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Full Length Article

Research, Application Demonstration of Key Technology for Microbial Remediation of Saline-Alkali Soil

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Abstract

Land-degradation has become a serious problem for agriculture. The purpose of this research was to reclaim the saline-alkali soil, which has affected soil for plants and groundwater. Become a serious land-degradation problem. In this work, the saline soil was restored by halo-tolerant microbes. Four strains, with halo-tolerant ability, were isolated from saline soil and selected by enrichment culture and screening. Through morphological, physiological and biological characterization, the strains were separately identified as *Pseudomonas* spp., *Bacillus substilis*, *B. megaterium* and Yeast. All four strains were mixed together in the ratio of 1:1:2:2 and fermented to produce bio-organic fertilizer and used to restore the saline lands. The effect on improvement of soil fertility was investigated with growth assays of alfalfa. Result showed that these complex microbial agents could not only reduce the salt content and pH but also increase the organic content of the saline soil. Moreover, the seed germination rate, plant height and grain yield of alfalfa grew in the different treatments had different levels of increment. Overall, restoring saline soil by addition of biological organic fertilizer fermented by halo-tolerant complex agents seems to be a promising strategy for saline soil remediation with simultaneous improvements of nutrient/organic matter contents. © 2018 Friends Science Publishers

Keywords: Halo-tolerant; Biological organic fertilizer; Saline soil; Complex agents; Alfalfa

Introduction

Saline soils are widely distributed on the earth (Shi *et al.*, 2018a). It has been reported that there are about 99.13 million hectares of saline-alkaline land area in China, which are mainly distributed in different areas such as the low terrain, high underwater table, semi-humid, semi-arid and arid inland or coastal areas (Liu *et al.*, 2017a). Once the soli is degraded to bare saline-alkaline land, toxic ions become harmful to the plant survival, or inhibitory to crop growth and impede the local agricultural sustainable development (Yu *et al.*, 2018). This problem has attracted the attention of scientist all over the world. Experts have conducted studies on the degradation of saline-alkaline soil (Yu *et al.*, 2018). Wang (2017) tried different strategy for saline soil remediation by using microbiology in saline soil to change the physical, chemical and biological properties of soil.

Saline–alkaline soil exhibits a specific type of extreme habitat for the development of a halo-alkaliphilic prokaryotic community that grows luxuriantly at high electrical conductivity (EC) and alkaline pH. The arid and semi-arid areas are increased due to high salinity and alkalinity in local depressions because of more evaporative climate. In this study, an attempt was made to rehabilitate the saline soil by using halo-tolerant microbes. After isolation and screening, the selected strains were mixed together and fermented for fertilizer production. Moreover, the effectiveness of adding biological organic fertilizer to a saline soil in Weinan halogen Saline Park Beach area, near Huanghe river, China was studied over a period of five months.

Materials and Methods

Test Soil

Test soil was collected in Weinan Halogen Saline Park Beach area, Shaanxi Province, China. Soil mainly contained tidal soil and saline soil ingredients. Ionic content in the soil was more than 1%, in addition, organic matter content in the soil was low and viscosity was high, with more chloride and a few content sulphates. The major physical and chemical properties are shown in Table 1.

Design of Field Experiment

The field experiment lasted for five months. There were three treatments in the field experiment: Control group (without fertilizer or cow manure); Test group A (with cow

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manure of 10000 kg/hm^2); and, test group B (with fertilizer of 10000 kg/hm^2). Each treatment area was 50 m^2. Fifteen days after the treatment, alfalfa was planted on the disposed soil. During the Alfalfa planting, the land preparation, the watering, the weeding, the sowing and the planting density were all consistent with each other in the four treatment group. Five months later, the effect on improvement of soil fertility was investigated with seed germination, plant height and yield assays of alfalfa.

