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Isolation and Resistance Analysis of Algae of Alkali Spot Soil

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Abstract Algae can survive in a very wide range environment and have high adaptability for a variety of adversity. In present study, we selected alkali spot soil in Anda City, Heilongjiang Province as the algae research plots. In addition, we selected garden nursery soil in Northeast Forestry University as the control. With streak plating method, we isolated 30 algae species eventually, 29 of which had been purified. After resistance screening of 29 algae species by NaCl stress and $NaHCO_3$ stress, we found their growth conditions were different from each other at different stress or different stress concentration gradient. Algae species of alkali spot soil showed stronger resistance than those of nursery soil. Moreover, some algae were strongly inhibited by lower concentration of sodium bicarbonate, but some were promoted.

Keywords Alkali spot soil; Soil algae; Salt-stress; Isolation and identification; Resistance

Background

Algae can survive in a very wide range environment, they can be found whether in soils, rocks and caves, or in permanent snow and ice fields, even on living animals and plants, algae are almost everywhere (Hoffmann, 1989). Soil habitats are the most important non-aqueous ecosystems for algae (Zenova et al., 1995). Soil algae include the algae living in water-terrestrial, true-terrestrial, native, hidden and algae on rock (Metting, 1981).

Since 1990s, more and more scientists are doing research on the ecology and application of soil algae; (Mazor et al., 1996); Current research focus on the use of soil algae as bio-fertilizer, pesticides testing organism and the research on soil improvement and desertification control (Whitton and Potts, 2000); In addition, in order to explore the possibility of life beyond the earth and the origin of life, polar ecology and the resistance mechanisms of pioneer algae species are conspicuous, and have made outstanding achievements (Scherer et al., 1988; Davey and Rothery, 1993; Young and Fuank, 1996; Gorton et al., 2001; Holzinger and Lütz, 2006; Remias et al., 2010; Tanabe et al., 2011).

Although there are many studies on soil algae (Johansen, 1993; Sukala and Davis, 1994; Tsujimura et al., 2000; Lukešová, 2001; Neustupa, 2001; Zancan, 2006; El-Gamal et al., 2008), the studies on the diversity and resistance of algae of alkali spot soil in northeast China are few. The salinity volume of the saline soil in Anda City, Heilongjiang Province, is 0.3% to 1.5%, and the soil pH is around 10, and the dominate soil soluble salt are bicarbonate and carbonate. Because of the high soluble salt content, general biology can hardly survive. However, some algae are able to grow and multiply in such environment. Algae have potential resistance-related genetic resources, the aim of this study on the isolation and resistance-identification of the algae of alkali spot soil was to filter out the algae of high resistance, which is the foundation of the screening of the salinity-related genes.

1 Results and Analysis

1.1 The algae species from identification and purification

We isolated 30 algae species eventually, 22 of which were isolated from alkali spot soil, recorded as

JB1~JB22 in turn. 21 algal species have been purified except JB12; 8 algae species were isolated from garden nursery soil, recorded as CK1~CK8 in turn.

Most of the 30 algae species are single cell type, but there are also filaments type (JB9), group type (such as JB22); Cell morphology varied a lot, for example, globe (such as CK5), ellipse (eg., CK1), ovate (such as CK3), cylindrical (JB9), drum-type (JB10), spindle (JB5), crescent (CK2); Some algae species had significant flagella (eg., JB2, JB4, JB7, CK8); Most of them are green, but some are yellow-green (JB9 and JB10), brown (JB13, JB18), blue-green (JB15). Morphological identification of algae is a long-term and pain staking work, therefore, the algae species were just indicated by numbers in this study.

1.2 Resistance analysis of algae

In order to study the resistance of algal, 29 algae species (except JB12) purified were cultured in BBM solid medium with different concentrations of NaCl and $NaHCO_3$.

In the blank medium and the BBM medium with 50 mmol/L NaCl stress, all of the algae could grow; When the concentration of NaCl was 550 mmol/L, algae species were subject to suppression and could not grow except CK1, CK2, JB6, JB9, JB14, JB16, JB17, JB19; When in 1 mol/L NaCl, only JB6 and JB19 could survival (Figure 1).

