BIOMEDICAL AND ENVIRONMENTAL SCIENCES **20**, 274-278 (2007)

Screening and Characterization of a Bioflocculant Produced by *Aeromonas* sp.

XIAO-MING LI[1], QI YANG, KAI HUANG, GUANG-MING ZENG, DE-XIANG LIAO, JIN-JING LIU, AND WEN-FANG LONG

College of Environmental Science and Engineering, Hunan University, Changsha 410082, Hunan, China

Objective To isolate the bioflocculant-producing bacteria from activated sludge and investigate the flocculating characteristics of the newly isolated bioflocculant. **Methods** Bacteria were screened from activated sludge samples to isolate bioflocculant-producing bacteria. Flocculating activity was used as a measure of the flocculating capability of the bioflocculant. **Results** A novel bioflocculant-producing bacterium was isolated, which was identified to belong to genus *Aeromonas* and named as *Aeromonas* sp. N11. Flocculating activity increased in the presence of K^+, Na^+, or Ca^{2+}. The highest flocculating activities for kaolin suspension were obtained in acidic pH ranges, and optimum pHs for it were 3.0, 4.0, and 5.0 with 1 mmol/L K^+, Ca^+, and Na^+ present, respectively. The highest flocculating activities for soil suspension were observed at pH 8.0. The bioflocculant had a good flocculating activity and could achieve a flocculating activity of 92.4% for kaolin suspension at a dosage of only 1 $mg \cdot L^{-1}$, and its activity in kaolin suspension was decreased by only 9.2% after heating at 100℃ for 60 min. **Conclusion** The bioflocculant produced by *Aeromonas* sp. N11 has strong flocculating activity and high stability, which affords high possibility of its practical use.

Key words: *Aeromonas* sp.; Bioflocculant; Biopolymer; Flocculation

INTRODUCTION

Flocculating agents are widely used in industrial processes including wastewater treatment, downstream processing, food and fermentation processes. Flocculants are generally classified into three major groups: (a) inorganic flocculants such as aluminum sulfate and polyaluminum chloride; (b) organic synthetic polymeric flocculants, such as polyacrylamide derivatives and polyethylene amine; and (c) naturally occurring flocculants, such as chitosan and sodium alginate, and microbial flocculants[1-2].

Among the flocculants, synthetic chemical polymeric flocculants are commonly used because of their effectiveness and low cost. Although synthetic flocculants, such as polyacrylamide derivatives, are frequently used, there is evidence that the acrylamide monomer is a strong carcinogen and neurotoxic to humans[3-4]. Moreover, some reports indicate that aluminum, as the main component of polyaluminum chloride, may induce Alzheimer's disease[2]. In contrast, the microbial bioflocculant can be nontoxic, harmless and without secondary pollution. Because of these concerns, the use of microbial bioflocculant is expected to increase in various fields[5]. Oh Hee-Mock reported that a bioflocculant could successfully harvest *Chlorella vulgaris* from culture broth[6].

In this paper, the isolation and characterization of a new bioflocculant are reported.

MATERIALS AND METHODS

Isolation and Growth of Bioflocculant-producing Bacteria

Many types of bacteria were screened from activated sludge samples. The composition of the medium for screening was as follows: 20 g glucose, 2 g KH_2PO_4, 5 g K_2HPO_4, 0.1 g NaCl, 0.2 g $(NH_4)_2SO_4$, 0.5 g carbamide, and 0.5 g yeast extract dissolved in 1 L deionized water with the initial pH adjusted to 8.0. After sterilization and inoculation of the medium, the bacterium was cultured on a rotary shaker at 170 rpm for 3 days at 30℃. Kaolin suspensions at a

[1]Correspondence should be addressed to Xiao-Ming LI. Tel: 86-731-8823967. Fax: 86-731-8822829. E-mail: xmli@hnu.cn

Biographical note of the first author: Xiao-Ming LI, male, born in 1966, Ph. D., Professor at Hunan University, research interest in environmental biotechnology and water pollution control engineering.

0895-3988/2007
CN 11-2816/Q
Copyright © 2007 by China CDC

concentration of 5000 mg/L were then used to evaluate the flocculating capability of a series of the culture broths.