Isolation and Screening of the Halo-tolerant Strains

The halo-tolerant strain were isolated from saline-alkaine soil (Weinan, Shaanxi province, China) through enrichment (Gerhardt *et al.*, 2017) and screened in LB plate containing 20 mg /mL salinity with pH of 8.5 (Gong *et al.*, 2017). The microbes were cultured on mineral plate with 2% ionic strength and at a pH of 8.5.

Morphological and Biochemical Characteristics of Selected Strains

The morphological and microscopic characteristics of the strain were observed under a light microscope. Gram staining was determined according to the standard microbiological procedures. Spore formation was determined by malachite green staining of cells. After the strain was streak-inoculated onto the plate and incubated at 37°C for 24 h. The morphology, texture, borderline, color, and optical property of the strain's colonies were observed and recorded.

Catalase and oxidase activity assays, sugar fermentation experiment, Vogues–Proskauer (VP) test, amyl hydrolysis assay, casein hydrolysis assay, citrate utilization test, nitrate deoxidize test, salt endurance test, whether grew on NA plate (pH=5.7) or not, H_2S and indole production tests, and cellulose decomposition test were carried out using standard procedures (Kumari and Singh, 2017). All experiments were conducted in triplicate.

Mixture of Strains and Production of Bio-organic Fertilizer

After the antagonistic experiment, the four strains were mixed in different proportions. Maximum temperature and the maximum duration at the maximum temperature were noted to determine the optimum mixing up ratio. All four strains were mixed together with the optimum ratio and prepared to be complex microbial agents. Then the complex microbial agents were added into cow manure, at the rate of 3% percent of the inoculation, for fermentation and bio-organic fertilizer production.

Improvement of Saline Soil Properties

The soil pH was tested by pH meter (EA-940), soil organic matter content was measured by using proposed modification of the chromic acid titration method (Liu *et al.*, 2017b). After measuring the height of alfalfa, the alfalfa seed germination rate was calculated according to the following formula:

Alfalfa seedling germination rate %= emergence number/seeding number×100%

Statistical Analysis

All the experiments were conducted in triplicate and the data was analyzed by SPSS19.0 with the n=0.05. Data are means of three replicates; Mean within a column followed by different letters are significantly different at p=0.05 using DMRT.

Result

Isolation and Screening of the Halo-tolerant Strains in Lab

After the enrichment and screening, 12 strains were grown on LB plated containing 2% saline (Table 2). The selected strains showed different growth and halo-tolerance ability. Four strains (NUU24, NUU36, NUU56 and NUU58), whose colony size were significantly larger than other strains, were selected for further study.

Characterization of Strains

The individual and colonial morphologies of the four selected strains were observed and the results are shown in Table 3. The physiological and biochemical characterization of four strains were also carried out (Table 4). Basing on these results, combined with common bacterial identification system manual and Bergey's bacterial identification manual the strains NUU24 was identified as *B. megaterium*. Strain NUU36 was identified as *B. subtilis*, strain NUU56 was identified as *Pseudomonas sp.* strain NUU58 was identified as yeast.

Mixture of Strains and the Production of Bio-organic Fertilizer

The result of antagonistic experiment showed that there wasn't any antagonistic activity between every two strains (data not shown). The four strains were mixed in different proportions. After detecting the maximum temperature and the max duration at the maximum temperature, the optimum mixed ratio was determined. The best ratio of four strains was 1:1:2:2 to ferment and product bio-organic fertilizer, which was used in saline soil improvement (Table 5).