In 100 mmol/L $NaHCO_3$, the control algae species were suppressed seriously except CK2, while those of alkali spot soil tended to raise except JB2, JB5, JB11, JB21, JB22; But in 200 mmol/L $NaHCO_3$, CK2 was inhibited, while the others changed little; Under 400 mmol/L $NaHCO_3$ stress, CK1 and CK2 turned yellow, show- ing mortality trends, JB6, JB19 showed strongest resistance, followed by JB13, JB14, JB16 and JB17 (Figure 2).

In conclusion, JB19 and JB6 showed strongest resistance to both NaCl stress and $NaHCO_3$ stress, which could tolerate 1 mol/L NaCl stress and more than 400 mmol/L $NaHCO_3$ stress, while the control algae species CK1 and CK2 could only tolerate 550 mmol/L NaCl stress and 200 mmol/L $NaHCO_3$ stress.

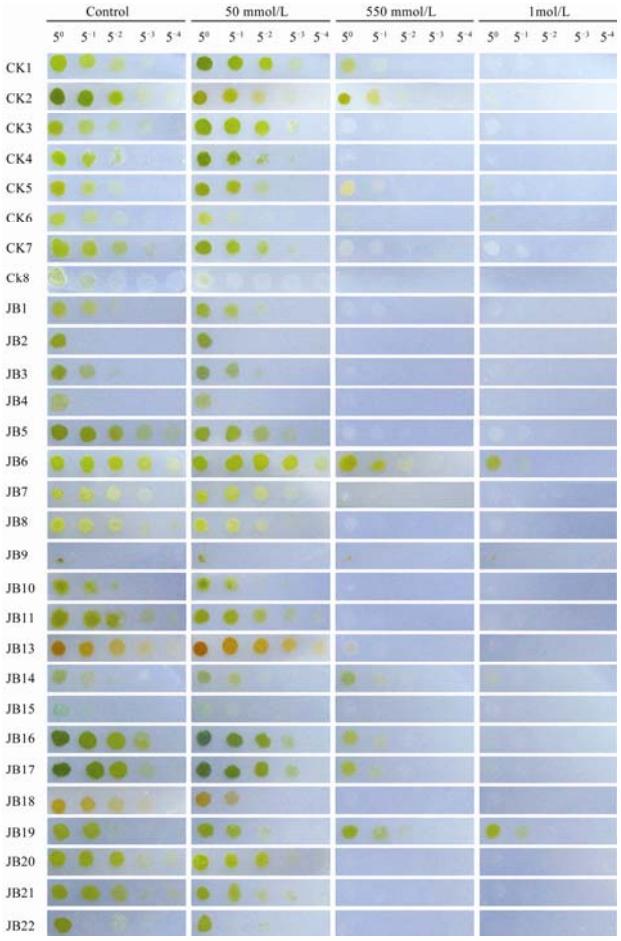

Figure 1 Analysis of algae expressed in different NaCl stresses
Note: Algae species included JB1~JB22 (except JB12) and CK1~CK8, incubated as described in Materials and Methods; Serial dilutions were spotted onto solid BBM medium supplemented without or with additional NaCl (50 mmol/L, 550 mmol/L and 1 mol/L); Growth was observed one week later

2 Conclusions

(1) We isolated 30 algae species eventually, 22 of which were isolated from alkali spot soil in northeast China, and 8 algae species from garden nursery soil. 29 algae species had been purified.

(2) After resistance screening of 29 algae species by NaCl stress and $NaHCO_3$ stress, we found their growth conditions were different from each other at different stress or different stress concentration gradient. Algae species of alkali spot soil showed stronger resistance than those of nursery soil.

(3) Some algae were strongly inhibited by lower concentration of sodium bicarbonate, but some were promoted.

