



Classification and Salt-tolerance of Actinomycetes in the Qinghai Lake Water and Lakeside Saline Soil

Yan Cai

College of Resources and Environment
Northwest Sci-Tech University of Agriculture & Forestry
Yangling 712100, China

College of Resources and Environment
Sichuan Agriculture University
Ya'an 625014, China
Tel: 86-835-2885-776 E-mail: caiyya@126.com

Quanhong Xue (Correspond author)

College of Resources and Environment
Northwest Sci-Tech University of Agriculture and Forestry
Yangling 712100, China
Tel: 86-29-8709-1321 E-mail: xueqhong@public.xa.sn.cn

Zhanquan Chen & Rong Zhang

Institute of Soil and Fertilizer
Qinghai Academy of Agriculture and Forestry
Xining 810016, China

Abstract

Mesophilic and cryogenic actinomycetes were isolated from water, quagmire soil, lakeside soil and saline wasteland soil in the Qinghai Lake by spreading samples on three kinds of agar media, in which content of NaCl was 70 g/L. They were characterized according to routine methods, and detected their salt-tolerance. The results indicated that there were salt-tolerant, mesophilic and cryogenic actinomycetes in quagmire and lakeside soil of the Qinghai Lake and in saline wasteland soil nearby the Qinghai Lake. The amount in wasteland soil was the largest, followed by lakeside and swamp soil. All of the salt-tolerant actinomycetes identified were *Streptomyces* (92.6 % of total), and they were all albsporus. And 47.6 %, 19.0 %, and 33.3 % of the tested actinomycetes could tolerate NaCl concentration at 70 g/L, 100 g/L, and 150 g/L respectively.

Keywords: Actinomycetes distribution, Salt-tolerant, Qinghai Lake

Actinomycetes is an important class of microbial resources, they are important producers of antibiotics and other important bioactive substances. So far, about two-thirds of the world's antibiotics were secreted by actinomycetes (Liu Zh. H., 2002; Liu Zh. H. and Jiang Ch. L., 2004). As the strains with biological activity were found in large numbers, we separated active actinomycete strains more and more difficult from conventional environment (Jiang Ch. L. and Xu L. H., 1997). So that the efficiency of the discovery of new compounds reduced. Halophilic or salt-tolerant actinomycetes are a kind of extreme environment actinomycetes, which are increasingly concerned around the world as research materials of microbial physiology from adverse circumstance. At present, Mikami Y. (1982), Sato M. *et al.* (1983) Mikami Y. *et al.* (1985) put on a certain studies of Alkaliphilic and alkali-tolerant actinomycetes and found several antibiotics and enzyme. Wang D. Zh. (1989), Wang L. F. *et al.* (1993), Liu D. R. *et al.* (1998) and Liu T. H. *et al.* (1999) also conducted a preliminary study on actinomycetes in saline-alkali soil, but study on the plateau halophilic or salt-tolerant actinomycetes reported very few. This article had conducted the preliminary study on the actinomycetes in Qinghai Lake and lakeside saline wasteland soil, where altitude was 3 200 meter. The purpose was to explore the

actinomycete ecological distribution characteristics in extreme environment of plateau saline soil, and provide a scientific basis for research, use of the plateau extreme environmental actinomycete resources and protection of ecological environment.

1. Materials and Methods

1.1 Overview of sampling point

Qinghai Lake is in the Qinghai-Tibet Plateau, where the altitude is 3 200 meter and the average annual temperature is around 0 °C. It is the natural closure of inland water, and rich in salts material. As the soil salinization, lakeside vegetation is sparse, and mostly *wormwood grass*, *ice grass* and *Chinese small iris* and other herbs. pH and salt content of samples analyzed by conventional methods. Table 1 is sampling point overview and samples basic properties.

