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Algicidal and denitrification characterization of *Acinetobacter* sp. J25 against *Microcystis aeruginosa* and microbial community in eutrophic landscape water

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ABSTRACT

Acinetobacter sp. J25 exhibited good denitrification and high algicidal activity against toxic *Microcystis aeruginosa*. Response surface methodology (RSM) experiments showed that the maximum algicidal ratio occurred under the following conditions: temperature, 30.46 °C; *M. aeruginosa* density, 960,000 cells mL^{-1}; and inoculum, 23.75% (v/v). Of these, inoculum produced the maximum effect. In the eutrophic landscape water experiment, 10% bacterial culture was infected with *M. aeruginosa* cells in the landscape water. After 24 days, the removal ratios of nitrate and chlorophyll-a were high, 100% and 87.86%, respectively. The denitrification rate was approximately 0.118 mg NO_3^-–N·L^{-1}·h^{-1}. Moreover, the high-throughput sequencing result showed that *Acinetobacter* sp. J25 was obviously beneficial for chlorophyll-a and nitrate removal performance in the eutrophic landscape water treatment. Therefore, strain J25 is promising for the simultaneous removal of chlorophyll-a and nitrate in the eutrophic landscape water treatment.

1. Introduction

Cyanobacterial blooms, which could pose a threat to aquatic ecosystems and human health, are a frequent and harmful phenomenon in freshwater lakes and estuaries worldwide (Carey et al., 2012). In particular, blooms caused by toxic cyanobacteria, such as *Microcystis*, *Anabaena*, and *Cylindrospermopsis*, produce microcystin, which affects ecosystem functioning and creates a significant water quality problem (Yang et al., 2014a, 2014b; Zhu et al., 2014). As a fast and efficient method, chemical agents such as copper sulfate, potassium permanganate, hydrogen peroxide, and ozone are used for eutrophication control (Fan et al., 2013; Matthijs et al., 2012). However, chemical methods induce secondary pollution, which is potentially dangerous in aquatic ecosystems (Qu and Fan, 2010; Tang et al., 2012). Meanwhile, physical methods will also more easily result in secondary pollution to water (Paul and Pohnert, 2011). Thus, there is a need to explore ecologically safe ways to control harmful cyanobacterial blooms.

At present, although many algicidal bacteria, such as *Pseudoalteromonas*, *Sphingomonas*, *Staphylococcus*, *Bacillus amyloliquefaciens*, and *Cytophaga*, have been reported and exploited by researchers, each algicidal bacterium has its specific host algae (Kang et al., 2008; Kim and Lee, 2006; Yang et al., 2014a, 2014b). Therefore, use of algicidal bacteria to control algal bloom and red tide is more effective, ecological, and environmentally friendly (Bährs et al., 2012; Choi et al., 2005).

Recently, close associations between bacteria and microalgae have been reported, and bacteria living in the phycosphere of microalgae have been suggested to affect algal population dynamics and toxicity (Rooney-Varga et al., 2005). However, several environmental factors have been associated with algicidal effects involved in the termination and decomposition of algal blooms, including low temperature, starvation, salinity, visible and ultraviolet (UV) light, and air pressure (Oliver, 2010). Zhou et al. (2013) indicated that potassium release is the main cause of cell breakage resulting from exposure to copper sulfate, hydrogen peroxide, diuron, and ethyl 2-methylacetoacetate. Besides, high-resolution Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) brings a major fraction of dissolved organic matter (DOM) into our analytical window, providing high-accuracy molecular-level information regarding elemental and inferred structural composition that can be related to source (Stubbins and Dittmar, 2014) and photochemical transformations of DOM (Gonsior et al., 2009; Stubbins et al., 2010). Furthermore, the presence of algae may also strongly affect denitrification. For example, readily degradable benthic algae, serving as a nitrate and organic carbon source, can facilitate denitrification (McMillan et al., 2010; Sirivedhin and Gray, 2006). Some algae (e.g., diatoms) even have a synergistic effect on denitrifying bacteria (Ishida et al., 2008).

