Study on the Degradation Efficiency of Nitrite by Strain Zl-1

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Abstract: Through experimental studies and measurements, the results and screened strains compared with other strains have obvious degradation of nitrogen strains and the advantages of harmful substances named ZL-1, the physiological and biochemical identification of the strains and 18SrDNA sequences sequencing, combined with the physiological and biochemical identification and phylogenetic analysis results of 18SrDNA sequences, determine the strain ZL-1 for Wickerhamomyces anomalus. The removal ability of nitrite was studied in this paper. The experimental results showed that the strain ZL-1 had the strongest growth and degradation ability to nitrite nitrogen and ammonia nitrogen at the temperature of 30℃, pH value of 6, glucose as carbon source, and C/N of 20:1.

1. Introduction

In aquaculture, nitrification and denitrification bacteria and anaerobic ammonium oxidation bacteria are the main biological denitrification strains. In addition to the above bacterial strains, yeast, as an excellent environmental contaminant degradation strain, has been studied by scholars at home and abroad. It not only plays an important role in the wine industry, but also has been more widely used in the treatment of all kinds of sewage, the utilization of waste and the remediation of contaminated soil. However, from the available patents and literature, there are few detailed reports of yeast as a nitrite removal strain.

Accumulation of nitrite is one of the main causes of aquaculture water pollution and an important environmental factor inducing aquatic animal explosive diseases. In aquaculture, in addition to the aquaculture animals, there are a large number of phytoplankton and algae, and only feeding on these plants is far from meeting the needs of high-density aquaculture animals. Therefore, artificial bait is needed to meet the nutritional needs of aquaculture animals. However, in order to pursue high profits in a short period of time, many breeding enterprises and farmers add too many artificial baits, which, combined with the accumulation of feces and secretions from breeding animals, leads to serious pollution of breeding water bodies. The most important nitrogen pollutants are ammonia and nitrite. Nitrite is an intermediate that converts ammonia to nitrate. Once the nitrification process is stopped, nitrites accumulate rapidly in aquatic ecosystems. The high content of ammonia nitrogen and nitrite has seriously affected the survival and growth of aquatic organisms and caused huge economic losses to aquaculture.

2. The Materials and Methods

2.1 The Materials and Reagents

Glucose; $NaNO_2$; KH_2PO_4; $MgSO4{\cdot}7H_2O$; $FeSO_4{\cdot}EDTANa_2$; AGAR; Peptone; NaOH; HCl.

2.2 Instruments and Equipment

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Constant temperature and humidity incubator; Double layer thermostatic shaker; Double cleaning table (SW-CJ-2D;)Vortex mixer; UV-visible spectrophotometer; Digital display constant temperature water bath; Vertical automatic electric heat pressure steam sterilization cooker; Electronic Balance (FA134S); Electric heating constant temperature blast drying oven; High speed centrifuge; PHB-4 pH meter; Glass colorimetric dish.

2.3 The Action Characteristics of Nitrite Degrading Strains

In order to better solve the problem of excessive content of harmful nitrogen-containing substances in aquaculture circulation and discharge water, it is necessary and of great significance to study the degradation characteristics of harmful substance degrading bacteria. So far, studies have shown that temperature, pH value, C/N ratio, carbon source and the concentration of the bacteria in the water are all important factors affecting the degradation effect of the bacteria. Different types of degrading bacteria, under different temperature, pH value, C/N ratio, carbon source, the concentration of bacteria and other conditions, the above factors on the denitrification effect is also different.

2.4 Screening of Nitrite Degrading Strains

The 5%(V/V) sample was enriched in a 250mL sterile conical flask containing nitrite enrichment medium (Table 1) for 28℃ for 72h. Add 5% fresh medium daily to survive the fittest.

Table 1 Required Medium Composition Table

composition	Enrichment medium	Separation medium	Liquid separation medium
Glucose (g)	5.0	5.0	5.0
$NaNO_2$ (g)	1.0	0.5	0.5
KH_2PO_4 (g)	0.5	0.5	0.5
$MgSO_4·7H_2O$(g)	0.1	0.1	0.1
$FeSO_4$·$EDTANa_2$ (mL)	2.0	2.0	2.0
agar (g)	0	20.0	0
distilled water (mL)	1000	1000	1000
pH	6	6	6

2.5 Isolation of Nitrite Degrading Strains

The inoculated strains were diluted 10 times, coated on the surface of the isolation medium (Table 1), and cultured at 30℃ for 48h.