<<Table 1 Basic properties of samples and overview of sampling point

1.2 Separation, counting and purification>>

Dilution plate spreading method was used to separate and count (Cheng L. J. and Xue Q. H., 2000). Separation culture medium were starch casein agar, glycerol asparagine agar and starch ammonium agar, which all added 70 g / L NaCl in particular (Ruan J. Sh. 1992, Cheng L. J. and Xue Q. H. 2000). The growth temperature and time respectively were 4 °C for 3 months and 28 °C for 1 month. Actinomycetes separated were preserved on the corresponding incline after purified by plate line method.

1.3 Actinomycete identification

According to the shape and the culture characteristics (Ruan J. Sh., 1992), Actinomycetes were identified to genus. Mycelium shape was observed by buried glass method, and culture characteristics was observed by Gao 1 agar culture medium.

1.4 Determination of actinomycete salt-tolerance

Purified actinomycetes were inoculated on medium, in which NaCl concentrations were 0, 50, 70, 100, 150, 180, 200, 250 and 300 g / L respectively, and observed their growth conditions. The highest NaCl concentration that the strain could tolerant expressed the strain salt-tolerance.

2. Results and analysis.

2.1 Number of actinomycetes in lakeside soil and lake quagmire

Although the plateau ecological environment had cold characteristics in general, but numbers of mesophilic actinomycetes in four samples were still far higher than those of cryogenic actinomycetes (from table 2). Average number of actinomycetes from saline wasteland soil was the maximum, whose mesophilic actinomycetes were 1.86×10^6 cfu/g (at 28 °C) and cryogenic actinomycetes were 8.55×10^4 cfu/g (at 4 °C), followed by lakeside soil and quagmire, whose mesophilic actinomycetes were respectively 1.03×10^3 cfu/g and 2.75×10^2 cfu/g. Number of mesophilic actinomycetes in saline wasteland soil was 1 805 times higher than that in lakeside soil, and was 6 763 times higher than that in quagmire. At 4 °C and 28 °C culture conditions, Water of Qinghai Lake were not isolated salt-tolerant actinomycete. You C.F. (1986) believed that actinomycetes liked dry environment, which was possibly an important reason for no actinomycete in water of Qinghai Lake. This study did not isolated cryogenic actinomycetes, in addition to saline wasteland soil.

<<Table 2 Number of actinomycetes in lakeside soil, quagmire, water of Qinghai Lake and saline wasteland soil in Qinghai Lake township>>

2.2 Classification and composition of actinomycetes in lakeside soil, quagmire and saline wasteland soil

According to the classification principle of actinomycete, the 27 representative actinomycete strains, which had been separated and purified, were identified and classified. The results were as follows: 25 strains were identified as *Streptomyces*, and all were albosporus, which were 92. 6% of the representative strains. 2 strains of actinomycete had no sporulation structure in the test medium and were unable to determine their classification status, which were 7.4 % of the representative strains.

So the actinomycete composition of lakeside soil and quagmire of Qinghai Lake and saline wasteland soil in Qinghai Lake township were all sole, that just tallied with their alpine, cold, and high-salt extreme environment. Liu D. R. and Li X. H. (1998) studied on actinomycetes of salt lake in Yuncheng in Shanxi, and included the separated strains in 1 familia and 4 genus, and classified *Streptomyces* into 11 groups. Lv Zh. T., Zhang L. P., Li Y. H. *et al.* (2006) studied salty samples from Cangzhou city Hebei province. They isolated 33 moderately halophilic actinomycetes strains and 13 halotolerant actinomycetes strains, which belonged to *Nocardiopsis*, *Streptomyces* and a novel genus of Cellulomonadaceae. The actinomycete composition of lakeside soil, saline wasteland soil and quagmire of Qinghai Lake were far simpler than other places, which noted that actinomycete composition in saline soil of plateau ecosystems was different with that of low elevation area. Li W. J., Tang Sh. K., Wang D. *et al.* (2004) studied biodiversity of

moderately halophilic actinomycetes in Xinjiang and Qinghai area, and also found that the variety of halophilic actinomycetes from Xinjiang was much higher than that from Qinghai area.