Pyrosequencing, developed by Roche 454 Life Science (Branford, CT, USA), is a high-throughput analytical method that can produce a large amount of DNA data through parallel sequencing-by-synthesis

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approach (Margulies et al., 2005). More than thousands of operational taxonomic units (OTUs) could be identified to investigate the microbial diversity in various environmental samples (Hu et al., 2012; Lu et al., 2012; Zhang et al., 2012). Pyrosequencing can provide better insights into the evolution of microbial community.

In this study, *Acinetobacter* sp. J25 was inoculated in the landscape water to evaluate the efficiency of algicidal and denitrification characteristics. Meanwhile, response surface methodology (RSM) analysis was then used to determine the optimum conditions (*Microcystis aeruginosa* density, temperature, and inoculum) of the strain J25. Factors affecting the performance of *Acinetobacter* sp. J25 with algae lysing were also comprehensively evaluated based on RSM analysis under the optimum conditions. 454 high-throughput pyrosequencing was used to analyze the bacterial communities and investigate the algae-lysing and denitrification performance and their community structure. Furthermore, this study would reveal the relationship between bacterial community structure, chlorophyll-a, and nitrate removal performance in a reactor.

2. Materials and methods

2.1. Algal and bacterial culture

M. aeruginosa was obtained from the Freshwater Algae Culture Collection of Institute of Hydrobiology (FACHB), Chinese Academy of Sciences (Wuhan, China). Before being used as an inoculant, it was cultured for 7 days to reach the log phase under the following conditions: sterilized BG11 media (Rippka et al., 1979); 3300 lx white light, light:dark = 12:12 h; 28 °C. The BG11 media used in this study comprised the following reagents in the amounts given per liter: $NaNO_3$ 1.5 g; K_2HPO_4 0.04 g; $MgSO_4 \cdot 7H_2O$ 0.075 g; $CaCl_2 \cdot 2H_2O$ 0.036 g; citric acid 0.006 g; ferric ammonium citrate 0.006 g; ethylenediaminetetraacetic acid (EDTA) 0.001 g; Na_2CO_3 0.02 g; and A5 (trace element) solution 1 mL. The ingredients of A5 solution per liter were as follows: H_3BO_3 2.86 g, $MnCl_2 \cdot 4H_2O$ 1.81 g, $ZnSO_4 \cdot 7H_2O$ 0.22 g, $CuSO_4 \cdot 5H_2O$ 0.079 g, $Na_2MoO_4 \cdot 2H_2O$ 0.039 g, and $Co(NO_3)_2 \cdot 6H_2O$ 0.049 g.

Strain J25 (GenBank accession number KT023013) (Su et al., 2016) was isolated from a eutrophic Qu Jiang lake, Xi'an, China. In order to analyze algicidal characteristics, the strain J25 was grown in sterilized Luria–Bertani (LB; 10 $g \cdot L^{-1}$ of tryptone, 5 $g \cdot L^{-1}$ of yeast extract, 10 $g \cdot L^{-1}$ of NaCl, pH 7.2) medium at 30 °C and reached logarithmic growth phase.

2.2. Box–Behnken design for optimizing the environmental factors

The RSM is a statistical experimental design for the optimization of biological processes. This method can build models, evaluating the effects of factors and searching for optimum condition of factors for desirable responses. RSM was used to investigate the algae-lytic effect of J25 bacteria on *M. aeruginosa* at the given conditions of temperature, inoculum, *M. aeruginosa* density.

The RSM was used to optimize and evaluate the main effects, interaction effects, and quadratic effects of the independent variables. The experimental design was performed with three factors at three levels (+1, −1, 0): temperature (25, 30, and 35 °C); inoculum (v/v: 10%, 20%, and 30%); and *M. aeruginosa* density (4×10^5, 12×10^5, and 20×10^5 cells mL^{-1}). Levels of these three independent variables were defined according to the Box–Behnken design, and 17 experiments were required for the procedure (Table 1). Design expert software was designed using the Minitab program (version 16, Minitab Inc., USA).