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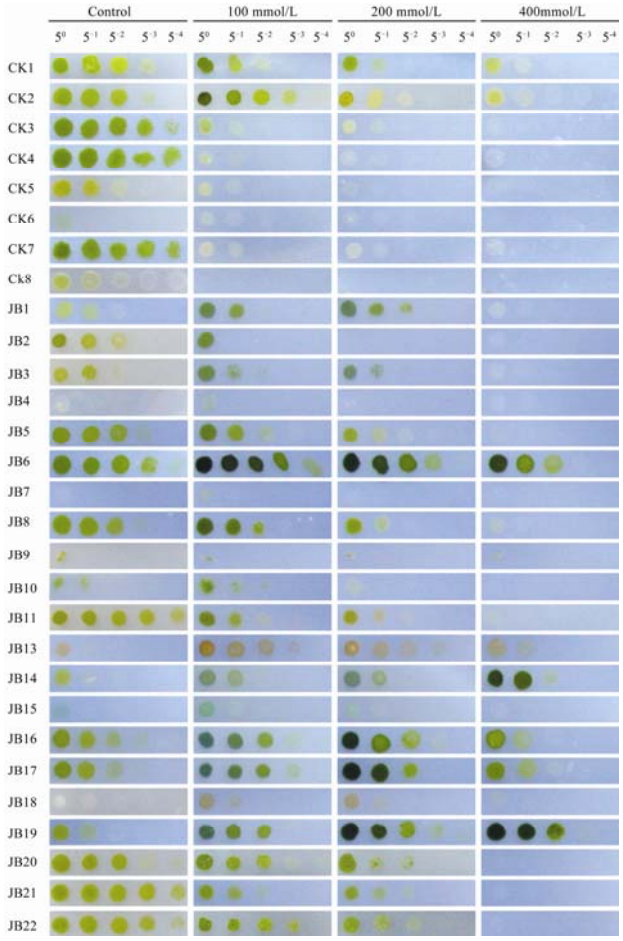

Figure 2 Analysis of algae expressed in different $NaHCO_3$ stresses

Note: Algae species included JB1~JB22 (except JB12) and CK1~CK8, incubated as described in Materials and Methods. Serial dilutions were spotted onto solid BBM medium supplemented without or with additional $NaHCO_3$ (100 mmol/L, 200 mmol/L and 400 mmol/L). Growth was observed one week later.

The isolation and screening of special resistance-related algae species as the foundation of resistance-related genes have an important theoretical and practical significance on soil salinity remediation, ecological restoration and the promotion of resistance-related genetic engineering of plants.

3 Materials and Methods

3.1 Materials

Soil samples were collected from alkali spot soil in May and September, 2009, in Anda area, Heilongjiang Province, In addition, we selected garden nursery soil in Northeast Forestry University as the control.

Bold's Basal Medium (BBM): $NaNO_3$ 0.250 g, NaCl 0.025 g, K_2HPO_4 0.075 g, KH_2PO_4 0.175 g, H_3BO_3 0.011 g, EDTA 0.050 g, KOH 0.031 g, $Co(NO_3)_2{\cdot}6H_2O$ 0.490 mg, $CuSO_4$ 1 mg, $MnCl_2{\cdot}4H_2O$ 1.440 mg, $ZnSO_4{\cdot}7H_2O$ 8.820 mg , MoO_3 0.710 mg, $MgSO_4{\cdot}7H_2O$ 0.075 g, $CaCl_2{\cdot}2H_2O$ 0.025 g, $FeSO_4{\cdot}7H_2O$ 5 mg, distilled water 1 L, and the pH of the medium adjusted to 8.0.

3.2 Methods

3.2.1 Identification and purification of the algae

5 g of soil was weighed and placed in 150 mL Erlenmeyer flask containing 50 mL sterile water, and then incubated in the culture room, the light intensity is 4 000~6 000 lux, photoperiod is 14 h: 10 h, 24℃. When the algae appeared, we picked a fluid with a inoculating loop, and then streaked on the BBM solid medium until individual colonies grown on the plates, and single colonies were purified more than 8 times.

3.2.2 Resistance analysis of algae

Single colonies of algae species purified was picked into liquid BBM medium, when the algae solution was dense, took some vigorous solution, centrifuged, abandoned the supernatant, then added a certain amount of sterile deionized water, make sure the initial of the cell concentration was 100 mg/mL, recorded as 5^{-0}, and then diluted to 5^{-1}, 5^{-2}, 5^{-3}, 5^{-4} in turn. 4 μL of serial dilutions (JB9, which is filament-tous, was only picked filaments on the solid medium with no concentration gradient) were spotted onto solid BBM medium supplemented without or with additional NaCl (BBM medium whose pH was adjusted to 8.0 as the basic culture and the control medium) and $NaHCO_3$ (BBM medium whose pH was not adjusted as the basic culture and the control medium). Growth was observed one week later.

Authors' contributions

JW and WS designed and conducted this experiment; TT participated the experiment design and data analysis; SKL is the person who takes charge of this project, including experiment design, data analysis, writing and modifying of the manuscript. All authors have read and approved the final manuscript.

