysaccharide significantly enhanced their germination rate. Probably, the immersion of seeds in these filamentous matrices buffered the osmotic disequilibrium promoting the water-absorbing capacity of seeds and favoring their germination.[29,1,30]

Addition of *Nostoc* sp. UP2 cells to rice seeds positively affected the lengths both in shoot and root ($P<0.05$). Particularly, the addition of a high concentration of *Nostoc* sp. UP2 cells (36.4, 72.2, 144.4 and 288.8 $g.L^{-1}$) showed a very strongly stimulating growth effect of the lengths of rice seedling compared with both controls (Fig 1a). Similar trend was also found in the weights of seedling. *Nostoc* sp. UP2 cells showed higher fresh and dry weights than PS and controls ($P<0.05$) (Fig 1b). Cyanobacteria cells involved in the production of metabolites and phytohormones like auxins, cytokinin and gibberellins.[31] These metabolites play an important role in the regulation of seedling, increasing the growth of root and shoot tissues.[32]

Table 2. Effects of *Nostoc* sp. UP2 cells and PS on germination percentage (GP), germination energy (GE), speed of germination (SG) and seedling vigor index (SVI).

Treatment	Seed germination parameters (Mean±SD[a])			
	GP (%)	GE (%)	SG (%)	SVI
DI	96.67±5.77[a]	56.67±4.71[b]	58.52±10.32[c]	401±19.09[h]
Zeolite 7.8 g.L^{-1}	95.00±5.77[a]	70.00±0.00[ab]	72.83±10.10[bc]	405±17.88[h]
Zeolite 15.6 g.L^{-1}	93.33±5.77[a]	86.67±6.94[a]	92.59±12.83[a]	565±16.97[ef]
Cell UP2 4.5 g.L^{-1}	93.33±5.77[a]	90.00±0.00[ab]	89.26±11.13[ab]	417±22.72[h]
Cell UP2 9.1 g.L^{-1}	93.33±5.77[a]	90.00±7.07[a]	88.89±7.86[ab]	556±20.51[ef]
Cell UP2 18.2 g.L^{-1}	90.00±0.00[a]	85.00±3.54[a]	94.44±7.86[a]	618±38.68[de]
Cell UP2 36.4 g.L^{-1}	90.00±0.00[a]	85.00±3.54[a]	83.33±7.86[ab]	815±24.75[d]
Cell UP2 72.2 g.L^{-1}	93.33±5.77[a]	90.00±7.07[a]	94.44±7.86[a]	952±29.70[c]
CellUP2 144.4g.L^{-1}	90.00±0.00[a]	90.00±5.77[a]	100.00±11.11[a]	1,026±31.49[b]
CellUP2 288.8g.L^{-1}	96.67±5.77[a]	85.00±3.54[a]	85.00±7.07[ab]	1,185±9.90[a]
PS UP2 15 mg.L^{-1}	90.00±0.00[a]	76.67±6.94[ab]	94.44±7.86[a]	478±17.52[g]
PS UP2 30 mg.L^{-1}	93.33±5.77[a]	76.67±2.36[ab]	94.44±7.86[a]	550±19.80[f]
PS UP2 60 mg.L^{-1}	93.33±5.77[a]	80.00±5.77[ab]	90.00±12.24[ab]	662±14.14[e]
PS UP2 120mg.L^{-1}	96.67±5.77[a]	93.33±5.18[a]	100.00±0.00[a]	675±22.63[d]
PS UP2 240mg.L^{-1}	90.00±0.00[a]	66.67±5.43[ab]	83.33±7.86[ab]	571±27.58[ef]

[a]Mean ± standard deviation followed by the same letter are not significantly different at $p<0.05$, according to Duncan's Multiple Range Test (DMRT). (GP: Germination Percentage (%), GE: Energy Germination (%), SG: Speed of Germination (%), SVI: Seedling Vigor Index)

