盐度对稀释平板法研究红树林区土壤微生物数量的影响

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摘要:在使用稀释平板法分离潮间带红树林及其对照光滩土壤微生物以及计数时,多数情况下使用陈海水制作培养基和稀释水,很少考虑培养基和稀释水的盐度对最终计数结果的影响。使用稀释平板法研究了盐度对福建九龙江口红树林区与深圳福田红树林保护区土壤微生物平板计数的影响,结果表明培养基与稀释水盐度对微生物数量有明显的影响。统计分析显示细菌的海水稀释效果优于淡水,而放线菌与真菌则刚好相反($P<0.05$,一个例外)。海水不适合配制红树林区土壤微生物平板计数的培养基,从 0 ~ 35,高盐度的平板培养基会降低微生物的数量,尤其是放线菌的数量,尽管培养基的盐度对真菌影响无规律,但细菌数量在低盐度时比在高盐度和不加氯化钠时要多。根据盐度效应,提出了稀释平板技术应用于潮间带的红树林及其相应光滩时的优化方法,认为细菌应该用海水作无菌稀释水,而放线菌和真菌则应用淡水作稀释水;包括光滩在内的红树林区土壤微生物分离与计数的培养基宜控制较低盐度范围。

关键词:盐度; 微生物数量; 土壤; 红树林; 潮间带
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Effect of salinity on microbial densities of soil in the dilution plate technique applied in mangrove areas

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Abstract: When the soil microbial densities are determined in mangroves and corresponding mudflat at the same tidal level by the dilution plate method, the agar media and dilution water are generally made up of aged seawater in most cases, and effects of salinity in agar media and dilution water on the enumeration of microbes is seldom taken into consideration. The effects of salinity on soil microbial counting from the samples in mangrove areas in Jiulongjiang Estuary of Fujian, and Futian Mangrove Nature Reserve of Shenzhen, China, were tested by dilution plate technique. The results showed that the soil microbial densities in mangroves and mudflat were significantly influenced by the salinity of dilution water and agar

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media. For the bacteria, the seawater served as sterilized dilution water was significantly ($P < 0.05$) more benefic to the enumeration on the plates than the freshwater, but in reverse for the actinomycetes and fungi. The increasing salinity of media within 35 significantly decreased microbial colonies on the plates, especially for the actinomycetes, in spite of the fact that the effect of salinity of media on fungal numbers was not indefinite. The bacterial colonies were more abundant on the agar plates with low salinity than with high salinity or without any NaCl. It was proposed that some methodological improvements were needed when the dilution plate technique was applied to microbial counting in the samples of mangrove forest and mudflat at the same tidal level in inter-tidal zone. The sterilized dilution water should be prepared with seawater for the bacteria, but with freshwater or low saline water for the actinomycetes and fungi. The salinity of agar media should be low for the microbial isolation and enumeration of soil samples from the mangrove areas including mudflats.

Key Words: salinity; microbial densities; soil; mangroves; inter-tidal zone

1 Introduction

Mangroves are woody plant communities in the inter-tidal zone[1]. Mangroves and their corresponding mudflat occupy a littoral habitat, characterized almost invariably by salt or brackish water and coastal silt. The salinity is one of the most important ecological factors in this coastal wetland, which is significantly different from other aquatic environments. Many researchers have addressed the effect of salinity in mangrove ecosystem[2-4]. Beyond all doubt, the microbes in this habitat must be able to tolerate the conditions characteristic in this kind of special ecosystem. The salinity influences generation time, morphological and physiological characters of bacteria and fungi in aquatic saline habitat[5]. Similarly, microbial distribution is easily influenced by the habitat salinity in mangrove ecosystem[6]. A study from Leano et al[7] revealed that three isolates from mangroves identified as *Halophytophthora vesicular* grew at a salinity range of 0 — 60 normally, but vigorously at 15 — 25 and the optimum salinity levels for sporulation were different among them. Nakagiri and Ito[8] also reported that the ascospore appendages formed by *Aniptodera salsuginosa* isolated from mangroves were functional only when they were submerged in brackish water. Moreover, there was a gradual decrease in the populations of soil nitrogen-transforming microorganisms in Bhitarkanika mangrove forest due to deforestation and adverse effect of increased salinity in deforested land and salty land respectively[9]. It is obvious that the salinity is an extensive ecological factor controlling microbes in mangrove ecosystem.