Determination of Flocculating Activity

Flocculating activity was used as a measure of the flocculating capability of the bioflocculant. A 0.5 g of kaolin clay (average diameter 4 μm) was suspended in 100 mL deionized water, and 2 mL of the bioflocculant (culture broth) was added to the kaolin suspension. The mixture was stirred vigorously for 20 s and left without shaking for 10 min. The absorbance of the supernatant and blank control without bioflocculant was measured at 660 nm (as OD_{660} and OD_{blank}, respectively) with a spectrophotometer. The flocculating activity (η) was defined and calculated as follows:

$$\eta = (OD_{blank} - OD_{660})/OD_{blank} \times 100 \quad (1)$$

Partial Purification of Bioflocculant

The bioflocculant was simply purified. The culture broth was centrifuged at 8000 r/min for 30 min. Then two volumes of cold ethanol were added to supernatant of the culture broth to precipitate the bioflocculant and the mixture was left overnight at 4℃. The precipitate obtained was dissolved in distilled water again. After lyophilization, a crude bioflocculant was obtained.

RESULTS

Screening and Identification

Five bioflocculant-producing bacteria were selected among more than 100 mucous colonies isolated from activated sludge samples from a municipal wastewater treatment plant. A strain with high flocculating activity was selected after rescreening. The selected isolate was an aerobic, Gram-negative, oxidase-positive and catalase-positive, non-endospore forming rod, colonies of which were viscous, smooth, and cream-colored. From these taxonomic characteristics, according to classical identification methods of bacteria[7], the bacterium was assigned to genus *Aeromonas* and named as *Aeromonas* sp. N11.

Bioflocculant-producing Properties

First, an optimum culture medium for production of bioflocculant was investigated. Among the carbon sources such as glucose, starch, maltose, fructose and glycerol, sucrose was favorable for bioflocculant production of *Aeromonas* sp. N11. Among the nitrogen sources, multiple nitrogen sources were better than the single nitrogen source and yeast extract was beneficial to bioflocculant production. NaCl could increase the flocculation activity of the bioflocculant produced. After orthogonal experiment, by measuring the amount and flocculating activity of the bioflocculant, the optimum culture medium was determined to be as follows: 20 g sucrose, 0.8 g yeast extract, 0.5 g carbamide, 0.5 g $(NH_4)_2SO_4$, 7 g NaCl in 1 L deionized water (data not shown). Optimum initial pH of the medium was 8.0.

Next, optimum culture time for production of the bioflocculant was investigated. Figure 1 shows the time courses of cell growth and flocculating activity of culture broth of strain N-11 cultured aerobically with the optimum culture medium on a rotary shaker at 170 rpm for 84 h at 30℃.

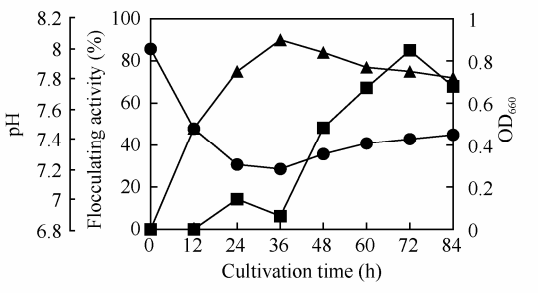

FIG. 1. Time course of the growth (OD_{660}, ▲), flocculating activity (■), pH (●) of culture broth of strain N-11 on a rotary shaker at 170 rpm, 30℃ for 84 h.

Bioflocculant Characterization

Bioflocculant characterization was examined in a kaolin suspension. The influence of cations on flocculating activities was studied and compared. The result is presented in Fig. 2 showing the flocculating activity of the bioflocculant in the kaolin suspension containing 0.1 mmol/L or 1 mmol/L cations. Of the various cations tested on the flocculating activity of bioflocculant, the strongest stimulating cations were K^+, Na^+, and Ca^{2+}.

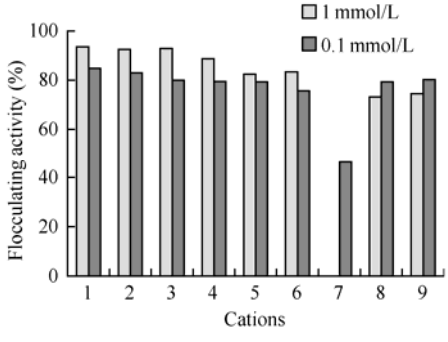

FIG. 2. Effect of cations on flocculating activity. 1 K^+; 2 Na^+; 3 Ca^{2+}; 4 Mg^{2+}; 5 Al^{3+}; 6 Fe^{2+}; 7 Fe^{3+}; 8 Zn^{2+}; 9 Mn^{2+}.

Since K^+, Na^+, and Ca^{2+} are effective for the stimulation of the flocculating activity, the effects of the flocculant concentration on the flocculating activity were examined in a kaolin suspension containing K^+, Na^+, and Ca^{2+}, respectively and the results are depicted in Fig. 3.