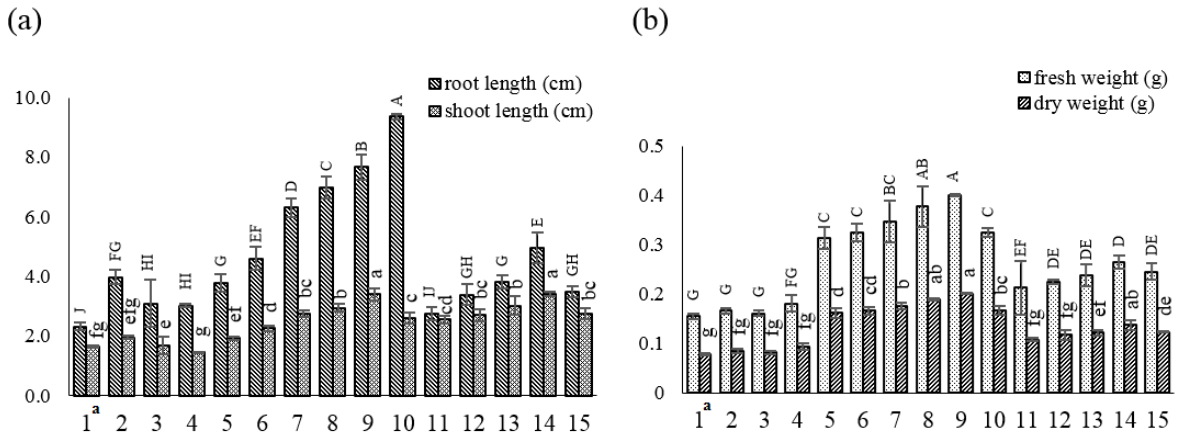

Figure 1. Effect of *Nostoc* sp. UP2 cells and PS on shoot and root lengths (a) and fresh and dry weights (b) at 7 days after the application.

[a]1: Deionized water	2: Zeolite 7.5 g.L^{-1}	3: Zeolite 15 g.L^{-1}	4: Cell UP2 4.5 g.L^{-1}
5: Cell UP2 9.1 g.L^{-1}	6: Cell UP2 18.2 g.L^{-1}	7: Cell UP2 36.4 g.L^{-1}	8: Cell UP2 72.2 mg.L^{-1}
9: Cell UP2 144.4 mg.L^{-1}	10: Cell UP2 288.8 mg.L^{-1}	11: PS UP2 15 mg.L^{-1}	12: PS UP2 30 mg.L^{-1}
13: PS UP2 60 mg.L^{-1}	14: PS UP2 120 mg.L^{-1}	15: PS UP2 240 mg.L^{-1}	

Note; Different letters above bars indicate a significant difference ($p<0.05$) within root length, fresh weight (uppercase letters) and shoot length, dry weight (lowercase letters)

Effect of the cells and PS from Nostoc sp. UP2 on the total number of bacteria and Nostoc in soil

The total number of bacteria was increased with increasing the day of application in all treatments. Both *Nostoc* sp. UP2 cells and PS clearly tended to give the higher total number of bacteria than zeolite and control at 7 days after application. Interestingly, comparing between the soil treated with *Nostoc* sp. UP2 cells and PS, it was found that PS gave the higher total number of bacteria than *Nostoc* sp. UP2, especially in the soil treated with 240 mg.kg^{-1} of PS (29.97×10^{8} cfu.g^{-1}) on 21st day of experiment (Fig 2a). Considering the total number of *Nostoc* sp., it was revealed that the number of *Nostoc* sp. in soil increased with increasing the applied *Nostoc* sp. UP2 cells concentration. The maximum total number of *Nostoc* sp. was found on day 14th (11.77×10^{7} cfu.g^{-1}) and the declining trend was found on day 21st of the experiment (Fig 2b). Inoculation of cyanobacteria enhanced soil biological activity in terms of increasing the total number of bacteria and providing some nutrient sources for rhizobacteria colonization.[33]