In order to predict the optimal point, a second-order polynomial function was fitted to correlate the relationship between three factors and the relatively algicidal ratio.

The function for the three factors is

$$Y = c_0 + a_1X_1 + a_2X_2 + a_3X_3 + \beta_{11}{X_1}^2 + \beta_{22}{X_2}^2 + \beta_{33}{X_3}^2 + X_1X_2\beta_{12} + X_1X_3\beta_{13} + X_2X_3\beta_{23},$$

Table 1
Box–Behnken experimental design and the corresponding responses.

Treatment	Temperature (°C)	*M. aeruginosa* density (cells mL^{-1})	Inoculum (v/v, %)	Algicidal ratio (%)
1	30	12×10^5	20	84.74
2	35	4×10^5	20	68.4
3	30	20×10^5	10	36.85
4	30	12×10^5	20	85.7
5	25	12×10^5	10	30.6
6	30	4×10^5	30	70.01
7	30	12×10^5	20	83
8	30	12×10^5	20	83.22
9	30	4×10^5	10	27.9
10	35	20×10^5	20	36.28
11	35	12×10^5	30	70.52
12	35	12×10^5	10	35.44
13	25	12×10^5	30	48.69
14	30	12×10^5	20	83.77
15	30	20×10^5	30	62.59
16	25	20×10^5	20	43.84
17	25	4×10^5	20	46.8

where Y is the predicted response; c_0 is constant; X_1, X_2, and X_3 are independent factors; a_1, a_2, and a_3 are linear coefficients; β_{12}, β_{13}, and β_{23} are cross-product coefficients; and β_{11}, β_{22}, and β_{33} are quadratic coefficients.

2.3. Algicidal efficiency assessment

In order to evaluate the efficiency of algicidal and denitrification characteristics, the 10% (v/v) inoculum of sample J25 was added to 5 L of eutrophic landscape water obtained from a eutrophic Qu Jiang lake (34.202534° to 34.211416° N, 108.989245° to 108.993611° E) in Xi'an, China. Meanwhile, the same volume of sterilized LB medium was also added to eutrophic landscape water as a control. The same *M. aeruginosa* cultures were added to eutrophic landscape water in both treatment and control, and the initial *M. aeruginosa* density was 300,000 cells mL^{-1}. This experiment was conducted at a certain temperature range (25–35 °C). Nitrate and chlorophyll-a concentrations of the samples were measured from days 0–24 every 2 days.

2.4. Microbial community analysis

2.4.1. DNA extraction, PCR amplification, and high-throughput sequencing

Both samples Z1 and Z2 were collected after 24 days in treatment and control (Section 2.3). Bacterial genomic DNA was extracted using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) following the manufacturer's protocols. For construction of gene libraries, the PCR amplification (Supplementary materials) used the universal bacteria primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), which were targeting the V1 and V3 hypervariable regions (Liao et al., 2013). Then, the pyrosequencing procedure was performed similarly to our previous approach (Hao et al., 2013).

2.4.2. Sequence analysis and phylogenetic classification

The sequence analysis followed the method (Supplementary materials) described in our previous study (Hao et al., 2013). The OTU, rarefaction curves, and diversity indices (Ace and Chao 1) were determined by Mothur ver. 1.17.0. Taxonomic classification of the sequences was performed using the RDP Classifier (Version 2.2) with a set confidence threshold of 80%.

2.5. Analytical measurements

Briefly, culture samples were centrifuged at 5000 rpm for 8 min for chemical analysis. Chlorophyll-a was extracted with 90% alcohol, and the whole procedure was carried out in darkness at −20 and −4 °C.