International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 07 Issue 04 April 2024
DOI: 10.47191/ijcsrr/V7-i4-23, Impact Factor: 7.943
IJCSRR @ 2024
www.ijcsrr.org

Bioremediation of Saline Soils with the Consortium of Halophilic Bacteria

Shavadze Beka
Department of agrarian technologies of the faculty of Agrarian Technologies and Biosystems Engineering of Georgian Technical University, Tbilisi, Georgia.
Georgian Technical University, Guramishvili st.17, 0114, Tbilisi, Georgia

ABSTRACT: 88 strains of bacteria were isolated and purified from the naturally saline soils of Kumisi, Sagarejo and Alazani valley (Eastern Georgia). Based on the initial identification all strains belong to the genus *Bacillus*. Two strains - *Bacillus* spp. 8(3)1) and *Bacillus* spp. 7(5)4, resistant to chloride, sulfate and mixed (chloride-sulfate) salinity, as well as possessing high cellulase and amylase activities were selected as a result of successive screenings among the isolated bacteria. After the antagonism between potential members of the consortium has been excluded, the consortium of halophilic bacteria was created. Compost was made and applied to moderately saline soils using the halophilic consortium. The bioremediation of saline soils of Alazani was especially successful: the halophilic consortium has reduced the salt content in the soil by 41% during the 21 days of composting.

KEYWORDS: consortium, bioremediation, composting, halophilic bacteria, halotolerance, soil degradation

INTRODUCTION

United Nations declared soil degradation and desertification as a global problem directly related to ecosystem change, and adopted the "Convention to combat desertification". Soil salinization is one of the main causes of desertification. According to experts, approximately 20% of agricultural lands in the world are unusable for agricultural activities due to salinization which from its side is due to incorrect irrigation policy, irrational use of fertilizers, burning and other factors; moreover, this figure is constantly increasing. The forecasts are very alarming: it is estimated that by 2050, about 50% of the agricultural lands will be degraded due to salinization; this, in turn, will reduce the volume of agricultural products and aggravate the food shortage. [1, 2, 3].

The reclamation technologies, including the closed cycle technology of water use; rainfall method and sprinkler technologies are considered as the best among the measures to reduce soil salinity. However, current water and soil resource management practices have not ensured the efficient use of salt-degraded soils for agro-cultivation. All existing methods of reducing and regulating salinity - agrotechnical, irrigation-drainage or engineering - are quite time-consuming, long and expensive; therefore, the search for economically effective and ecologically safe alternative ways of restoring saline soils, in particular development of the technologies based on the use of haloresistant organisms, is becoming more and more popular [4, 5]. Plant inoculation with growth-promoting bacteria, considered as one of the cost-effective options among environmental measures, is recommended to alleviate salinity stress [5]. Particularly promising is the combined use of halophyte plants and halophilic microorganisms, which can be the basis of phytoremediation technologies for restoring salt-affected soils [3, 6].

The aim of the present study was to investigate the possibility of restoration of saline soils using a consortium of halophilic bacteria, which involved the sequential implementation of the following tasks: isolation of halophilic bacteria from saline soils, including the detection of cultures resistant to high salt concentrations, creation of a halophilic consortium with selected strains, preparation of compost using the consortium and restoration of salt-degraded soils (in laboratory conditions) by composting method.

MATERIALS AND METHODS

Isolation and identification of bacteria from saline soils was carried out using standard methods [7, 8]. Incubation of primary seedlings was carried out on Petri dishes, on nutrient agar (BioLife, Italy), at 28-30 °C in a thermostat, for three days; sterilization mode 121 ºC, 15 min. Selection of resistant to high concentrations of chloride bacterial strains was also carried out on a Petri dish, with nutrient agar medium, containing different (4% and 8%) concentrations of sodium chloride. strains resistant to sodium sulfate were selected using the similar approach; and the screening of strains resistant to mixed salinity was carried out on nutrient agar medium, containing the mix of sodium chloride (4%) and sodium sulfate (4%), in a thermostat, at 30 °C, for 3 days. Halotolerance was evaluated visually, according to the intensity of bacterial growth, with our 5-point system: 1 - reduced growth, 2 - weak growth, 3 - medium growth, 4 - good growth, 5 - abundant growth.

cellulase producers were selected on Čapek nutrient medium, containing 1% microcrystalline cellulose as the only carbon source. 50 ml of medium was poured in 250 ml Erlenmeyer flasks and was places on a shaker (180 rpm) for 4 days at 30 °C. Similar approach was used to select amylase producers with the difference that 3% starch was included as the only source of carbon in the nutrient medium. Total cellulase and α-amylase activity was determined by standard methods [9].