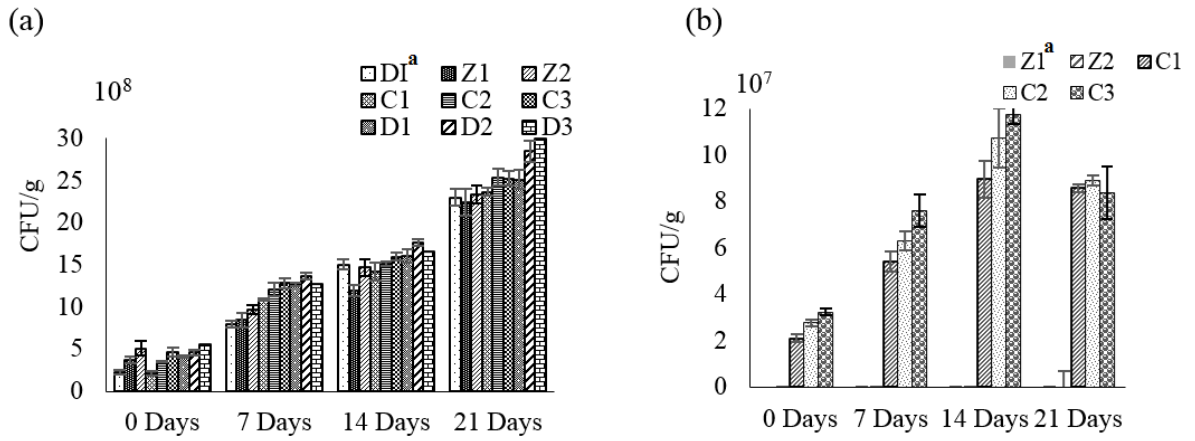

Figure 2. The total numbers of bacteria (a) and *Nostoc* sp. UP2 (b) in soil after addition of the different level of concentrations of *Nostoc* sp. UP2 cells and PS.

[a]D1: Deionized water Z1: Zeolite 7.8 g.kg^{-1} Z2: Zeolite 15.6 g.kg^{-1} C1: Cell UP2 36.4 g.kg^{-1} C2: Cell UP2 72.2 g.kg^{-1} C3: Cell UP2 144.4 g.kg^{-1} D1: PS UP2 60 mg.kg^{-1} D2: PS UP2 120 mg.kg^{-1} D3: PS UP2 240 mg.kg^{-1}

Effect of the cells and PS from Nostoc sp. UP2 on seedling growth and some biochemical contents of rice seedling after 21 days of application

All treated substances did not pose a significant difference on the shoot length. However, a high concentration of PS resulted in a longer shoot length compared with zeolite and control. Whereas, the highest concentration of *Nostoc* sp. UP2 cells (144.4 g.kg^{-1}) and PS (240 mg.kg^{-1}) significantly increased the root length (P<0.05). Nevertheless, fresh and dry weights of rice seedling were not significantly affected by all treated substances.

Considering the biochemical parameters, it was found that the highest concentration of *Nostoc* sp. UP2 cells and all concentrations of PS posed a significant higher total sugar content than those zeolite and control. Hussian *et al*. (2016) demonstrated that *Nostoc* cells and its polysaccharide treated on rice seedling increased the total sugar with the content ranged

between 24 and 40 mg.g^{-1}FW, which was 2-3 times lower than this experiment.[34] Similar trend was found in total free amino acid content; all concentrations of *Nostoc* sp. UP2 cells and PS showed a significantly positive effect on rice seedling. For total chlorophyll content, *Nostoc* sp. UP2 cells and PS did not show a significant effect on the seedling compared to zeolite. However, a significant higher content of total chlorophyll than the control was observed. Cyanobacterial cells and their polysaccharide pose the stimulating growth effect on seedling root and shoot. The longer roots directly influenced the nutrient absorption capacity, resulting in higher photosynthesis and its metabolites i.e. sugar content, amino acids, and chlorophyll.[35]

Table 3. Effect of *Nostoc* sp. UP2 cells and PS on shoot and root lengths, fresh and dry weights, total sugar, total free amino acid and total chlorophyll contents of rice seedling after 21 days of application.