The dilution plate method was, whether for isolation or enumeration of bacteria and fungi, often utilized in previous microbiological investigations in mangrove ecosystem[10-12]. However, in most cases, the salinity of dilution water and plate media was hardly considered in this method applied in the mangrove areas, although Garg[10] discovered that the colonies and occurrence frequency of fungi decreased with the increasing salinity of media at a gradient of 0, 30, and 60. The few salinity gradients and last two higher ones probably led to his experimental results because the high salinity of media above the habitat one by far would stress the fungal growth and development. Besides a few of bacteria, no actinomyctes and fungi were detected when dilution plate technique was utilized to investigate microbial distribution in Dongzhaigang Mangroves Reserve, Hainan of China[13], in which the dilution water and plate media were prepared with the seawater. The similar results were obtained when the same method was used to investigate microbial community in Xiamen West Sea Area, China[14]; Rare actinomyctes and fungi in actual habitat and the investigative method might be responsible for it. However, a great number of actinomycete colonies appeared on the agar plates as soon as the media salinity declined[14], implying that the salinity of agar media could influence the final number of colony. The inter-tidal zone including mangrove area is an ecotone from the land to the sea, where soil habitat is both terrestrial and marine, but is not completely different from

any one of them in some degrees. Should this area be taken as the terrestrial or marine one, and what range of salinity should be controlled when the dilution plate technique is applied to measure the microbial density of soil? Based on the findings above-mentioned, in this study, the effects of salinity of dilution water and agar media on colony counting of heterotrophic bacteria, actinomycetes and fungi on the plates were examined when this technique was used to investigate soil microbial densities in two mangrove areas of China.

2 Materials and methods

2.1 Sampling stations

Two mangrove areas investigated in this study locate at Jiulongjiang Estuary, Fujian Province, China, and Futian Mangrove Nature Reserve, Guangdong Province, China, respectively, where soil samples were collected from *Kandelia candel* forests, *Avicennia marina* forests and their corresponding mudflats. In Jiulongjiang Estuary, sampling stations J1 (*K. candel* forest) and J2 (mudflat) locate at Caoputou village (24°24′ N, 117°55′ E), Fugong Town, Longhai City, and J3 (*A. marina* forest) and J4 (mudflat) at Dongyu Village (24°31′ N, 118°03′ E), Haicang Town, Xiamen City. The mudflat stations are at least 100 m away from their corresponding *K. candel* forest and *A. marina* forest at the same tidal level and without any vegetation. In Futian Mangrove Nature Reserve, sampling stations F1 (*K. candel* forest) and F2 (*A. marina* forest) locate at a bird observation zone near Chegongmiao (114°05′E, 22°32′N), which has been described by Chen *et al.* [15] and Lin *et al.* [16], and no sample was taken from their corresponding mudflats because of administerial restriction. The climate in Jiulongjiang Estuary belongs to southern subtropical maritime climate. The *K. candel* forest in Jiulongjiang Estuary mainly occupies middle and high inter-tidal zone, and is a zonal distribution (about 40 m in width) along the southern coast of this estuary, with a small quantity of *Aegiceras corniculatum* trees and *A. marina* trees at the forest fringe. The forest form is tidy with the tree height from 5.5 m to 6.0 m and crown density over 0.9. The *A. marina* forest locating at Dongyu village is also a pure forest, with the tidy canopy, green physiognomy, and simple community structure. The average tree height is about 1.2 m in *A. marina* forest, but the density of tree is so great (reaching 15 indiv·m^{-2}) that it is difficult to go through when sampling there. In addition, there are plenty of seedlings in this forest (above 31 ind·m^{-2}), and many finger·like pneumatophores (about 35 ind·m^{-2}) rise out of the surface of mudflat about 10 cm high. The entire tidal flat is smooth.

2.2 Collection of soil samples

Soil samples were aseptically collected at random from topsoil of 20 cm depth at the middle inter-tidal zone with a sterile PVC pipe at low tide. Multiple samples collected at each plot were mixed together uniformly and kept in sterile polyethylene bags after the roots were removed. The mixed samples were then taken to the laboratory for analysis immediately.

