Microbiology

Selection of Halotolerant Cellulose and Starch Degrading Microscopic Fungi Strains for Composting Remediation of Saline Soils

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A total of 58 strains of microscopic fungi were isolated from saline soils of Kakheti and Kvemo Kartli (East Georgia). The strains are identified as representatives of the following genera: *Alternaria, Aspergillus, Culvularia, Fusarium, Cladosporium, Mucor, Epicoccum, Bipolaris, Penicillium* and *Trichoderma*. On the basis of successive screenings, strains, tolerant to mixed (chloride-sulfate) salinity (8%), and expressing high cellulase and amylase activities were selected. A significant remediation of saline soils has been demonstrated by the wheat bran compost inoculated with selected microscopic fungi strains consortium in model experiments. The best results were obtained on the soils collected in Sagarejo municipality, expressed in the 96% reduction of the salinity during the 21 days of exposure. The efficiency of the bioremediation depends on the concentration of salts in the soil. *© 2024 Bull. Georg. Natl. Acad. Sci.*

halophylic consortium, microscopic fungi, bioremediation, composting, soil degradation

Soil salinization is one of the most acute global ecological problems among the plant affecting abiotic stresses. For this reason the World Land Fund has lost about two billion hectares of once productive land, which is significantly greater than the current area of cultivated land [1].

Existing methods of preventing and reducing soil salinity related to management of irrigation-drainage systems are time-consuming and expensive. Therefore, the search for economically effective and ecologically safe alternative ways for the rehabilitation of salt-degraded soils is urgent. Bioremediation with the use of halotolerant microorganisms, such as bacteria and archaea have shown to be very effective for this purpose [2, 3], while the data on the potential of microscopic fungi in this direction are scarce.

Composting bioremediation due to intensity of the microbial activity within a composting matrix is considered to have significant potential for quicker and cheaper remediation of degraded soils [4]. The aim of the present paper is selection of halotolerant microscopi fungi strains, with expressed high cellulase and amylase activities for composting remediation of saline soils.

Materials and Methods

Soil samples were collected from naturally saline locations of Sagarejo, Gardabani and Sighnaghi municipalities (east Georgia). Isolation of fungi from saline soils and their identification was carried out using the standard methods [5]. The selection of strains tolerant to chloride salinity was carried out on Petri dishes, on universal, agarized nutrient medium with wort, containing different concentrations of sodium chloride (4% and 8%), pH 5.5-6.0 (sterilization mode – 121°C, 15 min); incubation in a thermostat, at 28-30°C for 10 days. Analogously, the nutrient media with different concentration of sodium sulfate was used to select the sulfate-tolerant strains. The selection of strains resistant to mixed cloride-sulfate salinity was carried out on the nutrient media containing both, sodium chloride (4%) and sodium sulfate (4%). Salt tolerance was evaluated with a 5-point system. Cellulase producers were selected on the nutrient medium containing 1% microcrystalline cellulose as the sole carbon source [6], while the amylase producers were selected on the media containing 3% starch as the sole carbon source [7]. Total cellulase and α-amylase activities were determined by the standard methods [8, 9].

To prepare the consortium of halophylic microscopic fungi, the preliminary selected strains were cultivated stationary, in a thermostat at 30°C during 10 days, in 250 ml Erlenmeyer flasks containing 50 ml of the following composition nutrient medium (g/l): $NaNO_3$ – 3.0; KH_2PO_4 – 1.0; $MgSO_4 \times 7H_2O$ – 0.5; KCl – 0.5; $FeSO_4 \times H_2O$ – 0.02; Glucose – 15; malt sprouts – 1.0; pH 5.5- 5.8; sterilization mode – 121°C, 15 min. At the end of cultivation, equal amount (6-7 g) of the biomass of each strain was mixed together, 150 ml of tap water added and homogenized until a homogeneous suspension – fungi consortium was obtained.

Wheat bran in amount 140 g was inoculated with 150 ml of fungi consortium suspension, in a 2-liter Erlenmeyer flasks and incubated in a thermostat at 30°C for 10 days for ripening. The effectiveness of the obtained wheat bran compost on bioremediation was studied in a following way: 15 g of the compost were mixed with 150 g different saline soil samples, placed in plastic containers and moistened; exposition at room temperature during 21 days. Treatments without the compost served as controls for each soil type.

The content of soluble salts in the soil samples was determined according to Gartley [10].

Results and Discussion

A total of 58 strains of microscopic fungi were isolated and purified from saline soils of Gardabani, Sagarejo and Sighnaghi municipalities. Based on the study of cultural-morphological characteristics, the strains isolated from the soils were identified to belong to the following genera: *Alternaria, Aspergillus*, *Culvularia, Fusarium, Cladosporium, Mucor, Epicoccum, Bipolaris, Penicillium* and *Trichoderma*. As the soils, selected for the study are characterized by chloride and sulfate salinization [11], screening of the isolated strains according to their resistance to this type of salinity was carried out. The isolated 58 microscopic fungi strains were screened for resistance to different concentrations of chloride and sulfate (4% and 8%), as well as to their mixture (chloride 4% + sulfate 4%). Nine strains revealed high resistance to both salts as well as to their mixture: *Penicillium s*p. 1-1-2, *Mucor* sp. 2-1-1, *Aspergillus* sp. 2-2-3, *A. niger* 3-1-3, *A. flavus* 5-3-3, *Fusarium sp*. 9-2-4, *Aspergillus sp*. 11-1-1, sp. 2-6-1 and *sp*. 3-1-10 (Table 1).