The relationship between the bacterial strains selected for the consortium was studied using the agar diffusion method. For this purpose, one-day cultures of bacteria were sown on nutrient agar medium containing 3days old cultures placed in the form of a "lawn" [10].

The strains selected for the biopreparation of halophilic consortium were cultivated stationary, in a thermostat at 30 °C, for 3 days, in 250 ml Erlenmeyer flasks on a 50 ml Chapek nutrient medium, where additionally was included malt sprouts - 1%; pH of the medium 5.5-5.8; sterilization mode - 121 °C, 15 min. After the cultivation was ended, the biomass of each strain was mixed in equal amounts and added 150 ml of tap water. The prepared suspension represented a consortium of selected strains.

To prepare the compost, 140 g of wheat bran was added to 150 ml of the consortium suspension and placed in a 2 L Erlenmeyer flask. "Compost" was ripening for 3 days in a thermostat at 30°C.

For the purpose of bioremediation, 150 g of saline soil was moistened and placed in a plastic container, then was added 15 g of prepared from the bacterial consortium compost. Soil sample without compost served as the control variant. The content of soluble salts in the soil was determined after Gartley (2011)[11].

RESULTS AND DISCUSSION

It is known that microorganisms resistant to high salt concentrations are mainly found in saline soils and salt lakes. Locations near Alazani Valley, Sagarejo and Kumisi Lake were selected to isolate halophilic bacteria. The mentioned areas were selected with the idea that there has been a salinization problem for a long time and the probability that the microflora adapted to these soils will be tolerant to salts was high. The second reason for investigation of these locations was the fact that due to salinization main part of these soils is less productive or completely unusable for agricultural activities. In case of successful bioremediation, these soils may be effectively used for agriculture.

Isolation of bacteria from soil samples was carried out according to the standard scheme, which meant obtaining primary inoculants and then - pure cultures. 88 isolates of bacteria were separated and purified from samples of saline soils of Eastern Georgia, as a result of the microbiological research; according to morpho-physiological characteristics (colony shape, consistency, etc., cell shape, results of gram staining, presence of spores, dependence on oxygen, growth character in liquid nutrient medium, etc.) most of them can be attributed to the genus Bacillus by the primary identification.

As the selected locations are of chloride and sulfate salinity [12], evidently, the members of the consortium of halophilic bacteria should be characterized by the resistance to this type of salinity. Therefore, at the next step of the research screening of cultures resistant to high concentrations of chloride and sulfate (8%) separately, as well as to mixed salinity (chloride 4% + sulfate 4%) has been done. The halo-resistance was evaluated by the strain's growth intensity, with our 5-point system

Screening has revealed, 11 bacterial isolates highly resistant to chloride ion: 1(6)2, 2(4)2, 2(4)4, 3(1)2, 3(5)1, 7(5)1, 7(5)4, 8(3)1,8(4)2, 8(4)7, 11(1)2. From Table 1 is clear that bacteria isolated from saline soils were more resistant to chloride ion than to sulfate. Four strains - 2(4)4, 3(1)2, 8(3)1, 7(5)4 – have demonstrated high resistance to sulfate ion; and only two ones - 8(3)1 and 7(5)4 - were distinguished by high resistance to mixed salinity. The majority of bacteria were less resistance to mixed (chloride-sulfate) salinity.

The ultimate goal of the presented study was the bioremediation of saline soils by the composting method, using a consortium of halophilic bacteria. Relatively cheap and easily metabolizable raw material - wheat bran was chosen in order to make the technology profitable. For the biotransformation of this cellulose-containing substrate, it was necessary to select halophilic strains, able to degrade the bran biopolymers. Accordingly, at the next stage of experiment, cellulase and amylase producers were screened among the halophilic strains of bacteria. Table 2 demonstrates that the selected bacterial strains were not characterized by high amylase and cellulase activity; only two cultures - (8(3)1), 7(5)4 - were identified, which revealed amylase and cellulase activities on the studied carbon source (Table 2).