2.3 Media

(1) Bacterial medium (2216E agar by Zobell), peptone 5 g, yeast extract 1 g, $FePO_4$ 0.01 g, agar 15 g, water 1000 ml, and pH 7.2－7.4; (2) Actinomycetes medium: soluble starch 20 g, casein 1 g, KNO_3 1 g, K_2HPO_4 0.5 g, $MgSO_4\,7H_2O$ 0.5 g, agar 15 g, trace elemental solution 1 ml, water 1000 ml, pH 7.4, and $K_2Cr_2O_4$ solution as inhibitor supplemented into medium after autoclave at final concentration of 50 (μg·g^{-1}); (3) Fungal medium: glucose 10 g, peptone 5 g, K_2HPO_4 1 g, $MgSO_4\,7H_2O$ 0.5 g, agar 15 g, Bengal red solution (0.5%) 0.66 ml, water 1000 ml, medical chloramphenicol (as inhibitor) 2 ml, and pH 6.6.

2.4 Measurement of microbial densities

In this study, microbial densities of soil samples were determined using dilution plate method. Soil sample of 10 g was mixed with sterilized water of 90 ml. The mixture was vigorously shaken on a swirl mixer and then settled for

10 min. The overlying suspension containing microbes was further diluted with the sterilized water at a rate of 1:10. Then, the suspension was plated out on the agar media, respectively. Three appropriate serial dilutions were selected for the bacteria, actinomycetes and fungi respectively. Each dilution replicated three times.

The samples from J1 and J3 were diluted with aged seawater (SW, salinity 29.6) and freshwater (FW), respectively, to compare the effect of salinity on the final colony counting on the plates, but the samples from J2, J4, F1 and F2 were diluted only with freshwater. To evaluate the effect of salinity in the media on microbial enumeration, the diluted suspensions were plated out on the agar plates above-mentioned with different NaCl concentration from 0 to 35.

The plates were incubated at (28 ±1)℃. The formation of colonies was observed regularly every day and the enumeration was performed after incubation for 2 −3 d for the bacteria, 10 d for the actinomycetes and 3 −5 d for the fungi. CFU (colony-forming unit) per gram of wet soil was calculated for each sample, but the final results were expressed as CFU per gram dry weight($CFU \cdot g^{-1} \cdot dw$) based on the water coefficient of samples.

2.5 Analysis of main physical-chemical characteristics of soil

The soil samples were air dried naturally, grounded to fine powder and sifted. The sifted samples were used to analyze the physical-chemical characteristics, including soil texture by the densimeter method, organic matter (OM) by wet oxidation with potassium dichromate, total nitrogen (TN) by Kjeldahl nitrogen, total phosphorus (TP) by standard colorimetric methods after acid digestion, total potassium (TK) by inductively coupled plasma atom emission spectrum (ICP-AES), salinity by electric conductivity, pH value by potentiometric analysis with a pH meter according to the methods described in related reference by Nanjing Institute of Soil Science, Chinese Academic of Sciences[17].

2.6 Statistical analysis

Differences in microbial densities between the seawater dilution and freshwater dilution and between stations were assessed using nonparametric Wilcoxon test. The statistical analysis was performed in the SPSS for windows.

3 Results

3.1 Main physical-chemical characteristics of soil

In Jiulongjiang Estuary, the concentrations of OM and TN were lower at J1 than at J3, but TP was higher at J1 and its corresponding mudflat J2 than at J3 and its corresponding mudflat J4, respectively (Tab.1). The soil salinity and pH value were correspondingly higher at J3 and J4 than at J1 and J2. In Futian Mangrove Nature Reserve, the concentrations of OM, TN, and TP, and salinity were lower at F1 than at F2, while the pH value was higher at F1 than at F2.

Table 1 Main physical-chemical characteristics of soil at sampling stations in two mangrove areas

Mangrove area	Station	Soil texture	OM(%)	TN(%)	TP(%)	TK(%)	Salinity	pH
Jiulongjiang Estuary	J1	Light clay	3.206	0.143	0.066	0.725	15.14	6.55
	J2	Light clay	3.054	0.1344	0.064	0.634	11.56	7.08
	J3	Light clay	3.600	0.148	0.057	0.851	24.30	7.00
	J4	Middle clay	2.139	0.117	0.055	1.121	21.25	7.18
Futian Mangrove Nature Reserve	F1	Light clay	4.09	0.139	0.086	-	15.25	6.50
	F2	Light clay	6.72	0.363	0.099	1.25*	19.30	5.88

* Cited from Lin *et al.* [16]

3.2 Effect of salinity of dilution water

The salinity of dilution water significantly influenced the final numbers of colony for the samples from J1 and J3 (Table 2). The statistical analysis indicated that the bacterial colonies were significantly ($p < 0.05$) more abundant

when the seawater was used as dilution water, but the actinomycetes and fungi were significantly ($p < 0.05$) more when the samples were diluted with the freshwater, except the fungi at J3 (Table 3). Therefore, the seawater was more suitable for dilution of bacterial suspension than the freshwater, with the reverse results for the actinomycetes and fungi.

