PLANT ASSOCIATED MICROORGANISMS WITH SILICA SOLUBILIZATION POTENTIAL

MICROORGANISME BENEFICE PLANTELOR CU EFECT DE SOLUBILIZARE A SILICIULUI

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Abstract. *Silicon is the second abundant element on Earth. Commonly, it is found as silica and silicates, or in biology as mineral constituent of microorganisms, protozoa and plants. Although silicon it is not considered an essential nutrient for plants, it has been noticed that available silicon positively influences plants' growth, mechanical strength, and resistance to several biotic and abiotic unfavorable conditions, such as fungal phytopathogens, herbivores and adverse chemicals. Our study presents several microbial strains able to solubilize silicon from different biological and mineral substrates. Some of these microorganisms were isolated from plant material with high content of mineral silicon like horsetail, wheat straw, rosemary and nettle. Moreover, microbial supernatant obtained on horsetail broth increased hypocotyl and roots length of cowpea Vigna unguiculata (L.) Walp.*
Key words: plant beneficial microorganisms, silica solubilization

Rezumat. *Siliciul este cel de-al doilea element de pe pământ din punct de vedere al abundenței. Acesta se regăsește cel mai adesea sub formă de silicați și silice, sau constituent mineral în microorganisme, protozoare și plante. Deși siliciul nu este considerat un nutrient esențial pentru plante, s-a observat că prezența siliciului în forme solubile influențează în mod pozitiv creșterea plantelor, rezistența mecanică și îmbunătățește rezistența la factorii biotici și abiotici nefavorabili, cum ar fi atacul fungilor fitopatogeni, erbivorele și substanțele chimice adverse. Studiul de față prezintă o serie de microorganisme capabile să solubilizeze siliciul prezent în diferite substraturi organice sau minerale. Unele dintre acestea fiind izolate din material vegetal bogat în siliciu, precum coada calului, paiele de grâu, rozmarinul și urzica. Mai mult decât atât, s-a observat că supernatantul microbian obținut pe decoct de coada calului stimulează creșteria plăntuțelor de fasoliță Vigna unguiculata (L.) Walp.*
Cuvinte cheie: microorganism benefice plantelor, solubilizarea siliciului

INTRODUCTION

Silicon (Si) is an abundant element in nature. In plants growth, it is considered as a non-essential element. However, it is accumulated in up to 1% in

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dicotyledonous plants,up to 5-10% in monocots (like rice) and wetland grasses, or it can reach 25% in *Equisetaceae* (Epstein, 1994). Some plants, such as green onion (*Allium cepa*), pepper (*Capsicum* sp.), radish (*Raphanus sativus*), and tomato (*Lycopersicon esculentum*), accumulate silicon in their roots, but have a low quantity of Si in their shoots (Tubana *et al.*, 2016).Silicon is absorbed by plants only as silicic acid (Luyckx *et al.*, 2017). However, plants ability to absorb this element is differentiated among species. This property seemsto be driven by the differencesin the cell wall types (Yokoyama and Nishitani, 2004) where Si has a structural importancein type II cell walls, compared to the type I cellwalls of dicots. Moreover, studies carried out by Deshmukh *et al.* (2015) revealed that a specific type of plant aquaporins, with a precise distance of 108 amino acids between the asparagine-proline-alanine domains, enable Si absorption in species that possess this NIP-III aquaporins (Deshmukh *et al.*, 2015).

It has been demonstrated that available silicon positively influences plants' growth, mechanical strength, and resistance to several biotic and abiotic unfavorable conditions. Studies regarding this aspect evidenced that silicic acid primes the defense response in both Si-accumulators and non-accumulator plants (Detmann *et al.*, 2012, 2013; Ghareeb *et al.*, 2011; Luyckx *et al.*, 2017).

Due to the proven effectsof Si in plant growth and stress mitigation, the microorganisms that can convert an insoluble silicon source into silicic acid are of great interest.

Microorganisms able to solubilize phytosilica are relatively widespread (Vasanthi *et al.,* 2016). In the case of fungi and bacteria, it has been noticed that can grow in extremely nutrient deficient environments, including nitrogen sources, when silica compounds (such as silicagel) are present. An explanation of their growth in such conditions can be due to the ability of silicon compounds to absorb gases and volatile compounds that can then be used as sources of carbon or nitrogen by microorganisms (Wainwright *et al.*, 1997).

The aim of this study was the selection and characterization of some microbial strains with silica solubilization abilities.