2.3 Salt-tolerance of actinomycetes in lakeside soil, quagmire and saline wasteland soil

Salt-tolerance of 21 actinomycete strains were determined, and the results were as follows: 10 strains could grow on the 70 g / L NaCl medium, which were 47.6% of the tested strains and scheduled for mild salt-tolerant actinomycetes tentatively; 4 strains could grow on the 100 g / L NaCl medium, which were 19.0% of the tested strains and scheduled for moderate salt-tolerant actinomycetes tentatively; 7 strains could grow on the 150 g / L NaCl medium, which were 33.3 % of the tested strains and scheduled for mild halophilic actinomycetes.

Wang D. Zh.(1989), Liu D. R. and Li X. H. (1998) believed that the growth of extreme halophilic bacteria requested a high concentration of NaCl (at least 120 g / L), and the optimal NaCl concentration was 200 ~ 250 g / L, and even in saturated NaCl solution they could grow. Optimal growth salinity of the 21 tested actinomycete strains was 0 ~ 70 g / L. 7 tested actinomycete strains could endure 150 g / L NaCl and called mild halophilic actinomycete. To sum up, certain amount of salt-tolerant actinomycetes and mild halophilic actinomycete were in lakeside soil, quagmire and saline wasteland soil of Qinghai Lake.

3. Conclusions

There were some mesophilic and cryogenic actinomycetes in lakeside soil, quagmire of Qinghai Lake and saline wasteland soil of Qinghai Lake township; Actinomycetes were not detected in Water sample of lake. the salt-tolerance actinomycetes identified were all *Streptomyces*. 47.6 %, 19.0 % and 33.3% of the tested actinomycetes could tolerate the highest NaCl 70 g / L, 100 g / L and 150 g / L respectively. Certain amount of salt-tolerant actinomycetes and mild halophilic actinomycetes were in lakeside soil, quagmire and saline wasteland soil of Qinghai Lake.

4. Discussion

In this paper, isolated identified actinomycetes were all *Streptomyces* by conventional separation culture medium, and quantity of rare actinomycetes (other actinomycetes in addition to *Streptomyces*) was small or none. For decades, researchers found that rare actinomycetes produced many bioactive substances, including antibiotics (erythromycin, rifampicin, extremadura, foreign erythromycin and so on), enzymes, vitamins and so on. Some antibiotics have been commercialized, and resulted in tremendous social value and economic value (Jiang Ch. L. and Xu L. H.,1997; Liu Zh. H. ,2002; Liu Zh. H. and Jiang Ch. L.,2004). Isolation of rare actinomycetes is the first and the most important step of actinomycetes resource development. Isolation and Screening of rare actinomycetes should choose a suitable medium in addition to choice of special ecological environment as the separation of outside sources. Tang Sh. K., Jiang Y., Zhi X. Y. *et al.*(2007) thought that separation medium fitting for halophilic actinomycetes was improved ISP5 medium, starch ammonium medium, modified starch ammonium medium. They also thought that we must give full consideration to micro-environment of medium, and simulate salt environmental conditions (including a certain degree of concentration, a certain proportion of complex salt, the special nutrition needs and so on) as far as possible. Only in this way it would be possible to isolate more number of rare actinomycetes.