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 07 Issue 04 April 2024
DOI: 10.47191/ijcsrr/V7-i4-23, Impact Factor: 7.943
IJCSRR @ 2024
www.ijcsrr.org

Table 1. Isolated from saline soils bacterial strains resistant to chloride, sulfate and mixed salinity

N	Strain number	Growth intensity				
		NaCL		Na_2SO_4		NaCL+Na_2SO_4
		4%	8%	4%	8%	8%
1	1(6)2	5	4	3	2	2
2	2(4)2	5	4	2	2	1
3	2(4)4	5	4	4	3	2
4	3(1)2	5	5	4	3	2
5	3(5)1	5	4	3	2	2
6	7(5)1	5	4	3	2	2
7	7(5)4	5	5	4	5	4
8	8(3)1	5	5	4	5	4
9	8(4)2	5	4	2	2	1
10	8(4)7	5	4	2	2	2
11	11(1)2	5	4	3	2	2

Table 2. Screening of cellulase- and amylase producing bacteria

N	Number of bacterial strain	Cellulase activity U/ml	α-amylase activity U/ml
1	1(6)2	0,05	
2	2(4)2	-	-
3	2(4)4	-	-
4	3(1)2	-	0,2
5	3(5)1	0,01	-
6	7(5)1	-	0,3
7	7(5)4	0,28	0,9
8	8(3)1	0,35	0,7
9	8(4)2	-	-
10	8(4)7	-	0,45
11	11(1)2	-	-

While selecting bacterial strains for the consortium, those halophiles with high resistance to high mixed salinity and at the same time possessing high cellulase and amylase activity were chosen (Table 2) With this approach, two strains of bacteria: 8(3)1) and 7(5)4 were selected.

Before creating a halophilic consortium, it was necessary to exclude the antagonistic relationship between the selected cultures, to avoid mutual inhibition of growth and development during the bioremediation process. Based on the standard test, an antagonistic relationship between the bacterial strains selected for the consortium was excluded. Thus, successive experiments allowed us to create a halophilic bacterial consortium.

Application of lyophilized, commercial microbial preparations is recommended in agriculture, to improve the physical characteristics of the soil, enrich it with microflora, increase its fertility, etc. In our experiments, a homogeneous concentrate-suspension of a consortium of halophilic bacteria was used to prepare compost; the last was obtained by mixing the raw biomass accumulated under the conditions of submerged cultivation of selected strains. Using this suspension, wheat bran compost was prepared, which was ripening for 3 days in a thermostat at 30 °C. Before the beginning of bioremediation, the content of soluble salts in the test soil samples was determined. The selected locations were found to be of medium salinity (electrical conductivity 2-4dsm/m), the most saline was Kumisi soil, and relatively less - Alazani one.

Bioremediation of the experimental soils was carried out in plastic containers for 21 days at room temperature in summer (end of August - beginning of September).

Bioremediation of Alazani saline soils with a bacterial consortium gave very interesting results: during 21 days of composting the halophilic consortium reduced the salt content in the soil by 41% (from 2,69 to 1,58 dsm/m. Thus, the average salinity soil became weakly saline, indicating to the effectiveness of the composting method (Fig. 1).

The remediation of Sagarejo and Kumisi soils was relatively less effective: the bacterial consortium reduced the salt content in the soil only by 22%.

