

The isolation, identification , physiological and biochemical properties of salt-tolerant strains

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Abstract : [Objective] By screening salt-tolerant strains , provide support for salted wastewater.[Method]With high salted water high efficiency salt activated sludge acclimation salt high efficiency cultivation as the source of strain , isolated and purified salt-tolerant strains , by measuring the salt-tolerant strains to biological growth OD₆₀₀ of different salinity , 3 dominant strains SP1、 SP3、 SP7 were selected for salt-tolerance. Combining the basic shape , physiological and biochemical characteristics and 16S rRNA sequence analysis , identification of these 3 are salt tolerant strains *Zymomonas*(*Halomonas* sp.) . [Result] The growth of salt-tolerant strains were the best when the temperature was 30 , the pH value was 7-8 and the salt concentration was 15% , under this condition, the removal rate of COD and ammonia nitrogen reached 75% and 62% respectively. When the salinity reached 18%, the salt tolerance declined and the growth of salt-tolerant strains were not adapted to the salt tolerance.

Introduction

Salted wastewater with high salt, high organic matter and the features of high nitrogen and phosphorus, biological treatment is difficult, especially for the removal of nitrogen and phosphorus which become difficult. In addition, salted wastewater of high pollution (COD, BOD, SS) and high salt content, we can not use the general method of biological degradation or flocculation and other general physico-chemical treatment process in the treatment process^[1,2,3,4]. Salt tolerant strains with good performance and can effectively remove organic pollutants, the existence of a large number of common salt tolerant strains can improve the process capability of salinity. Therefore, screening of salt tolerant strains from the high salinity environment will be conducive to improve the effect of high salinity wastewater treatment biological technique and the stability of the process operation.

Based on this, the subject of salted water were studied experimentally, through the screening, physiological and biochemical properties of the dominant strains and the study on the processing performance of the preserved wastewater, to provide the biological basis for the adjustment of production process and the determination of the parameters of the production process.

Experimental materials

Source of experimental wastewater. In this study, salted water from Shenyang Yuyuan industrial food factory.

Strain source. In this study, bacteria from the high salted water efficient culture salt domesticated activated sludge.

Medium. In this study, there tolerant bacteria complete medium, medium salt nitrification bacteria, denitrifying bacteria salt medium 3 media.

Experimental methods and results

Experimental methods

Isolation and purification of strains. Separation by dilution coated tablet method for microorganism in wastewater. The salted water according to the concentration gradient diluted 10^{-1} - 10^{-7} , then plate culture, placing 3 kinds of different media, and in 30 °C constant temperature incubator. Single cells were isolated initially pick emulated purified microorganism, repeated several times, until the purified strains were isolated.

Salt strain detection and screening. The screened 8 strains were screened, detection their biological growth in the salinity of 5%, 9%, 15% and 18%^[5,6,7,8], determine their respective salt resistance and screen out superiority strains.

Morphological and biochemical characteristics of the strain. Superiority strains for gram staining and individual morphological observation^[9,10]. From the contact enzyme test, glucose oxidation fermentation, methyl red test, VP test, test starch, nitrate test, denitrification test, test production of ammonia, indole test, gelatin liquefaction, etc., to explore the biochemical properties of the reaction of each strains^[11].

Full sequencing and analysis of 16S rRNA. PCR amplification was carried out on the salt tolerant strains, in order to follow-up 16 s rRNA gene sequence analysis. PCR reaction conditions were: 94 °C denaturation for 5min, after 30 cycles. Wherein, 94 °C denaturation 40s, 55 °C annealing 40s, after 72 °C for 1.5min, 30 cycles, and then in 72 °C for 7min.

Dominant strains of COD, ammonia nitrogen removal capacity. Detecte the removal efficiency of COD and ammonia nitrogen of the 3 dominant strains at the salinity of 6%, 9% and 15%.

Experimental results

Isolation and purification of strains. By isolation and purification, 8 strains were obtained. Figure 1 and 2 respectively show 2 typical strains after gram staining under optical microscope images, respectively, short rods gram negative bacterium (Figure 1) and globular gram positive bacteria (Figure 2). The most suitable culture conditions of the strain were determined by the influence factor of the strain: pH was 7-8, the temperature was 30.

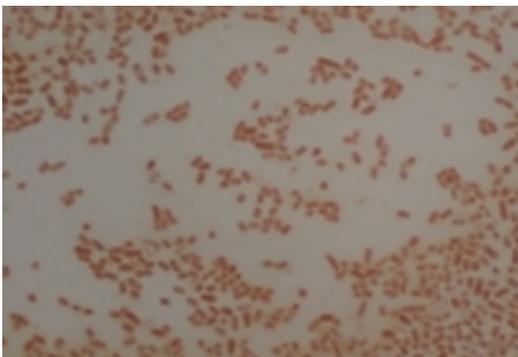


Figure1. Gram stain of Sp3 strain (15 x 100 times)



Figure2. Gram stain of Sp7 strain (15 x 100 times)

Rescreening and growth characteristics of Strain

Rescreening of Strain. From figure 3, it can be seen that the growth of strain 1, 3 and 7 in different salinity is particularly prominent, which shows that the 3 strains are better able to bear salt, and can obtain higher growth rate under high salinity. As a result, 3 strains were selected as the dominant strain, and the 3 strains were named as SP1, SP3 and SP7, to do further research.