Treatment	Seedling physical and biochemical parameters (Mean±SD[a])						
	Shoot length	Root length	Shoot and root fresh weights	Shoot and root dry weights	Total sugar	Total free amino acid	Total chlorophyll
	cm		g			mg Fw^{-1}	
DI	23.22±0.50^{a}	9.50±1.08^{b}	0.265±0.028^{a}	0.050±0.004^{a}	66.65±1.43^{de}	8.69±1.46^{c}	1.75 ±0.01^{b}
Zeolite 7.8 g.kg^{-1}	22.88±1.81^{a}	8.80±0.6^{b}	0.253±0.040^{a}	0.051±0.011^{a}	71.45±1.15^{c}	12.54±0.18^{c}	1.87±0.04^{ab}
Zeolite 15.6 g.kg^{-1}	23.45±1.32^{a}	8.88±0.64^{b}	0.251±0.010^{a}	0.055±0.004^{a}	74.27±0.18^{c}	12.93±0.08^{c}	1.95±0.02^{a}
Cell 36.4 g.kg^{-1}	21.47±0.95^{a}	11.22±0.38^{b}	0.320±0.042^{a}	0.024±0.003^{a}	65.91±1.92^{e}	19.08±0.23^{b}	1.85±0.23^{ab}
Cell 72.2 g.kg^{-1}	23.59±0.58^{a}	10.91±0.13^{b}	0.313±0.060^{a}	0.028±0.003^{a}	70.60±2.01^{cd}	20.80±3.00^{ab}	1.89±0.05^{ab}
Cell 144.4 g.kg^{-1}	22.25±2.40^{a}	14.01±0.53^{a}	0.252±0.021^{a}	0.055±0.003^{a}	82.73±1.20^{b}	22.60±3.19^{ab}	1.94±0.18^{a}
PS 60 mg.kg^{-1}	23.70±2.67^{a}	10.05±1.31^{b}	0.270±0.023^{a}	0.059±0.004^{a}	81.77±6.43^{b}	19.59±0.18^{b}	1.84±0.08^{a}
PS 120 mg.kg^{-1}	24.91±2.74^{a}	11.39±2.54^{b}	0.316±0.037^{a}	0.028±0.001^{a}	85.91±0.66^{b}	25.03±0.12^{a}	1.84±0.02^{ab}
PS 240 mg.kg^{-1}	25.15±0.37^{a}	15.15±1.70^{a}	0.317±0.066^{a}	0.027±0.006^{a}	97.64±2.09^{a}	26.09±0.07^{a}	1.95±0.23^{a}

[a]Mean ± standard deviation followed by the same letter are not significantly different at $p<0.05$, according to Duncan's Multiple Range Test (DMRT)

Effect of Nostoc sp. UP2 cells and its PS on the properties of soil

The soil pH, ranging between 6.13-6.25, was not significantly different among the treatments and. While, the EC of soil treated with PS (34.04 µs.cm^{-1}) was significantly lower than the control. Addition of *Nostoc* sp. UP2 cells and PS significantly increased the CEC; however, it was slightly lower than the zeolite. Considering the OM and total N, no significant differences were found between the test substances and the control. *Nostoc* sp. UP2 cells and PS tended to increase the available Fe compared with zeolite and control. Whereas, the available P were not significantly different among the treatments (Table 4). In addition to the exchangeable K^{2+}, Ca^{2+}, Mg^{2+} and Na^{2+}, no significant differences were found among all treatment. However, the PS tended to increase all of those values, especially in exchangeable K^{2+} and Na^{2+} (Table 5). In general, soil microbes play an important role in the supply of nutrients for plants as the decomposers of organic materials and transformers of various elements.[37] The major intracellular cation in organisms, including bacteria and fungi, is accumulated inside the cells. Therefore, it can be expected that soil microbial biomass is a significant element pool.[36]

Table 4. Effect of *Nostoc* sp. UP2 cells and PS on pH, electrical conductivity (EC), cation exchange capacity (CEC), organic matter (OM), total nitrogen (N), available iron (Avai Fe) and available phosphorus (Avai P) in soil after 14 days of the application.

Treatment	Soil chemical properties (Mean±SD[a])						
	pH	EC ($\mu s.cm^{-1}$)	CEC ($meq.100g^{-1}$)	OM (%)	Total N (%)	Avai Fe ($mg.kg^{-1}$)	Avai P ($mg.kg^{-1}$)
DI	6.25±0.01[a]	44.01±2.14[a]	14.9±0.2[c]	2.19±0.08[a]	0.110±0.004[a]	85.00±1.77[ab]	11.08±1.89[a]
Zeolite 15.6 $g.kg^{-1}$	6.13±0.04[a]	42.52±8.88[ab]	19.9±0.3[a]	2.00±0.04[a]	0.100±0.002[b]	83.38±2.51[b]	10.08±0.28[a]
Cell UP2 144.4 $g.kg^{-1}$	6.33±0.22[a]	46.41±1.85[a]	16.8±0.1[b]	2.18±0.03[a]	0.110±0.001[a]	92.98±5.62[a]	9.80±0.87[a]
PS UP2 120 $mg.kg^{-1}$	6.23±0.02[a]	34.01±1.56[bc]	17.0±0.7[b]	2.16±0.13[a]	0.106±0.04[ab]	88.48±1.45[ab]	11.15±1.44[a]

[a]Mean ± standard deviation followed by the same letter are not significantly different at $p<0.05$, according to Duncan's Multiple Range Test (DMRT)

Table 5. Effect of *Nostoc* sp. UP2 cells and PS on exchangeable K^{2+}, Ca^{2+}, Mg^{2+} and Na^{2+} contents in soil after 14 days of the application.