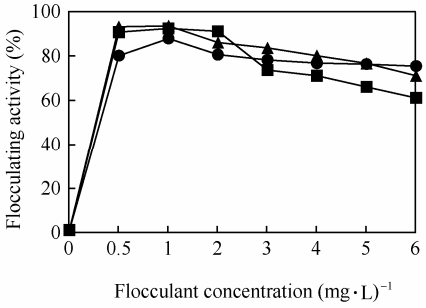

FIG. 3. Effect of flocculant concentration on flocculating activity in kaolin suspension containing 1 mmol/L K^+ (■), 1 mmol/L Na^+ (▲), 1 mmol/L Ca^{2+} (●), separately.

The flocculant was also found to be an effective stimulator with low dosage requirements. As shown in Table 1, compared with other bioflocculants, the bioflocculant produced by *Aeromonas* sp. N11 obtained high flocculating activity at a relatively low dosage.

Figures 4 and 5 show the effects of pH on flocculating activity in kaolin suspension and soil suspension containing 1 mmol/L K^+, 1 mmol/L Na^+, and 1 mmol/L Ca^{2+}, respectively. The effects of pH on flocculating activity varied with suspensions.

Thermal stability of flocculant was also examined. Figure 6 shows the heat-stability curve of bioflocculant. The flocculant solution was heated at 100℃ for indicated time and the flocculating activity for kaolin suspension was measured.

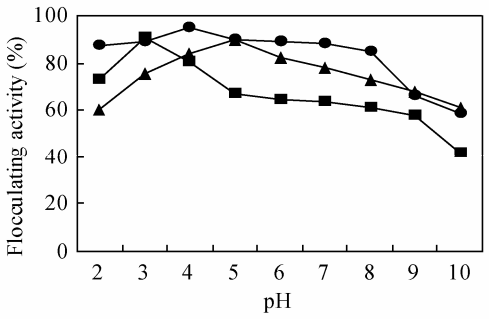

FIG. 4. Effect of pH on flocculating activity in kaolin suspension containing K^+(■), Na^+ (▲), Ca^{2+} (●), separately. Final concentrations: flocculant, 1 mg·1^{-1}; cationss, 1 mmol/L. The pH of the suspended kaolin solution was adjusted by addition of 0.1 mol/L HCl or 0.1 mol/L NaOH.

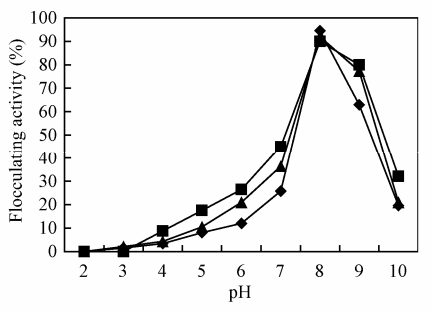

FIG. 5. Effect of pH on flocculating activity in soil suspension containing K^+(■), Na^+(▲), and Ca^{2+}(●), separately. Final concentrations: flocculant, 1 mg·L^{-1}; cations, 1 mmol/L. The pH of the suspended kaolin solution was adjusted by addition of 0.1 mol/L HCl or 0.1 mol/L NaOH.

TABLE 1

Dosage of Different Bioflocculants for Flocculating Kaolin Suspension

Bioflocculant-producing Bacteria	Isolation Source	Optimum Flocculant Concentration (mg·L^{-1})	Flocculating Activity (%)	References
Aeromonas sp. N11	Activated Sludge	1	92.4	This Work
Bacillus sp. NOC-1	Soil	20	33[a]	Takeda *et al*. 1991[8]
Pseudomonas sp.	Soil	20	11[a]	Yokoi *et al*. 1998[9]
Bacillus sp. PY-90	Soil	20	15[a]	H. Suh *et al*. 1997[10]
Bacillus firmus	Soil	4	65[a]	Salehizadeh *et al*. 2002[11]
Klebsiella sp.	Activated sludge	15	1.4[a]	Dermlim *et al*. 1999[12]
Bacillus coagulants AS101	Activated sludge	30	92	Salehizadeh *et al*. 2000[13]
Nannocystis sp. NU-2	Coastal soil	10	90	J. Zhang *et al*. 2002[14]

Note. [a]Flocculating activity=$1/OD_{blank}$-$1/OD_{550}$.