Result of Field Experiment

Effect of different treatments on pH and organic content of saline soil: The saline soil was separately disposed by three different treatments. The processing time lasted for

Table 1: Main physical and chemical properties of soil

Organic matter (g/kg)	Total nitrogen (g/kg)	Alka-hydr-olyzed nitrogen (mg/kg)	Available phosphorus (mg/kg)	Available Potassium (mg/kg)	Water soluble salt (g/kg)
2.21	0.66	43.25	7.68	113.70	46.76

Table 2: Isolation and screening results in the plate

Strain	Colony diameter (cm)
NUU1	1.58±0.021c
NUU5	1.72±0.032c
NUU13	1.76±0.030bc
NUU16	1.80±0.034b
NUU38	1.86±0.036b
NUU42	1.88±0.030b
NUU48	1.92±0.036b
NUU52	1.96±0.030ab
NUU24	2.10±0.022 a
NUU36	2.12±0.033 a
NUU56	2.08±0.036 a
NUU58	2.10±0.032 a

Table 3: Individual and colonial morphologies results of four strains

strains	Cell shape	Cell size	Gram stain results	Whether form spore
NUU24	rod	1.0μm×0.6 μm	+	+
NUU36	rod	1.1μm×0.8 μm	+	+
NUU56	rod	2.3μm×0.5 μm	-	-
NUU58	oval	3.3 μm	+	-

Table 4: Physiological and biochemical identification results of four strains

strain	NUU24	NUU36	NUU56	NUU58
Acid produced from glucose	+	+	+	+
Methyl red test	+	+	-	+
Voges-Proskauer test	+	+	-	+
Activity of Catalase	+	+	+	+
Activity of Oxidase	-	-	+	+
Growth on sole carbon source of citrate	+	+	+	+
Hydrolysis of Starch	+	+	-	-
Hydrolysis of Gelatin	-	+	+	-
Nitrate reduction	-	+	+	-
nitrogen source L-tyrosine	+	+	-	-
11% NaCl	+	+	+	+
Growth on 50 degrees Celsius	+	+	+	+

Note: +/- shows the physiological and biochemical identification of positive/negative reactions. +: positive; -: negative

Table 5: Compounding agents plan design

Strains Plans	NUU24	NUU36	NUU56	NUU58
Plan1	1	1	1	1
Plan2	2	1	1	1
Plan3	1	2	1	1
Plan4	1	1	2	1
Plan5	1	1	1	2
Plan6	2	2	1	1
Plan7	1	1	2	2
Plan8	1	2	2	1

150 days. The pH, total salt content and organic matter of each treatment was measured every 30 days (Table 6). Along with the increase of fertilization time and fertilization amount, the pH value, the jonic content and the organic matter changed to different degree, which was mainly characterized as follows: for the same test group, the longer treated, the better effect could be obtained; For the different group, with the addition of microbial agents, the test B was more effective than test A and control in improving the saline soil. The results indicated that: bio-organic fertilizer has better effect on reducing pH value, jonic content and organic matter of the saline soil.

Plants growth under different soil treatments: Alfalfa was planted on the disposed soil 15 d after the treatment. Twenty days after the planting, the seed germination was tested and five months later, the plant height and yield assays of alfalfa were tested. With the addition of microbial agents, alfalfa grew better in group B than group A and control (Table 7 and Fig. 1).

Discussion

Weinan Halogen Saline Park Beach area was surrounded by salt marshes in Shaanxi Province (Li *et al.*, 2017; Ma *et al.*, 2017). The climate was very dry with an excessive evaporation and little rainfall. It has brought a series of problems, whose major effects were soil salinization, water shortages, dust storms, sinking ground, poor water quality and decreased biological resources (Cao *et al.*, 2017). Soil is practically useless for agriculture and animal growth. Therefore, remediation and reclamation of the saline soil is required to solve the problem.

In this work, the saline soil was restored by halo-tolerant microbes. Four strains, with halo-tolerant ability, were isolated from saline soil and separately identified as *Pseudomonas* sp., *B. substilis*, *B. megaterium* and *Yeast* by morphological, physiological and biological characterization. After the optimum mixed ratio was determined, they were mixed together with the ratio of 1:1:2:2 and fermented in the cow manure to produce bio-organic fertilizer. The effect on improvement of soil fertility was investigated with growth assays of alfalfa. As was shown in the result, these complex microbial agents could not only reduce the salt content and pH but also increase the organic content of the saline soil. Moreover, the seed germination rate, plant height and grain yield of alfalfa was better in different treatments. The field experiment result indicated that restoring saline soil by addition of biological organic fertilizer fermented by halo-tolerant complex agents seems to be a promising strategy for saline soil remediation (Liu *et al.*, 2017a, b; Lei *et al.*, 2018; Shi *et al.*, 2018b).