2.6 Purification of Nitrite Degrading Strains

After the samples were dried, the petri dishes were placed upside down in a constant temperature incubator at 30℃ for culture. A total of 8 representative strains were isolated according to morphological characteristics and dominant degree of colonies.

According to the color and morphological characteristics of the colony, the representative single bacterial colony was selected, and the culture medium plate was purified until the single bacterial colony was obtained. The isolated and purified strains were transferred to AGAR medium and stored at 4℃.

2.7 Molecular Identification of Strain 18srdna

Specifically, the 18SrDNA of the strains isolated and purified above was amplified by PCR using the genomic DNA of the strains as the template.

2.8 Effect of Temperature on the Degradation of Nitrite Nitrogen and Ammonia Nitrogen

In 18 (6×3) 250mL conical flask, 1.5×108cfu/mL ZL-1 5% (v/v) strain solution and 95% (v/v) B basal medium were added: $(NH_4)_2SO_4$ 0.1g, 0.05g KH_2PO_4, 0.01g $MgSO_4·7H_2O$, 0.5mL $FeSO_4$· $EDTANA_2$ (*1000) and sterile water, respectively. The pH value was 6.5. The C/N (glucose-C/sodium nitrate-N) in each conical flask was adjusted to 20:1. When glucose is the only

carbon source, the total capacity is 100mL.

The culture was repeated for 3 times in a shaking table at 180r/min for 48h. The temperature was set at 15, 20, 25, 30, 35 and 40℃; Under different conditions, each sample was sampled three times. Under different setting conditions, 0.1mL of sample liquid was taken and mixed into a 5mL test tube to supplement distilled water for the determination of nitrite nitrogen and ammonia nitrogen samples.

2.9 Effect of Ph on the Degradation of Nitrite Nitrogen and Ammonia Nitrogen

In a 250mL conical flask, 1.5×108cfu/mLZL-1 5% (v/v) strain solution and 95% (v/v) A basal medium were added :0.05g $NaNO_2$, 0.5g glucose, 0.05g KH_2PO_4, 0.01g $MgSO_4 \cdot 7H_2O$, 0.5ml $FeSO_4 \cdot EDTANA_2$ (*1000) and sterile water, so that the total capacity was 100mL and the temperature was 30℃. The culture was repeated for 3 times in a shaking table at 180r/min for 48h. PH set to 4, 5, 6, 7, 8, 9; Under different conditions, each sample was sampled three times. Under different setting conditions, 0.1mL of sample liquid was taken and mixed into a 5mL test tube to supplement distilled water for the determination of nitrite nitrogen and ammonia nitrogen samples.

2.10 Effects of Carbon Source Types on the Degradation of Nitrite Nitrogen and Ammonia Nitrogen

The carbon source was set to add: sodium acetate 0.5g, glucose 0.5g and starch 0.5g, so that the total capacity was 100mL, the temperature was 30℃, and the pH value was 6.5. The culture was repeated for 3 times in a shaking table at 180r/min for 48h. The carbon source is sodium acetate, glucose and starch. Under different conditions, each sample was sampled three times. Under different setting conditions, 0.1mL of sample liquid was taken and mixed into a 5mL test tube to supplement distilled water for the determination of nitrite nitrogen and ammonia nitrogen samples.

2.11 Effects of Different C/n on the Degradation of Nitrite Nitrogen and Ammonia Nitrogen

Adjust the C/N (glucose-C/sodium nitrite -N) in each conical flask to 10:1, 15:1, 20:1, 25:1, 30:1, and 35:1; The total capacity is 100mL, the temperature is 30℃, and the pH value is 6.5. The sample was incubated in a shaking table at 180r/min for 48h, repeated for 3 times. 0.1mL of sample liquid was taken and mixed into a 5mL test tube to supplement distilled water for the determination of nitrite nitrogen and ammonia nitrogen.

3. Results and Analysis

Through experimental research, the results and screened strains compared with other strains have obvious degradation of nitrogen strains and the advantages of harmful substances named ZL-1, the corresponding physiological and biochemical identification of the strains and 18SrDNA sequences sequencing, combined with the physiological and biochemical identification and phylogenetic analysis results of 18SrDNA sequences, it was determined that strain ZL-1 was *Wickerhamomyces anomalus*.