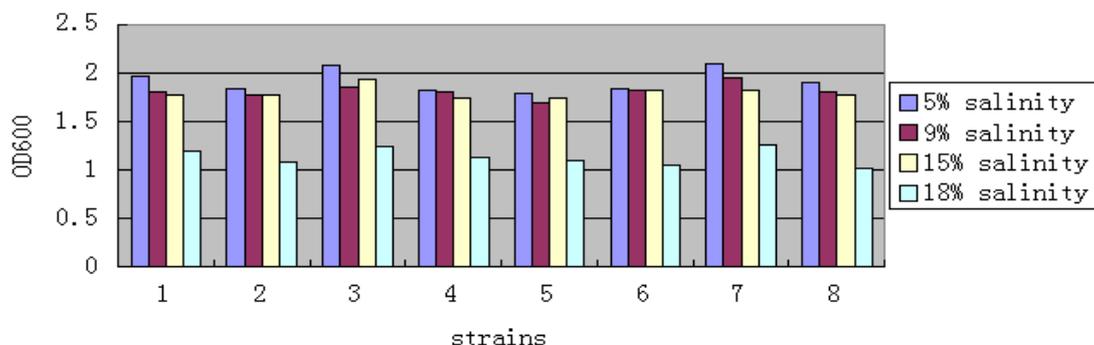


Figure3. The biomass growth of 8 strains of salt tolerant bacteria in different salinity

Growth characteristics of dominant salt tolerant strains. By Figure 4, 5, 6, it can be seen that the growth of 1,3,7(SP1,SP3,SP7) strain at the salinity of 9% and 15%, the growth is similar, can be more rapid after inoculation into the logarithmic growth period. But in the salinity of 18% and 21%, the strain grows more gentle whole, we need to go through 32h, 28h, 28h lag before they can enter the logarithmic growth phase after vaccination, respectively.

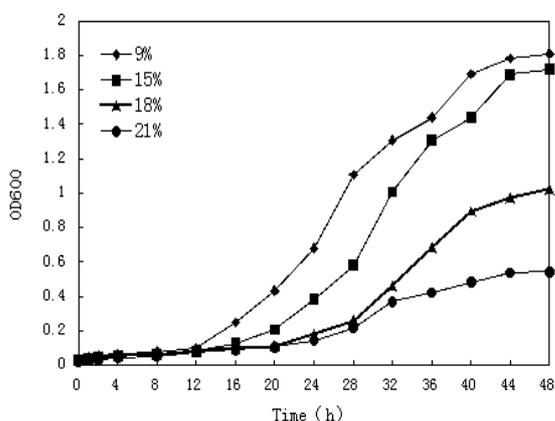


Figure4. Strain 1 growth curves at different salinity

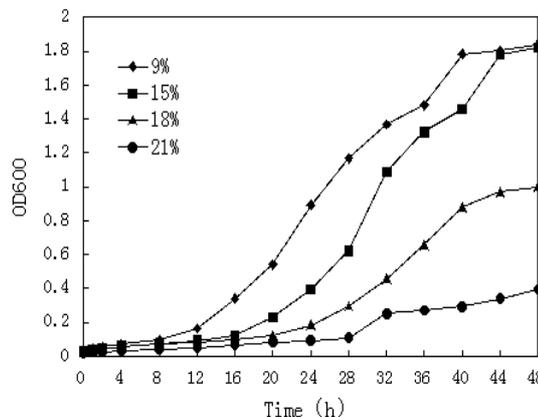


Figure5. Strain 3 growth curves at different salinity

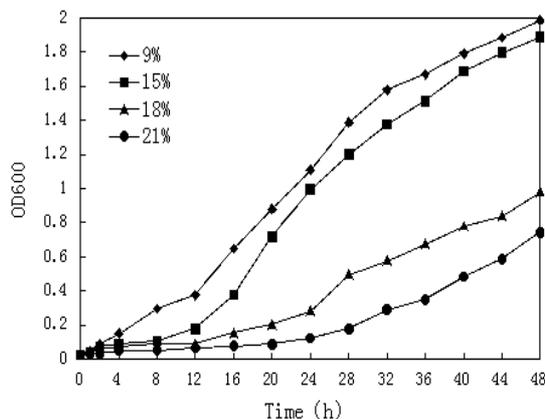


Figure6. Strain 7 growth curves at different salinity

Taking these 3 experiments, 3 strains could grow well at the salinity of 15%, when it reaches 21 %, the growth of strains has discomfort, indicating the degree of strain tolerance limit has been reached, namely to withstand salinity ,it should not exceed 18%-20%.

Morphological and biochemical characteristics of dominant salt tolerant strains.By visualizing the size of the colony, texture, shape, colour and lustre, by gram staining to observe the morphological characteristics in many aspects. Observations as shown in Table 1.