Table 2　Microbial densities of soil under salinity gradient of media in two mangrove areas*

Station	Microbial group	Dilution water	Concentration of NaCl in the media						
			0	5	10	15	20	25	30	35
J1	Bacteria	SW	68.17	77.61	79.57	69.06	23.32	19.76	16.73
J1	Bacteria	FW	13.88	11.93	9.79	11.21	9.79	8.90	6.76
J1	Actinomycetes	SW	22.07	18.33	14.95	9.97	5.52	1.96	0.71
J1	Actinomycetes	FW	39.69	23.5	16.38	12.99	11.93	3.92	1.25
J1	Fungi	SW	6.23	8.9	8.9	8.01	7.12	3.56	3.56
J1	Fungi	FW	38.27	38.27	32.04	35.6	31.15	32.93	26.7
J2	Bacteria	FW	26.38	32.40	26.38	22.12	21.92	11.83	13.58
J2	Actinomycetes	FW	28.13	25.41	19.01	10.86	7.95	3.10	0.97
J2	Fungi	FW	26.19	21.34	20.37	28.13	13.58	13.58	13.58
J3	Bacteria	SW	19.02	21.74	19.44	19.65	19.44	17.14	16.3
J3	Bacteria	FW	19.96	14.21	8.78	7.73	4.6	3.76	2.72
J3	Actinomycetes	SW	26.75	24.45	20.06	7.11	2.72	1.46	0.21
J3	Actinomycetes	FW	27.38	26.13	21.95	15.05	9.82	3.76	1.25
J3	Fungi	SW	5.23	8.36	5.23	8.36	6.51	4.18	5.23
J3	Fungi	FW	9.41	8.36	8.36	8.36	4.18	10.45	10.45
J4	Bacteria	FW	5.48	7.37	4.35	4.73	3.02	1.51	0.76
J4	Actinomycetes	FW	16.63	22.30	20.98	13.42	7.56	4.73	0.95
J4	Fungi	FW	0.95	3.78	0.95	4.73	3.78	1.89	3.78
F1	Bacteria	FW	11.64	14.32	14.32	6.27	9.85	6.27	3.58
F1	Actinomycetes	FW	20.59	14.86	11.10	6.44	3.22	0.72	0.18
F1	Fungi	FW	8.06	7.16	7.16	6.27	6.51	4.34	6.51
F2	Bacteria	FW	34.72	56.42	39.06	44.49	24.96	22.79	8.64
F2	Actinomycetes	FW	30.16	25.39	20.18	14.11	8.03	2.39	1.09
F2	Fungi	FW	45.57	51	27.22	39.06	56.42	52.08	56.42

* Bacteria 10^4 CFU·g^{-1} dw; Actinomycetes 10^3 CFU·g^{-1} dw; Fungi 10 CFU·g^{-1} dw; SW: seawater; FW: fresh water

Table 3　Nonparametric Wilcoxon test on variation of microbial densities in dilution water type

Microbial groups	Station	Comparison of dilution water	Z value of Wilcoxon test	p value
Bacteria	J1	FW-SW	-2.521	0.012*
	J3	FW-SW	-2.380	0.017*
Actinomycetes	J1	FW-SW	-2.380	0.017*
	J3	FW-SW	-2.366	0.018*
Fungi	J1	FW-SW	-2.527	0.012*
	J3	FW-SW	-1.753	0.080

* Significant at the level of $P < 0.05$

3.3　Effect of salinity of media

The colony numbers on the plates were significantly influenced by the salinity of media. The most bacterial colonies from the sample of J1 were found on the plates containing 10 salinity and the least at 35 , and the colonies were significantly more abundant at low salinity (0 — 15) than at high salinity (20 — 35) when the seawater was used as dilution water. However, such variation was not found when the freshwater was used as dilution water (Table

