In vitro evaluation of some halophilic bacterial isolates as biofertilizers

Samsonova E.A.¹, Ibrahim I.M.², Fedonenko Yu.P.^{1,3}, Konnova S.A.^{1,3}

¹Chernyshevsky Saratov State University, Saratov, Russia; ²Department of Agricultural Microbiology, Fayoum University, Fayoum, Egypt; ³Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, Saratov, Russia

E-mail: elena.smsnv@mail.ru

Key message. For six strains of halophilic bacteria, nitrogen fixation ability, production of phytohormones and siderophores, the solubilization of phosphates, and heavy metal resistance were revealed.

Keywords: halophilic bacteria, plant-growth promoting activity

Halophilic microorganisms are able to survive under salt and osmotic stresses, and are often adapted to alkalization, high temperatures, and other extreme environmental conditions. Plant-growth-promoting halophilic bacteria are promising agents for creation of effective biofertilizers, therefore, the aim of this study was to identify such activities among 15 halophilic strains isolated from the salt samples from Qarun and Elton salt lakes.

Bacteria were cultured and maintained under aerobic conditions in GRM broth and GRM agar (2%) media, supplemented with NaCl (5-10%, depending on the strain). The ability of bacteria to fix atmospheric nitrogen was determined using semi-liquid nitrogen-free Muromtsev medium. The content of indolyl-3-acetic acid (IAA) in the Muromtsev culture medium containing L-tryptophan as an inductor of auxin biosynthesis was determined by HPLC. The ability to mobilize inorganic phosphates was evaluated after cultivation in NBRIP medium for 72 hours. After precipitation of bacteria, the content of mobile forms of phosphorus was measured in the supernatant according to the Chirikov method. The resistance of microbial strains to heavy metals was evaluated after 3 day incubation in a liquid S-G liquid medium supplied with ZnSO₄·7H₂O, Cd(CH₃COO)₂·2H₂O, Pb(CH₃COO)₂·3H₂O, CuSO₄·5H₂O, NiCl₂·6H₂O (up 0 to 5 mM). For the assessment of the ability to secrete siderophores under conditions of iron starvation the bacterial suspensions (100 µl, OD₆₀₀ = 1) were added to the wells in M9 medium with chromazurol S dye (CAS agar). The ability of bacteria to grow on CAS agar with the formation of a radial zone and to change in the color of the medium were evaluated.

Of 15 studied isolates, six strains showed the most pronounced PGRP activities: two strains of Gram negative bacteria: *Chromohalobacter salexigens* EG1QL3, *Halomonas caseinilytica* EG33S7QL; and four strains of firmicutes: *Bacillus licheniformis* EG1QL30, *Bacillus subtilis* EGP5QL12, *Bacillus halotolerans* RU2EL4, *Halobacillus dabanensis* EG1HP4QL. These bacteria belong to different ecological groups: extreme halophiles – *C. salexigens* (>15% NaCl); moderate halophiles – *B. licheniformis* and *B. subtilis* (5-15% NaCl); weak halophiles – the other strains (0-5% NaCl). All isolates grew on nitrogen-free medium, which indicated the presence of nitrogen-fixing ability in these bacteria. IAA production was observed for *B. licheniformis* and *H. dabanensis* only, and its content in the cultivation medium was 0.15 and 0.19 µg / ml, respectively. During the cultivation of *B. licheniformis*, *H. dabanensis*, and *B. halotolerans* in Muromtsev medium, the concentration of phosphates increased 2.2, 1.6, and 0.4 fold as compared to the initial medium, respectively. Among the studied microorganisms, inter- and intra-strains variability of heavy metal sensitivity was revealed. The greatest inhibitory effect to the most strains was shown for salts of cadmium, zinc and lead. All studied strains produced siderophores with different activity at growth on CAS agar. *C. salexigens* and *H. caseinilytica* were the most active siderophore producers, while the activity of *H. dabanensis*, *B. licheniformis*, *B. subtilis*, and *B. halotolerans* was significantly lower.

Based on the studies, all selected strains can be recommended for further studies of their biofertilizing properties.