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References

Davey M.C., and Rothery P., 1993, Primary colonization by microalgae in relation to spatial variation in edaphic factors on Antarctic fell-field soils, Journal of Ecology, 81: 335-343 doi:10.2307/2261503

El-Gamal A.D., Ghanem N.A.E., El-Ayouty E.Y., and Shehata E.F., 2008, Studies on soil algal flora in Kafr El-Sheikh Governorate, Egypt, Egyptian J. of Phycol., 9: 1-23

Gorton H.L., Williams W.E., and Vogelmann T.C., 2001, The light environment and cellular optics of the snow alga Chlamydomonas nivalis (Bauer) Wille, Photochemistry and Photobiology, 73(6): 611-620 doi:10.1562/0031-8655(2001)073<0611:TLEACO>2.0.CO;2

Hoffmann, L., 1989, Algae of terrestrial habitats, The Botanical Review, 55(2): 77-105 doi:10.1007/BF02858529

Holzinger A. and Lütz C., 2006, Algae and UV irradiation: Effects on ultrastructure and related metabolic functions, Micron (Oxford England: 1993), 37(3): 190-207

Johansen J.R., 1993, Cryptogamic crusts of semiarid and arid lands of north America, Journal of Phycology, 29(2): 140-147 doi:10.1111/j.0022-3646.1993.00140.x

Lukešová A., 2001, Soil algae in brown coal and lignite post-mining areas in central Europe (Czech Republic and Germany), Restoration Ecology, 9(4): 341-350 doi:10.1046/j.1526-100X.2001.94002.x

Mazor G., Kidron G.J., Vonshak A., and Abeliovich A., 1996, The role of cyanobacterial exopolysaccharides in structuring desert microbial crusts, FEMS Microbiology Ecology, 21(2): 121-130 doi:10.1111/j.1574-6941.1996.tb00339.x

Metting B., 1981, The systematics and ecology of soil algae, The Botanical Review, 47(2): 195-312 doi:10.1007/BF02868854

Neustupa J., 2001, Soil algae from marlstone-substratum based biotopes in the Nature park Džbán (Central Bohemia, Czech Republic) with special attention to the natural treeless localities, Algological Stud., 101: 109-120

Remias D., Karsten U., Lütz C., and Leya T., 2010, Physiological and morphological processes in the Alpine snow alga Chloromonas nivalis (Chlorophyceae) during cyst formation, Protoplasma, 243(1-4): 73-86 doi:10.1007/s00709-010-0123-y PMid:20229328

Scherer S., Chen T.W., and Böger P., 1988, A new UV-A/B protecting pigment in the terrestrial cyanobacterium Nostoc commune, Plant Physiol., 88(4): 1055-1057 doi:10.1104/pp.88.4.1055 PMid:16666420 PMCid:1055714

Sukala B.L., and Davis J.S., 1994, Algae from nonfertilized soils and from soils treated with fertilizers and lime of northcentral Florida, Nova Hedwigia, 59(1-2): 33-46

Tanabe Y., Shitara T., Kashino Y., Hara Y., and Kudoh S., 2011, Utilizing the effective xanthophyll cycle for blooming of Ochromonas smithii and O. itoi (Chrysophyceae) on the snow surface, PLoS One, 6(2): e14690 doi:10.1371/journal.pone.0014690 PMid:21373183 PMCid:3044130

Tsujimura S., Nakahara H., and Ishida N., 2000, Estimation of soil algal biomass in salinized irrigation land: a comparison of culture dilution and chlorophyll a extraction methods, Journal of Applied Phycology, 12(1): 1-8 doi:10.1023/A:1008126232188

Whitton B.A., and Potts M., eds, 2000, The ecology of cyanobacteria: Their diversity in time and space, Dordrecht, Netherlands, Kluwer Academic Publishers, 1-504

Young A.J., and Fuank H.A., 1996, Energy transfer reactions involving carotenoids: Quenching of chlorophyll fluorescence, Journal of Photochemistry and Photobiology B, Biology, 36(1): 3-15 doi:10.1016/S1011-1344(96)07397-6

Zancan S., Trevisan R., and Paoletti M.G., 2006, Soil algae composition under different agro-ecosystems in North-Eastern Italy, Agriculture, Ecosystems and Environment, l12(1): 1-12 doi:10.1016/j.agee.2005. 06.018

Zenova G.M., Shtina E.A., Dedysh S.N., Glagoleva O.B., Likhacheva A.A., and Gracheva T.A., 1995, Ecological relations of algae in biocenoses, Microbiology, 64(2): 121-133

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