Treatment	Exchangeable cation ($mg.kg^{-1}$) (Mean±SD[a])			
	K^{2+}	Ca^{2+}	Mg^{2+}	Na^{2+}
DI	103.35±18.60[b]	1,888.75±88.74[b]	231.85±1.98[a]	66.63±25.28[b]
Zeolite 15.6 $g.kg^{-1}$	166.66±5.69[a]	2,164.75±165.11[a]	237.38±20.82[a]	113.76±7.25[a]
Cell UP2 144.4 $g.kg^{-1}$	100.88±5.48[b]	1,958.50±74.25[ab]	244.28±13.40[a]	89.19±7.65[ab]
PS UP2 120 $mg.kg^{-1}$	130.67±36.79[ab]	1,928.50±66.47[ab]	235.10±0.35[a]	96.67±5.75[ab]

[a]Mean ± standard deviation followed by the same letter are not significantly different at $p<0.05$, according to Duncan's Multiple Range Test (DMRT)

Interestingly, considering the physical parameters of the soil, it was found that soil moisture and soil porosity were significantly increased by the addition of *Nostoc* sp. UP2 cells. Despite the addition of PS, it did not affect soil moisture and soil porosity but demonstrated clearly increasing trend compared with the control. The similar trend also found in bulk density; the addition of *Nostoc* sp. UP2 cells and PS resulted in a higher bulk density than those of zeolite and control (Table 6). In consistent with this study, Saadatnia and Riahi (2009) revealed that the addition of Anabaena *spp.* cells significantly increased the moisture and porosity of soil, no positive effect was observed in bulk density of soil.[37]

Table 6. Effect of *Nostoc* sp. UP2 cells and its PS on soil moisture, bulk density and soil porosity after 14 days of the application.

Treatment	Soil physical properties (Mean±SD[a])		
	Soil moisture (%)	Soil porosity (%)	Bulk density (g/cm^3)
DI	48.01±1.29[b]	41.90±0.19[c]	0.8673±0.00[a]
Zeolite 15.6 $g.kg^{-1}$	54.22±2.39[ab]	43.23±4.12[ab]	0.8738±0.02[a]
Cell UP2 144.4 $g.kg^{-1}$	67.29±7.62[a]	60.94±2.89[a]	0.9242±0.04[a]
PS UP2 120 $mg.kg^{-1}$	51.42±5.52[b]	50.14±2.27[b]	0.9220±0.04[a]

[a]Mean ± standard deviation followed by the same letter are not significantly different at $p<0.05$, according to Duncan's Multiple Range Test (DMRT)

Conclusion

This study demonstrated that PS and *Nostoc* sp. UP2 cells did not affect the germination percentages, but they tended to increase the speed of germination, germination energy and seedling vigor index. Particularly, *Nostoc* sp. UP2 cells strongly posed a significant growth promoting effect on seedling length, resulting in the significantly higher SVI.

Considering the 21 days old rice seedlings, the PS *Nostoc* sp. UP2 cells tended to promote the length of shoot and root and fresh weight of rice seedling. The significantly higher contents of total sugar, total free amino acid and total chlorophyll were found in seedling treated with the highest concentration of PS and *Nostoc* sp. UP2 cells than zeolite and control.

The study of soil quality demonstrated that the pH, OM, total nitrogen and available Fe and P in soil were not affected by all concentrations of PS and *Nostoc* sp. UP2 cells. Whereas, cation exchange capacity, exchangeable K^{2+}, Ca^{2+}, Mg^{2+} and Na^{2+} were increased. Furthermore, the moisture content and porosity of the soil were also significantly positively influenced by PS and *Nostoc* cells.