Wheat bran – a relatively cheap and easily metabolizable raw material has been selected as a substrate for composting remediation. Therefore, further screening of the selected halotolerant strains have been carried out according to their ability to efficient transformation of biopolymers – cellulose and starch of wheat bran. Results of screening on

Table 1. Selected microscopic fungi strains tolerant to chloride, sulfate and mixed salinity

N	Culture	Growth intensity (by the 5 point scale)				
		NaCl		Na_2SO_4		$NaCl+Na_2SO_4$
		4%	8%	4%	8%	8%
1	*Aspergillus* sp. 11-1-1	**5**	**5**	**5**	**5**	**5**
2	*Aspergillus flavus* 5-3-3	**5**	**5**	**5**	**5**	**5**
3	*Aspergillus* sp. 2-2-3	**5**	**4**	**5**	**4**	**5**
4	*Aspergillus nig*er 3-1-3	5	5	5	5	5
5	*Fusarium* sp. 9-2-4	**4**	**4**	**5**	**5**	**5**
6	*Mucor* sp. 2-1-1	5	4	5	5	5
7	*Penicilium* sp. 1-1-2	5	5	5	4	4
8	sp. 3-1-10	**5**	**4**	**5**	**4**	**5**
9	sp. 2-6-1	5	4	5	5	5

Table 2. Screening of selected halotolerant microscopic fungi strains, expressing high cellulase and amylase activities

N	Microscopic fungi culture	Cellulase activity, U/ml	Amylase activity, U/ml
1	*Aspergillus* sp. 11-1-1	0.7	0.7
2	*Aspergillus flavus* 5-3-3	0.4	0.3
3	*Aspergillus* sp. 2-2-3	0.1	-
4	*Aspergillus nig*er 3-1-3	0.4	0.6
5	*Fusarium* sp. 9-2-4	0.1	-
6	*Mucor* sp. 2-1-1	0.4	0.4
7	*Penicilium* sp. 1-1-2	0.8	0.4
8	sp. 3-1-10	-	-
9	sp. 2-6-1	-	-
10	*Trichoderma viridae* 12-1-1	1.14	0.45

high cellulase and amylase activities of the halotolerant strains are represented in the Table 2.

Based on the successive screenings, 5 strains of halophylic microscopic fungi characterized by high resistance to the chloride and sulfate and simultaneously expressing high cellulase and amylase activities were chosen for the construction of desirable microscopic fungi consortium: *Penicillium s*p. 1-1-2*, Mucor* sp. 2-1-1, three strains of the genus *Aspergillu*s - *A. niger* 3-1-3, *A. flavus* 5-3-3, *Aspergillus sp*. 11-1-1. The strain *T. viridae* 12-1-1, characterized by resistance to low chloride-sulphate concentration mixture was also included in the consortium, since this species is known as one of the strong lignocellulose decomposer that contribute to the soil fertility [12].

The selected microscopic fungi consortium was used to compost the wheat bran as described in the materials and methods section. Obtained in this way compost was tested on bioremediation of the saline soils, collected in the above mentioned locations in model experiments. The results of the soluble salts content in the experimental soil samples before and after the composting bioremediation are presented in the Figure.

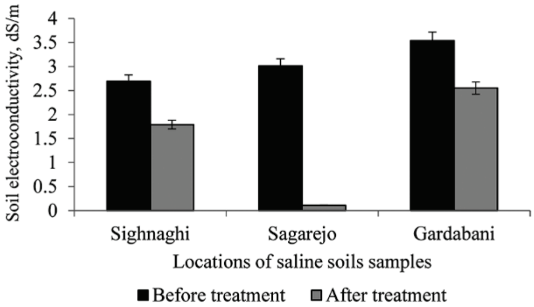

Fig. Content of soluble salts in saline soils before- and after treatment by the wheat bran compost, inoculated with selected microscopic fungi strains consortium in model experiments.

As seen, the selected locations were found to be of medium salinization (electrical conductivity 2-4 dS/m); among them the soil samples, collected in Gardabani municipality contained the salts at highest concentrations, and the soils, collected in Sighnaghi municipality, contained the salts at the least concentrations. Best bioremediation results were obtained in the case of Sagarejo saline soils (Fig.): application of the wheat bran compost, obtained using the consortium of selected halophylic fungi strains reduced the salt content in the soil by 96% (from 3.01 to 0.112 dS/m). Less effective was the composting bioremediation of Gardabani soils: the salt content in the soil reduced only by 28%. Thus, the efficiency of the composting bioremediation depends on the concentration of salts in the soil.