MATERIAL AND METHOD

For the isolation of phyosilica solubilizing microorganism we used water-based macerates of horsetail (*Equisetum arvense*), wheat straw (from *Triticum aestivum*), rosemary (*Rosmarinus officinalis*) and nettle (*Urtica dioica*), maintained at room temperature for three days. An amount of 100 µl suspension of each macerate wasinoculated on Bunt and Roviraagar medium containing 0.25% silica gel or talc, and also on glucose - agar medium supplemented with 0.25% silica gel or talc. After one week incubation at 28°C, the colonies emerged on this media were analyzed for their ability to solubilize silicon by revealing a clarified halo on Bunt and Rovira agar medium supplemented with 0.25% of silica gel, talc or diatomite. Bacterial strains revealing clear halos surrounding their colonies were identified through Biolog Gen III technique.

New isolated microorganisms as well as other bacterial and fungal strains were analyzed for their ability to solubilize silicon from silica gel, an insoluble Si-compound.

The amount of silicic acid released from the solubilization process was quantified using the Spectroquant kit (Merck). Microbial strains used in this experiment included five newly isolated strains of *Burkholderia fungorum* (Si1, Si4, Si17, Si18b and Si24), five strains of *Bacillus* sp. (BW, BIR, B5, OS15 and OS17), five strains of *Pseudomonas* spp. (Ps 8/2/3, P6, P8, P9, P22), and five fungal strains of *Trichoderma* spp. (Td2, TK14, TK20, TK27 and T36). All of these microbial strains were grown in liquid Bunt and Roviramedium supplemented with 0.25% silica gel. Centrifuged culture supernatant was then used to determine solubilized Si content as silicic acid. The quantitative determination was made in presence of sulfuric acid, when silicate and molybdate ionsare reduced to silicomolibdate, a blue compound determined spectrophotometrically at 400 nm.

In order to evaluate the effect of released phytosilica on the germination and growth of cowpea seedlings (*Vigna unguiculata*), five fungal strains of *Trichoderma* sp. (Td2, TK14, TK20, TK27 an d T36) were grown in 1% sterile *Equisetum arvense* herba prepared in distilled water. After 7 days of incubation at room temperature, culture supernatants were filtered through Whatman no.1 filter paper and sterile membranes of 0.22 µm porosity. Disinfected cowpea seeds were then placed on Water-Agar substrate supplemented with 5 and 10% microbial filtered culture-supernatant obtained on horsetail broth. Seeds germination was evaluated after 3 days of incubation at room temperature, and roots and hypocotyls were measured.

RESULTS AND DISCUSSIONS

A total number of 24 bacterial colonies and two fungal colonies were detected to be surrounded by clear halos on the silica gel medium (fig. 1).

A remarkable aspect among the bacterial isolates obtained from horsetail macerate is their high capacity to solubilize tricalcium phosphate from Pikovskaya agar medium (fig. 2).

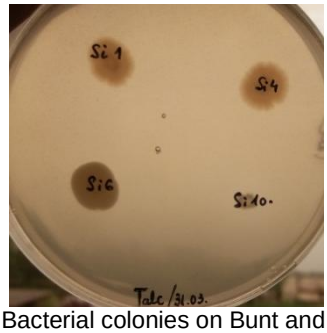

Fig. 1 Bacterial colonies on Bunt and Rovira medium with 0.25% talc. Light clear halos can be seen around Si1, Si4 and Si 10 colonies suggesting talc solubilizing activity

Fig.2 Phosphate solubilizing bacteria (isolates Si1, Si4 and Si10) onPikovskaya agar medium.

The identification of the newly isolated Si-solubilizing bacteria revealed that Si1, Si4, Si10, Si17, Si18b and Si24 strains belong to *Burkholderia fungorum*, with a probability of 95.5 to 96.8%, according to Biolog Gen III ID

system. The two fungal strains with Si-solubilizing activity were identified as *Fusarium* species, based on their colony morphology and microscopic aspect.

Among the newly isolated strains only five of them were maintained, due to their cultivation capacity. These strains of *Burkholderia fungorum* (Si1, Si4, Si17, Si18b and Si24), along with other ten bacterial strains of *Bacillus* spp. (BW, BIR, B5, OS15 and OS17), *Pseudomonas* sp. (Ps 8/2/3, P6, P8, P9, P22) and five fungal strains of *Trichoderma* sp. (Td2, TK14, TK20, TK27 and T36) were forwardly analyzed. Silicic acid was then quantified in the microbial culture supernatant (fig. 3), after 7 days of incubation on insoluble silica gel supplemented substrate.