Table1. Morphological characteristics of each colony

strains	colour	diameter (mm)	luster	shape	smooth	viscosity	dyeing shape	gram staining
1	brown	2	yes	convex round	yes	yes	short rod	negative
3	milky	8	yes	round	yes	yes	thick short rod	negative
7	yellow	1	yes	convex round	yes	yes	ball	Positive

The experimental results of the biochemical properties of 3 strains of dominant salt tolerant strains are shown in Table 2.

Table2. The results of biochemical properties of 3 strains of dominant strains

strains	1	3	7
contact enzyme test	+	+	+
glucose oxidation fermentation	+	+	+
methyl red test	+	+	+
V-P test	+	+	-
nitrate test,	+	+	+
test starch	-	-	+
denitrification test	-	+	-
test production of ammonia	-	+	-
indole test	-	+	+
gelatin liquefaction	-	+	-

Determination of 16S rRNA sequence of dominant salt tolerant strain.Combining the morphological characteristics, physiological and biochemical properties and 16S rRNA sequence analysis of the isolates,identification of the 3 tolerant strains SP1, SP3, SP7 belong salt Xanthomonas (Halomonas sp.)

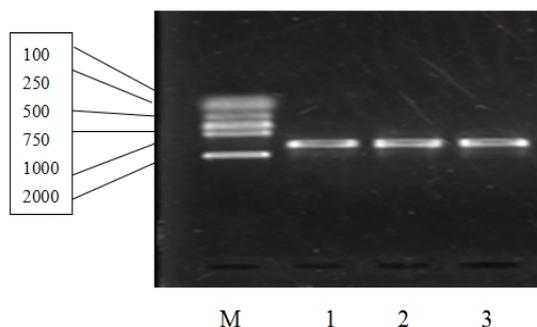


Figure7. 3 strains of dominant bacteria by PCR, horizontal electrophoresis results

Dominant strains of COD, ammonia nitrogen removal capacity. From figure 8, it can be seen that the removal rate of COD was more than 75% at 3 different salinity, these 3 strains of salt tolerant strains in the high salt load environment, and can effectively remove the organic pollutants in the process of obtaining a large amount of biomass.

From figure 9, it can be seen that the removal rate of ammonia nitrogen was more than 62% at 3 different salinity, these 3 strains of salt tolerant bacteria in the high salt load environment, in the same time to obtain a large amount of biomass can effectively remove the pollutants ammonia.

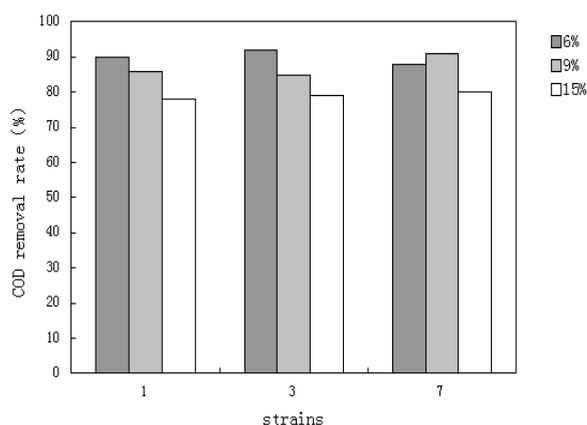


Figure8. Removal rate of COD at different salinity

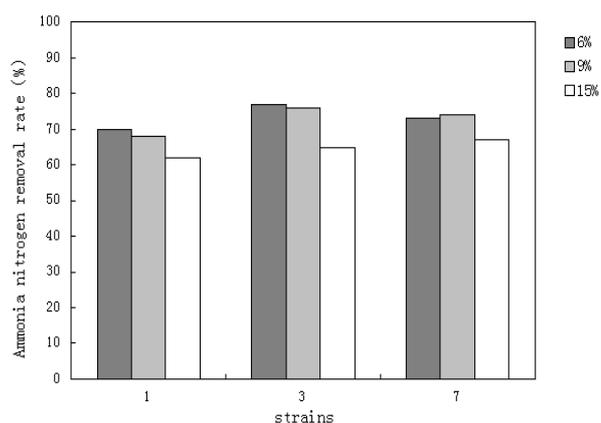


Figure9. Removal rate of ammonia nitrogen at different salinity

Experimental conclusion

(1) Through a series of screening high salt resistant strains 3 strains were isolated by SP1, SP3 and SP7, gram staining results were negative, negative, positive, combined with the strain morphological characteristics, physiological and biochemical properties and 16S rRNA sequence analysis, identification of the 3 strains of salt-tolerant strains belong salt *Xanthomonas* (*Halomonas* sp.)

(2) The 3 salt strains at a temperature of 30 °C, pH value was 7-8, the salt tolerance can reach 15%, when the salinity reaches 18%, the salt tolerance declined, and the growth of salt tolerant strains were not adapted.

(3) When the temperature was 30 °C, pH value was 7-8, under the salinity of 15%, the removal rates of COD and ammonia nitrogen can reach 75% and 62%, respectively.

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