The mass concentration of $NH4^+$-N and NO_2^--N were determined by the national standard (GB/T7493-1987) and the national standard (HJ535 -- 2009).

3.1 Efficiency of Nitrite Nitrogen and Ammonia Nitrogen Removal At Different Temperature Drops

Experimental results that strain degradation of nitrite nitrogen and ammonia nitrogen ZL-1 the optimum temperature range for 20-35 ℃, among them, when the temperature of 30 ℃, the experimental samples before and after vaccination in the content of ammonia nitrogen was 2.12 mu mu g and 0.36 g, nitrite nitrogen content before and after inoculation of 0.5 mu mu g and 0.07 g, strain WP - 1 for the degradation of nitrite nitrogen and ammonia nitrogen rate was the highest, were 86.9% and 82.9%, respectively. See Figure 1 for details.

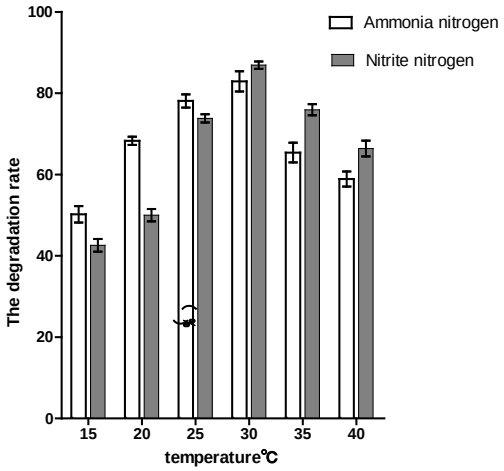

Fig.1 Effects of Different Temperatures on Degradation Efficiency

3.2 Efficiency of Dissolving Nitrite Nitrogen and Ammonia Nitrogen with Different Ph Decreases

The results showed that the optimal pH value for strain ZL-1 to degrade nitrite nitrogen and ammonia nitrogen was 5-8. When the pH value was 6, strain ZL-1 had the highest degradation rates of nitrite nitrogen and ammonia nitrogen, which were 83.8% and 77.7%, respectively. See Figure 2 for details.

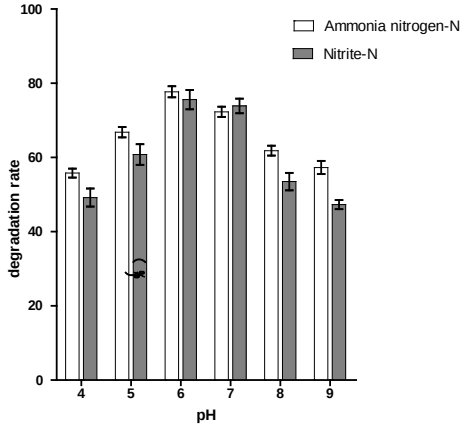

Fig.2 Influence of Different Ph on Degradation Efficiency

3.3 Different Carbon Sources Reduce the Efficiency of Nitrite Nitrogen and Ammonia Nitrogen

The results showed that the best degradation rate of nitrite nitrogen and ammonia nitrogen by strain ZL-1 was glucose, and the highest degradation rate of nitrite nitrogen and ammonia nitrogen by strain ZL-1 was 85.5% and 79.5%, respectively. Where a is sodium acetate, b is glucose and c is starch. See Figure 3 for details.

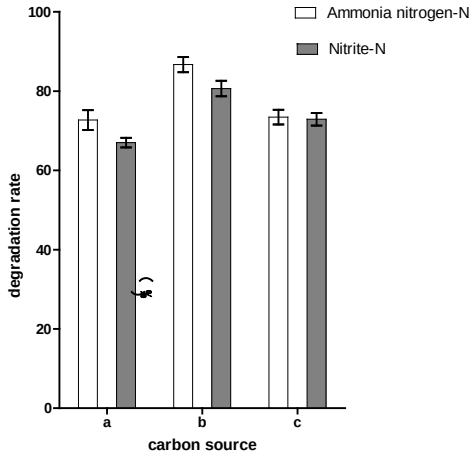

Fig.3 Influence of Different Carbon Source Types on Degradation Efficiency

3.4 Different C/n Decreases the Efficiency of the Decomposition of Nitrite Nitrogen and Ammonia Nitrogen

The results showed that the degradation rate of nitrite nitrogen and ammonia nitrogen by ZL-1 increased with the increase of C/N ratio when the C/N ratio range was 10-20.