Conclusion

Saline soil was reclaimed by halo-tolerant microbes. After being fermented to produce bio-organic fertilizer and used to restore the saline lands. The saline soil seemed to be restored by addition of biological organic fertilizer fermented by the halo-tolerant complex agents, which is a

Table 6: The pH, salt content and organic matter content in saline soil of different treatments

Characteristics		April 15	May15	June 15	July15	August 15
pH	CK	8.80±0.27a	8.81±0.12a	8.78±0.08a	8.79±0.13a	8.80±0.09a
	Test A	8.59±0.21b	8.45±0.08bc	8.17±0.16c	7.97±0.06cd	7.82±0.13d
	Test B	8.36±0.09bc	7.99±0.27cd	7.79±0.08d	7.56±0.11de	7.30±0.08e
Total salt (g/kg)	CK	14.36±0.02a	14.22±0.13a	14.19±0.13a	13.99±0.29a	13.99±0.26a
	Test A	12.59±0.05b	11.45±0.04b	10.40±0.47bc	9.60±0.04c	7.95±0.18d
	Test B	10.90±0.08bc	9.60±0.03c	7.92±0.09d	6.85±0.05E	6.48±0.08de
Organic matter (g/kg)	CK	11.26±0.10δ	11.22±0.20δ	11.12±0.18δ	11.20±0.18δ	11.10±0.24δ
	Test A	12.50±0.12γ	13.65±0.16γ	15.46±0.26β	15.82±0.25β	15.88±0.22β
	Test B	15.96±0.25β	18.32±0.25α	19.38±0.28α	20.66±0.22α	20.88±0.35α

Note: Different letters mean significant difference at 0.05 level, same as the table below

Table 7: Effect of different treatment on alfalfa seedling emergence, plant height and yield

Treatment	Seedlings emergence rate (%)	Plant height (cm)	Yield (kg/hm^2)
K	66.5±1.35	55.8±2.38	5577.6±133.6
Test A	78.6±1.66	68.1±1.66	7836.6±116.6
Test B	86.6±1.56	74.6±1.38	9036.8±105.8

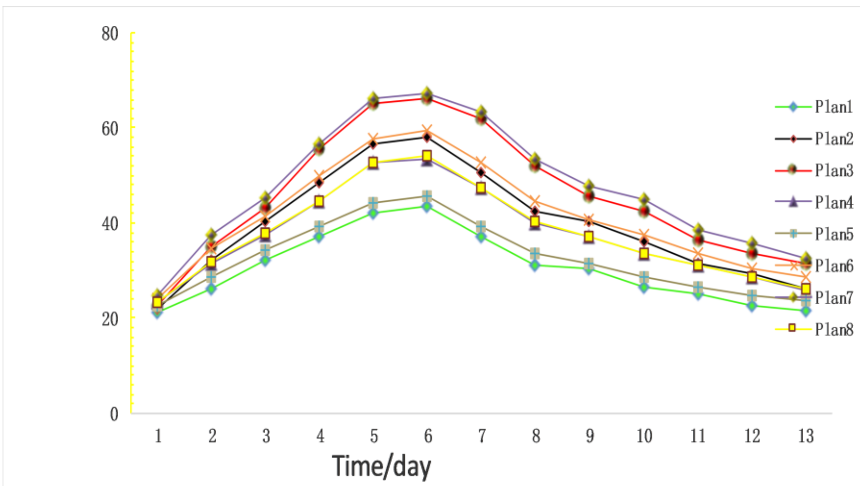

Fig. 1: Effect of different ratios of strains in complex microbial community on fermentation temperature transformation

promising strategy for saline soil remediation.

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