2). At J2, the bacterial densities decreased gradually with increasing salinity of media from 0 to 35 when the freshwater was used as dilution water, with the most densities at 5. At J3, the bacterial colonies were the most abundant at 5, and then decreased with increasing salinity of media when the samples were diluted with the fresh water (Table 2), but the difference between low and high salinities was much smaller at J3 than at J1. The bacterial densities at J3 sharply decreased with increasing salinity of media from 0 to 35 when in the freshwater dilution system. However, the bacterial densities decreased a little with the increasing salinity of media at J4 although the variation tendency was similar to that at J3 (Table 2) to some extent, which resulted in the fact that more bacterial colonies were counted at J3 than at J4.

For the actinomycetes in Jiulongjiang Estuary, the highest densities were almost found at 0, only with one exception at J4 in which the highest one emerged at 5 (Table 2). The actinomycetes densities at all stations declined sharply with the increasing salinity, and down to the least when the salinity rose up to the highest (35). Moreover, some blank plates were present at the highest salinity of 35. When the freshwater was used as dilution water, the difference in densities of actinomycetes between J1 and J2, and between J3 and J4 was not as large as that of the bacteria.

The densities of fungi in Jiulongjiang Estuary did not show well-regulated variation with the increasing salinity of media, except at J1 and J2 the fungi colonies were more abundant at low salinities than at high salinities when the samples were diluted with the freshwater (Table 2). However, much more fungi were counted in the samples from two mangrove stations than those from two corresponding mudflat stations when the freshwater was used as dilution water.

In Futian Mangrove Nature Reserve, the significant decreasing colony densities with the increasing salinity of media were also found in the bacteria and actinomycetes. However, the variation tendency of fungi was indefinite since at F2, the densities of fungi seemed to be higher at high salinity than those at low salinity, nor did the fungi at F1 (Table 2).

3.4 Difference in microbial densities between mangrove stations

The soil microbes were significantly ($p < 0.05$, with the exception of fungi) more abundant at J1 than at J2 in Jiulongjiang Estuary when the samples were diluted with suitable water. In Futian Mangrove Nature Reserve, for three groups of microbes, the higher microbial densities were all measured at F2 than at F1 ($p < 0.05$), and the biggest difference was found in the fungi (Table 2).

4 Discussion

Although the plate counting method remarkably underestimates true microbial densities, it is continually used for the isolation and enumeration of microbes, especially for the actinomycetes and fungi, in mangrove areas[6,10-13,18]. The bacterial density is generally determined by epiflourescence microscopy due to its accurateness, but the bacteria are also isolated and their densities are measured sometimes by dilution plate method[12,13,18], therefore few researcher cared about the effect of salinity of dilution water and media on bacterial abundance in this method. However, this study indicated that the bacterial colonies were more at the low salinity range of agar media whether in Jiulongjiang Estuary or in Futian Mangrove Nature Reserve, and the effects of seawater dilution were significantly better than the fresh water. Therefore, it is concluded that the bacteria are terrestrial in the mangrove areas including mudflats, but they probably adapt to the salt stress from these areas easily during a long evolution, and their growth and development are more vigorous under the certain salinity than without any salt stimulation.

The regulation that the abundance of fungal colony varied with the salinity of media was apparently indefinite in this study, which was to some extent relative to the fungal nature. Gray *et al.* considered that many mangrove fungi

were able to tolerate the great variations in the salinity of media with ease[19], which was in agreement with this investigation, while Garg discovered that the numbers and occurrence frequency of fungi decreased with the increasing salinity of media at a gradient of 0, 30, and 60[10]. We consider that the few salinity gradients and last two higher ones may be key factors leading to his finding. The statistical analysis showed that the fungal colonies with the fresh water dilution were significantly more than with the seawater dilution in this study; we think it is relative to the terrestrial origin of fungi and their nature with the resistant spore. It was confirmed that the fungi found in mangrove mud are all "terrestrial" species with individual exceptions[20].