Application of the selected halotolerant microscopic fungi strains consortium composted plant substrate is promising novel approach for the rehabilitation of salinization eroded soils in Georgia.

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მიკრობიოლოგია

ცელულოზის და სახამებლის დამშლელი ჰალოტოლერანტული მიკროსკოპული სოკოების შტამების სელექცია დამლაშებული ნიადაგების კომპოსტირების მეთოდით რემედიაციისათვის

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კახეთისა და ქვემო ქართლის ბუნებრივად დამლაშებული ნიადაგებიდან გამოყოფილი და გასუფთავებულია მიკროსკოპული სოკოს 58 შტამი. კულტურები იდენტიფიკაციის შედეგად მიეკუთვნა გვარებს: *Alternaria, Aspergillus, Culvularia, Fusarium, Cladosporium, Mucor, Epico-*

ccum, Bipolaris, Penicillium და *Trichoderma*. გამოყოფილ მიკროსკოპულ სოკოებს შორის თანმიმდევრული სკრინინგების საფუძველზე შერჩეულია შერეული (ქლორიდულ-სულფატური) დამლაშებისადმი (8%) რეზისტენტული შტამები, რომლებიც ხასიათდება მაღალი ცელულაზური და ამილაზური აქტივობებით. შექმნილია ჰალოფილური მიკროსკოპული სოკოების კონსორციუმი, რომლის გამოყენებით დამზადებულია ხორბლის ქატოს კომპოსტი. მოდელურ ცდებში ნაჩვენებია მიღებული კომპოსტის ეფექტურობა დამლაშებული ნიადაგების ბიორემედიაციაში. საუკეთესო შედეგი მიღწეულ იქნა საგარეჯოს მუნიციპალიტეტში აღებული ნიადაგის ნიმუშზე – 21 დღის განმავლობაში მასში მარილის შემცველობა 96%-ით შემცირდა. ბიორემედიაციის ხარისხი დამოკიდებულია ნიადაგში მარილის რაოდენობრივ შემცველობაზე. შერჩეული სოკოების შტამების კონსორციუმით კომპოსტირებული მენარეული მასალის გამოყენება ახალი პერსპექტიული მიდგომაა საქართველოს დამლაშებით ეროზირებული ნიადაგების რეაბილიტაციისათვის.

REFERENCES

1. Arora S., Singh A,K. Singh Y.P. (eds) (2017) Bioremediation of salt affected soils: an Indian perspective. Springer International Publishing AG, DOI: 10.1007/978-3-319- 48257-6.
2. Patel A.K., Singhania R.R., Albarico F.P.B., Pandey A., Chen C.-W., Dong C.D. (2022) Organic wastes bioremediation and its changing prospects. *Science of the Total Environment,* **824**(2):153889.
3. Dar S.A., Dijoo Z.K., Bhat R.A., Ali M.T. (2021) Halotolerant microorganism reclamation industry for salt-dominant soils. In: Hakeem K.R., Dar G.H., Mehmood M.A., Bhat R.A. (eds) Microbiota and Biofertilizers. Springer, Cham. https://doi.org/10.1007/978-3-030-48771-3_12
4. Lin C., Cheruiyot N., Bui, X.-T., Ngo H. (2022) Composting and its application in bioremediation of organic contaminants. *Bioengineering,* **13**(1):1073-1089. https://doi.org/10.1080/21655979.2021.2017624.
5. Kutateladze L., Zakariashvili N., Jobava M., Burduli T.. Sadunishvili T. (2016) Microscopic fungi spread in different types of soils in Western Georgia. *Annals of Agrarian Science*, **14**(3): 227-232.
6. Urushadze T., Khvedelidze R., Iashvili T. (2010) Biotechnologicaly important cellulases of microscopic fungi. *Bull. Georg. Acad. Sci.,* **7**(3):92-98.
7. Klyosov A.A. (1990) Biochemistry and enzymology of cellulose hydrolysis. *Biochemistry,* **55**:1731-1765.
8. Haki G.D. and Rakshit S.K. (2003) Developments in industrially important thermostable enzymes: a review. *Bioresource Technology*, **89**(1): 17-34.
9. Gartley K.L. (2011) Recommended methods for measuring soluble salts in soils. In: Northeastern Regional Publication No. 493, 3rd edition, 87-94.
10. Urushadze G., Bloom V. (2014). Soils of Georgia. 332p.Tbilisi, Mtsignobari.
11. Khvedelidze R., Urushadze T., Kutateladze L., Tsiklauri N., Zakariashvili N., Aleksidze T. (2017) Amilase-producing micromycetes isolated from soils of South Caucasus. *Annals of Agrarian Science*, **15**(3): 318-321.
12. Mona S.A., Hashem A., Abd_Allah E.F., Alqarawi A.A., Soliman D.W.K., Wirth S. and Egamberdieva D. (2017) Increased resistance of drought by *Trichoderma Harzianum* fungal treatment correlates with increased secondary metabolites and proline content. *Journal of Integrative Agriculture,* **16**(8): 1751-1757.

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