Therefore, it can be concluded that PS and *Nostoc* sp. UP2 cells had a growth stimulating effect on rice seedling. Moreover, it revealed a positively influence on both physical and chemical properties of soil.

Reference

1. Ruamchimplee T. J Grad RRU. 2014;1:75-80.
2. Savci S. Int J Biomed Eng Tech Procedia. 2012;287-292.
3. Kaushik BD. Proc Indian Natl Sci Acad. 2014;80(2):379-388.
4. Singh JS, Kumar A, Rai AN, Singh DP. Front Microbiol. 2016;529(7):1-19.
5. Obana S, Miyamoto K, Morita S, Ohmori M, Inubushi K. J Appl Phycol. 2007;19:641-646.
6. Saadatnia H, Riahi H. Plant Soil Environ. 2009;55:201-212.
7. Osman MEH, El-Sheekh MM, El-Naggar AH, Gheda SF. Biol Fertil Soils. 2010;46:861-875.
8. Potts M. Eur J Phycol. 1999; 34:319-328.
9. Huang Z, Liu Y, Paulsen BS, Klaveness D. J Phycol. 1998;34:962-968.
10. Dubios M, Gilles KA. Hamilton JK, Rebers PA, Smith F. Analyt Chem. 1956;28:350-356.
11. Miller GL. *Anal Chem.* 1959;31(3);426-428.
12. Chon SU, Choi SK, Jung S, Jang HG, Pyo BS, Kim SM. Crop Prot. 2002;62:1077.
13. Kim JD, Lee CG. J Microbiol Biotechnol. 2006;16(2):240-246.
14. Krishnaswamy V, Seshu DV. Seed Sci Technol. 1990;18:147-15.
15. Singh D, Khattar J, Gupta M, Kaur G. Pestic Biochem Physiol. 2014;110:63-70.
16. Pithukpakorn W, Mala T, Sinma K, Antarikanonda P. J Sci Tech. 2015;4:29-40.
17. Hamilton BP, Van Slyke DD. J. Biol Chem. 1943;19:231-250.
18. Costache MA, Campeanu G, Neata G. Res J Chem Sci. 2012;17(5):7702-7708.
19. Braden C, Kelly O. Drying Technology. 2004;22(7):1,767-1,776.
20. Burwell RE, Allmaras RR, Amemiya M. Soil Sci Soc Am J. 1963;27(6):697-700.
21. William EF, Page-Hanify DS. Soil Sci. 1959;88(3):168-171.
22. Chapman HD. J Am Soc Agron. 1965;9:891-900.
23. Bradstreet RB. *Anal Chem.* 1954;26(1):185-187.
24. Gelman F, Binstock R, Halicz L. Fuel. 2011;96:608-610.

25. Bray RH, Kurtz LT. Soil sci. 1945;59:39-45.
26. Lindsay WL, Norvell WA. Soil Sci. Soc. Am. J. 1978;42:421-448.
27. Temperli AT, Misteli H. Anal Biochem. 1969;27(2):361-363
28. Lu CK, Bray, Roger H. Soil Sci. 1951;72(6):449-458.
29. Wang SM, Wang QL, Li SH. Zhang JR. Acta Hydrob Sin.1991;15:45-52.
30. Xu Y, Rossi F, Deng S, De Philippis R, Chen L. Biol Fertil Soil. 2013; 49:143-152.
31. Lu Y, Xu J. Trends Plant Sci. 2015;20(5):273-282.
32. Priya H, Prasanna R, Ramakrishnan B, Bidyarani N, Babu S, Thapa S, Renuka, N. Am J Microbiol Res. 2015;171;78-89.
33. Hegazi ZA, Mostafa SMS, Ahmed HMI. Nat Sci. 2010;8(11):183-194.
34. Hussain A, Shah S, Rahman H, Irshad M, Iqbal A. Front in plant sci. 2016;6:1-9.
35. Ranjan K, Priya H, Ramakrishnan B, Prasanna R, Venkatachalam S, Thapa S, Shivay YS. Appl Soil Ecol. 2016;108:195-203.
36. Yamashita K, Honjo H, Nishida M, Kimura M. Asakawa S. J Soil Sci Plant Nutr. 2014;60:512-519.
37. Saadatnia H, Riahi H. Plant Soil Environ. 2009;55:201-212.

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