The actinomycetes seemed to be very sensitive to the salinity by reason of that their abundance on the plates declined rapidly with the increasing salinity of agar media, moreover, much more colonies were counted as the samples were diluted with the fresh water. So, the low salinity in dilution plate method is significantly more effective and helpful to the isolation and enumeration of actinomycetes. Takizawa *et al.*[21] reported that the actinomycetes densities reached $1.8 \times 10^2 - 1.4 \times 10^5$ CFU·ml^{-1} sediment when the media-starch casein agar was supplemented with NaCl at final concentration of 0.5%, and all sediment samples were diluted with sterilized 0.5% saline water. The same effect occurred at both mudflat stations and mangrove ones in this investigation, as meant that this method could identically be applied to the mangrove areas including mudflats. We think that the actinomycetes are also "terrestrial" like the bacteria and fungi in the mangrove areas including mudflats, as a result, it was more effective for the soil samples in the mangrove areas to be treated in a non-marine way or a terrestrial way.

There were few reports involved in the effect of salinity of dilution water and agar media on the microbial enumeration on the plates in the previous studies in spite of the finding by Garg[10]. Neither the salinity of dilution water nor that of agar media was mentioned or described clearly in a few of reports[10-12, 22] despite the reliable results were obtained in them. In practice, no or scarce colonies of soil actinomycetes[13, 22, 23] and fungi[11, 13, 22] were counted in some mangrove stations, which had to some extent relevant to this effect of salinity of dilution water and plate media in the majority of these reports. This study confirmed that the higher salinity would cut microbial numbers down with ease. Therefore, it should be considered carefully for the salinity of dilution water and agar media when the dilution plate method was employed in mangrove areas including mudflats.

Kohlmeyer found that the fungal densities decreased with the increasing habitat salinity from the inland zone to the seaward zone in mangrove forest[24]. The same phenomenon involved in the fungi, bacteria and actinomycetes occurred in mangrove area of Jiulongjiang Estuary, where although the concentration of OM and TN was higher at J3 (*Avicennia marina* forest) than at J1 (*Kandelia candel* forest) (Table 1), soil microbes were more abundant at J1 than at J3, similarly, the microbial densities were more at J2 than J4, because J3 and J4 were more seaward than J1 and J2. As a result, the time submerged by tide was longer, the soil salinity was higher (Table 1), and the redox potential① was lower at J3 and J4 than at J1 and J2 respectively. Lee and Baker[6] considered that organic matter content, nutrient level, oxygen content (equivalent of redox potential), pH and salinity were probably the most important environmental factors controlling the nature and distribution pattern of microfungi in mangrove swamp soil. But the time submerged by tide, soil salinity and redox potential seemed to be more important than other factors such as organic matter content, nutrient level among these stations in Jiulongjiang Estuary. However, this phenomenon did not occurred at two mangrove stations in the Futian Mangrove Nature Reserve, in respect that microbial densities were least at F1 (*K. candel* forest) than at F2 (*A. marina* forest), which was without question associated with

① Zhang Y L. Ecological studies on enzymatic activity and fine roots of soil in mangrove forest in Jiulongjiang Estuary. Doctor Thesis in Xiamen University, Xiamen, China, 1996. 27－40

special habitat. The concentration of OM and nutrient level in soil was lower at F1 than at F2, so did soil salinity, in spite of the fact that two stations have same tidal level[15,16]. Therefore, the organic matter and nutrient level should be considered as the leading factor at these two stations. Microbial distribution and horizontal difference is, in virtually, a complicated ecological question involved in habitat salinity.

On the basis of this study, some optimum improvements are proposed as follows when the dilution plate technique is employed to investigate the microbial density of soil in mangrove areas including corresponding mudflats:

①How to prepare sterile dilution water. Sterilized dilution water should be prepared with the seawater for the bacteria, and with the freshwater or low saline water (for instance 5) for the actinomycetes and fungi respectively at least in this study.

②The seawater is not suitable for preparing the agar media. The suitable salinity of bacterial media is at the low range of salinity (approximately 10 in this study) and appropriate salinity of media can be adjusted easily in the light of actual salinity in the habitat. The salinity of agar media for the actinomycetes should not be over 5, and that for the fungi should also be in low salinity although the effect of salinity of fungal media was indefinite.

③A better strategy is to measure the habitat salinity and provide preliminary experiments for some samples following the method supplied by authors. In this way, the salinity of media and the types of dilution water may finally be controlled and selected with ease.

If the study aims at obtaining halophilic microbes or real marine ones, both media and dilution water should be prepared with the seawater, and even the salinity of media should further be raised when necessary.

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说明:培养基 NaCl 浓度单位为 g/kg　The unit of concentration of NaCl in the media is g/kg.