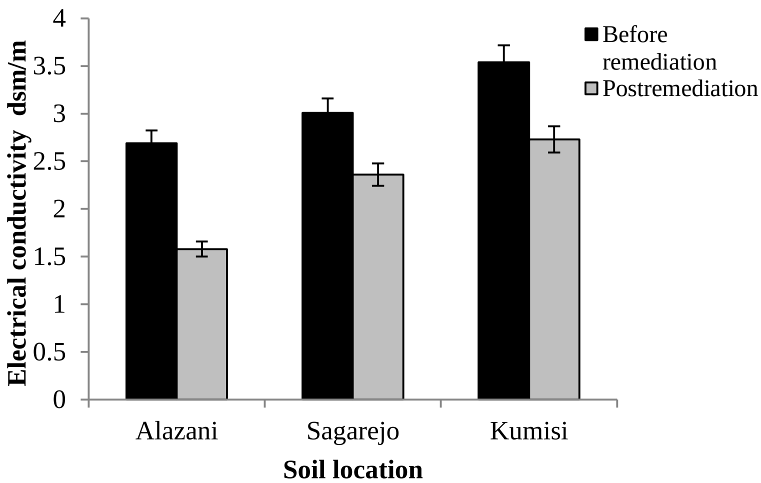

Fig. 1 Salinity degree of experimental soils before- and after remediation

The different results of bioremediation of the same (medium) salinity soils (Alazani, Kumisi, Sagarejo) clearly indicate that an individual approach is needed to restore a specific type of soil. Obviously, the “factor of the autochthonous microflora " of the soil should also be taken into account. To what extent the consortium introduced from the "outside" will "adapt" to the autochthonous microflora requires further research.

CONCLUSIONS

1. The possibility of bioremediation of salt-degraded soils using a consortium of halophylic bacteria has been established.
2. As a result of bioremediation using the composting method, the salt content in the moderately saline soil of Alazani valley was reduced by 41%.
3. The different results of bioremediation of soils with the same degree of salinity (Alazani, Kumisi, Sagarejo) clearly indicate that an individual approach is needed for the specific type soil restoration.

REFERENCES

1. Munns, R. and Tester, M. 2008 Mechanisms of Salinity Tolerance. Annual Review of Plant Biology, 59, 651-681.https://doi.org/10.1146/annurev.arplant.59.032607.092911
2. Gupta, B. and Huang, B. 2014 Mechanism of Salinity Tolerance in Plants: Physiological, Biochemical, and Molecular Characterization. International Journal of Genomics, Article ID: 701596. https://doi.org/10.1155/2014/701596
3. Arora, S. et al. (eds.). 2017 Bioremediation of Salt Affected Soils: An Indian Perspective. Springer International Publishing AG, DOI 10.1007/978-3-319- 48257-6_6
4. Qadir, M., Ghafoor, A., Murtaza, G. 2000. Amelioration strategies for saline soils. Land degradation and development, 11, 501-521.
5. Kumar, A. and Verma, J.P. 2018 Does plant—microbe interaction confer stress tolerance in plants: a review? Microbiol. Res., 207, 41–52. doi: 10.1016/j.micres.2017.11.004

6. 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
7. Gagelidze, N.A., Amiranashvili, L.L., Varsimashvili, KhI., Tinikashvili, L.M., Tolordava, L.L., Sadunishvili T.A. 2016 Selection of effective biosurfactant producers among *Bacillus* strains isolated from soils of Georgia J. Annals of Agrarian Science, 14 pp. 72-75
8. Steubing, P.M. 1993 Isolation of an unknown bacterium from soil. Proceedings of the 14th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), http://www.zoo.utoronto.ca/able/volumes/copyright.html,
9. Haki, G. and Rakshit, S. 2003 Determination of α-amylase activity was based on decreased staining value of blue starch-iodine complex. Developments in industrially important thermostable enzymes: a review. Bioresource Technology, 89(1):117–134.
10. Gritskecch E.R., Ikonnikova N.B., Buchenkov I.E., Rishkel O.S. 2017 Laboratory handbook in microbiology. Minsk, IVTs Minfin, 113p. ISBN 978-985-7142-96-5. (in Russian)
11. Gartley, K.L. 2011 Recommended Soil Testing Procedures for the Northeastern United States. Cooperative Bulletin, No. 493, chapter 10, 87-94.
12. Urushadze, T.F. and Bloom V. 2014. Soils of Georgia. Tbilisi, Mtsignobari. 332p. (in Russian).

Cite this Article: Shavadze Beka (2024). Bioremediation of Saline Soils with the Consortium of Halophilic Bacteria. International Journal of Current Science Research and Review, 7(4), 2187-2191