References

- Cheng L. J., Xue Q. H. (2000). *Microbiology Experiment Technology*. Publisher: World Books Publication Company, pp: 80-83, 104-105 (in Chinese).
- Jiang Ch. L., Xu L. H.(1997). *Microbial Resources*. Publisher: Science Press, pp:104-200 (in Chinese).
- Liu Zh. H.. (2002). *Modern Microbiology*. Publisher: Science Press, pp:3-35 (in Chinese).
- Liu Zh. H., Jiang Ch. L..(2004). *Modern Biology and Technology of Actinomycetes*. Publisher: Science Press, pp:10-50 (in Chinese).
- Li W. J., Tang Sh. K., Wang D. *et al.* (2004). A primary study on biodiversity of moderately halophilic actinomycetes in Xinjiang and Qinghai area. *Acta Microbiol. Sinica*, 44(1):1-7.(in Chinese).
- Liu D. R., Li X. H. (1998). Studies on actinomycetes population from saltlake of Yuncheng in Shanxi. *Acta Microbiol. Sinica*, 38(2):137-141. (in Chinese).
- Liu T. H., Zhou P. J.. (1999). Halophilic microbes. *Microbiology*. 26(3):232. (in Chinese).
- Lv Zh. T., Zhang L. P., Li Y. H. *et al.* (2006). Biodiversity of halophilic actinomycetes of Cangzhou salty environments. *Journal of Hebei University (Natural Science Edition)*, 26(1):1-6.
- Mikami Y., Miyashita K., Arai T.. (1982). Diaminopimelic acid profiles of alkaliphilic and alkaline-resistant strains of actinomycetes. *J. Gen. Microbiol*, 12(8):1 709-1 712.
- Mikami Y.. (1985- 1986). Alkaliphilic actinomycetes. *The Actinomycetes*. 19 :176 - 191.
- Ruan J. Sh.. (1992). Foundation of Actinomycete Classification. Publisher: Science Press, pp:18-109 (in Chinese).

Sato M., Beppu T., Arima K. (1983). Studies on antibiotics produced at high alkaline pH. *Agr. Biol. Chem*, 47(9): 2 019 -2 027.

Tang Sh. K., Jiang Y., Zhi X. Y.*et al.* (2007). Separation method of halophilic actinomycetes. *Microbiology*. 34(2): 390-392. (in Chinese).

You C. F.. (1986). Actinomycete of soil microorganisms. *Soil Science Progress*, (6): 1-5 (in Chinese).

Wang D. Zh.. (1989). Extreme environment microbes and application.*Chinese Journal of Applied Ecology*, 6(2):77 - 81. (in Chinese).

Wang L. F., Song Sh. Zh., Ruan J. Sh.. (1993). A study on classification of actinomycetes from saline-alkali soils. *Acta Microbiol. Sinica*, 33(6):393 - 399. (in Chinese).

Table 1. Basic properties of samples and overview of sampling point

| No. of samples | Sampling point | Altitude (m) | pH | Salt content (g·kg ⁻¹) | Vegetation |
|----------------|---|--------------|------|------------------------------------|---|
| 15-1 | Lakeside of Qinghai Lake | 3 230 | 9.38 | 1.90 | <i>wormwood grass</i> |
| 15-2 | Quagmire(0-20cm) of Qinghai Lake | 3 230 | 9.02 | 5.44 | |
| 15-3 | Water of Qinghai Lake | 3 230 | 9.02 | 4.33 | |
| 17 | Saline wasteland in Qinghai Lake township | 3 081 | 8.62 | 19.6 | <i>ice grass</i> , <i>Chinese small iris</i> |

Table 2. Number of actinomycetes in lakeside soil, quagmire, water of Qinghai Lake and saline wasteland soil in Qinghai Lake township(cfu/g)

| | No. of sample | Starch casein agar | Starch ammonium | Glycerol asparagine agar | Average |
|--|---------------|------------------------|------------------------|--------------------------|------------------------|
| Number of mesophilic actinomycete (at 28 °C) | 15-1 | 1. 82 ×10 ³ | 1. 01 ×10 ³ | 2. 70 ×10 ² | 1. 03 ×10 ³ |
| | 15-2 | 4. 12 ×10 ² | 1. 37 ×10 ² | 2. 75 ×10 ² | 2. 75 ×10 ² |
| | 15-3 | 0 | 0 | 0 | 0 |
| | 17 | 1. 38 ×10 ⁶ | 2. 02 ×10 ⁶ | 2. 18 ×10 ⁶ | 1. 86 ×10 ⁶ |
| Number of cryogenic actinomycete (at 4 °C) | 15-1 | 0 | 0 | 0 | 0 |
| | 15-2 | 0 | 0 | 0 | 0 |
| | 15-3 | 0 | 0 | 0 | 0 |
| | 17 | 1. 20 ×10 ⁵ | 5. 81 ×10 ⁴ | 7. 84 ×10 ⁴ | 8. 55 ×10 ⁴ |