When the C/N ratio was 20, strain ZL-1 had the highest degradation rate of nitrite nitrogen and ammonia nitrogen, which were 82.5% and 81.8%, respectively. See Figure 4 for details.

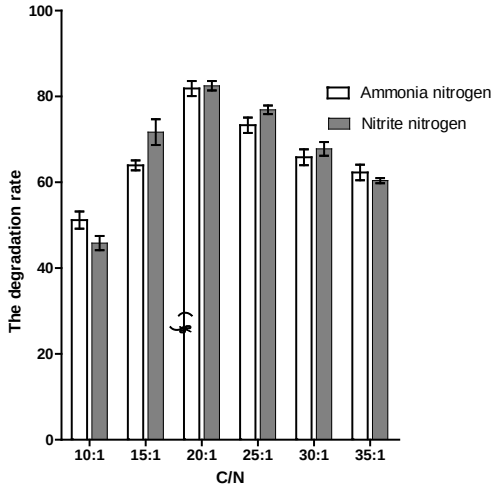

Fig.4 Influence of Different C/n on Degradation Efficiency

3.5 Discuss

According to the experimental results and relevant literature, in aquaculture, the degradation efficiency of nitrogen-containing harmful substance degrading strains in aquaculture water has a very important relationship with temperature, pH value, carbon source type and C/N ratio [1]. Gong Gangming et al[2]. showed that the optimal pH for lactic acid bacteria H2 to degrade nitrite was 6.5, and that when pH > 6.5 or pH < 6.5, its ability to degrade nitrite is further reduced; The experimental results of Gao Lei et al[3]. showed that the optimal C/N for the growth of Bacillus was 15. Wang Hui et al[4]. found that the optimal C/N ratio for the growth of strains XH1 and XH7 was 15. Liu Yuting et al. Experimental results show that the temperature of 25-40 °C, xylose-oxidized *Acrobacter* can grow normally, and maintain a high denitrification activity; At 30-35 °C, the growth and degradation of Xylose Oxidative *Acrobacter* were the best, and the degradation rate was over 99.5%[5].As is known to all, carbon source is one of the most important nutrient elements in the biological world, and is also an indispensable growth factor for microorganisms[6].

4. Conclusion

The optimal degradation conditions of the strain were as follows: When the temperature was 30℃, strain ZL-1 had the highest degradation rate of nitrite nitrogen and ammonia nitrogen, which were 86.9% and 82.9% respectively. The strain ZL-1 had the highest degradation rate of nitrite nitrogen and ammonia nitrogen, which were 87.3% and 86.7%, respectively. When the C/N ratio was 20, ZL-1 had the highest degradation rate of nitrite nitrogen and ammonia nitrogen, which were 82.5% and 81.8%, respectively. When pH value was 6, strain ZL-1 had the highest degradation rate of nitrite nitrogen and ammonia nitrogen, which were 83.8% and 77.7%, respectively.

References

[1] Wu Wei, Hu Gengdong, Qu Jianhong et al.Journal of Ecology and Rural Environment, 2007,23(4):37−40. (in Chinese with English abstract)

[2] Gong Gangming, Guan Shimin, Shao Hai et al.Isolation and identification of nitrite degrading lactic acid bacteria [J]. Food Industry, 2009(5): 12−13. (in Chinese with English abstract)

[3] GAO Lei, BAO Wei-yang, ZHANG Tian-wen, Shan Hong-wei, MA Xiao-xian. Effects of C/N ratio on the growth, antagonism and C/N ratio of Bacillus, Lactobacillus and Vibrio in water [J].Journal of Ocean University of China (Natural Science Edition), 2013, 43(01): 34-40. (in Chinese with English abstract)

[4] WANG H. Isolation and identification of ammonia-degrading bacteria and evaluation of water quality control effect of composite microbial agents [D].Shandong Agricultural University, 2017. (in Chinese with English abstract)

[5] Sun, FL, et al. Metagenomic Analysis of the Effect of Enteromorpha prolifera Bloom on Microbial Community and Function in Aquaculture Environment. Current Microbiology, 2020. 77(5): p. 816-825.

[6] Deng, M., et al., Community metagenomic assembly reveals microbes that contribute to the vertical stratification of nitrogen cycling in an aquaculture pond. Aquaculture, 2020. 520: p. 10.