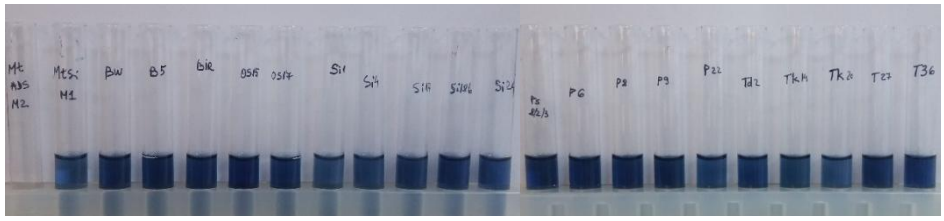

Fig. 3 Differences in silica gel solubility among the tested microbial strains (legend within the text)

Table 1

Fungal and bacterial strains capacity to solubilizeSi from silica gel

Crt no.	Microbial strain	µg / mL SiO_2
1	*Bacillus amyloliquefaciens* BW	2.96
2	*Bacillus sp.* B 5	5.024
3	***Bacillus sp.* BIR**	**6.856**
4	*Bacillus sp.* OS 15	5.536
5	***Bacillus amyloliquefaciens* OS 17**	**5.96**
6	*Burkholderia fungorum* Si 1	4.224
7	*Burkholderia fungorum* Si 4	3.904
8	*Burkholderia fungorum* Si 17	4.904
9	*Burkholderia fungorum* Si 18b	4.472
10	*Burkholderia fungorum* Si 24	4.584
11	*Pseudomonas fluorescens* Ps 8/2/3	4.568
12	***Pseudomonas* sp. P 6**	**7.704**
13	***Pseudomonas* sp. P 8**	**7.176**
14	*Pseudomonas* sp. P 9	5.2
15	*Pseudomonas* sp. P 22	3.88
16	*Trichoderma*sp.Td 2	4.416
17	***Trichoderma*sp. Tk 14**	**7.664**
18	*Trichoderma*sp.Tk 20	3.328
19	***Trichoderma*sp.T27**	**5.848**
20	*Trichoderma*sp. T36	4.832
	Control (uninoculated culture supernatant)	2.744

Among the twenty microbial strains tested, different amounts of silicic acid were quantified (tab. 1). The most effective strains in Si-solubilization were *Bacillus* sp. BIR and OS17, *Pseudomonas* sp. P6 and P8, *Trichoderma* sp. Tk14 and T27 strains. These results suggest that, in most cases, the ability of microorganisms to grow on media with insoluble silicon form is associated with silicon solubilization and extracellular silicate accumulation.

Regarding the effect of microbial released phytosilica on the germination cowpea seeds, no differences were noticed among the experimental variants. The germination percentage of cowpea seeds was 100%. However, when roots and hypocotyls were measured, results showed that all microbial treatments stimulated the vegetative growth of the cowpea seedlings (fig. 4), except for T27 strain (in both concentrations), and Tk20 strain (in 10% concentration).

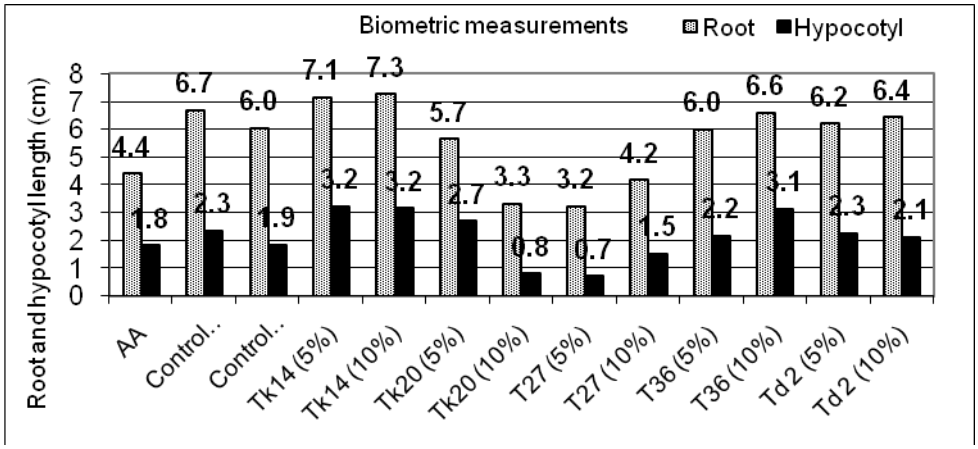

Fig. 4 Biometric evaluation of cowpea seedlings

Best results were obtained with Tk14 strain, with insignificant differences between the two tested concentrations.

CONCLUSIONS

New bacterial strains belonging to *Burkholderia fungorum* and *Fusarium* sp. were selected for their ability to solubilize phytosilica, silica gel, talc and diatomite.

Silicic acid quantification performed on 20 microbial cultures revealed the high Si-solubilizing activity of *Bacillus* sp. BIR and OS17 strains, *Pseudomonas* sp. P6 and P8 strains, and *Trichoderma* sp Tk14 and T27 strains. These microbial strains released 5.8 to 7.7µl SiO_2/mL.

Microbial solubilization of phytosilica from *Equisetum arvense* herba has a positive influence on cowpea seedlings growth.

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