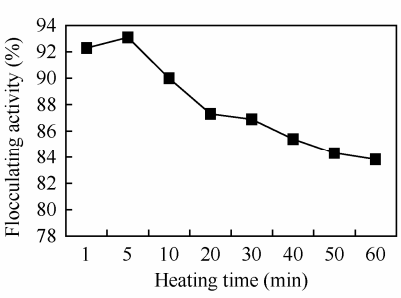

FIG. 6. Effect of heat treatment on flocculating activity. The flocculant solution was boiled in a water bath for indicated time and the flocculating activity was measured.

DISCUSSION

From Fig. 1 we can see that the rapid growth of strain N-11 in the first 36 h of cultivation of cultivation correlated with a decline of pH from 8 to 7.2. The production of the flocculant was not associated with cell growth, which was produced only during the stationary phase. The flocculating activity started to increase rapidly after 36 h of cultivation of cultivation while the growth ceased and reached its maximum after 72 h of cultivation. The amount of crude bioflocculant (2.25 g/L) was obtained after 72 h.

Figure 2 shows that among the various cations tested on the flocculating activity of bioflocculant, the strongest stimulating ones were K^+, Na^+, and Ca^{2+}. It is assumed that K^+, Na^+, and Ca^{2+} stimulate flocculation by accelerating bridge formation between suspended-particles and bioflocculant. Although it is known that the addition of cations to suspensions is necessary to induce the effective flocculation capability of bioflocculants, the effects of cations on the flocculating activity of the bioflocculant are different from other bioflocculants reported previously[15].

As shown in Fig. 3, the highest flocculating activity was obtained with the bioflocculant at 1 $mg \cdot L^{-1}$, and the activity initially increased with increasing flocculant dosage but then decreased as the adsorption of excess bioflocculant restabilized the kaolin particles; thus the attractive forces of other particles were reduced and flocculating activity decreased[16].

From Figs. 4 and 5, we can see that in kaolin suspension, the highest flocculating activities of bioflocculant were obtained in acidic pH ranges, and optimum pHs for its flocculating activity were 3, 4, and 5 with 1 mmol/L K^+, Ca^+, Na^+ present, respectively. In soil suspension, the highest flocculating activities of bioflocculant were observed at pH 8.0 and the flocculating activities decreased rapidly when the pH was adjusted to more or less than 8.

Figure 6 shows the heat-stability curve of bioflocculant. The bioflocculant maintained its stability and activity in kaolin suspension were decreased by only 9.2% after heating at 100℃ for 60 min, suggesting that the bioflocculant produced by strain N11 is thermo-stable.

It has been reported that bioflocculants from *R. erythropolis*[17] and *Bacillus firmus*[11] are also heat-stable, but flocculating activity remains only about 50% of the initial activity after heating for 15 min in boiling water.

In conclusion, a novel bioflocculant-producing bacterium has been isolated from activated sludge samples, which is identified to be in the genus *Aeromonas* and named as *Aeromonas* sp. N11.

The flocculating activity increases in the presence of K^+, Na^+, or Ca^{2+}. The highest flocculating activities for kaolin suspension are in acidic pH ranges. The optimum pHs for its flocculating activity are 3.0, 4.0, and 5.0 with 1 mmol/L K^+, Ca^+, and Na^+ present, respectively, and that for soil suspension is 8.0.

A flocculating activity of 92.4% for kaolin suspension can be reached at a dosage of only 1 $mg \cdot L^{-1}$ and its activity in kaolin suspension is decreased by only 9.2% after heating at 100℃ for 60 min, demonstrating that the bioflocculant has strong flocculating activity and high stable quality, which affords high possibility of its practical use.

REFERENCES

1. Kurane R, Matsuyama H (1994). Production of a bioflocculant by mixed culture. *Biosci Biotech Biochem* **58**, 1589-1594.
2. Kurane R, Hatamochi K, Kakuno T (1994b). Purification and characterization of lipid bioflocculant produced by *Rhodococcus erythropolis*. *Biosci Biotechnol Biochem* **58**, 1977-1982
3. Yokoi H, Natsuda O, Hirose J, *et al*. (1995). Characteristics of a biopolymer flocculant produced by *Bacillus* sp. PY-90. *J Ferment Bioeng* **79**, 378-380.
4. Yokoi H, Yoshida T, Mori S, *et al*. (1997). Biopolymer flocculant produced by an *Enterobacter* sp. *Biotechnol Lett* **19**, 569-573.
5. Salehizadeh H, Van Loosdrecht1 M C M, (2004). Production of polyhydroxyalkanoate (PHA) by mixed culture: recent trends and biotechnological importance. *J Biotech Advance* **22**, 261-279.
6. Oh H M, Lee S J, Park M H, *et al*. (2001). Harvesting of *Chlorella vulgaris* using a bioflocculant from *Paenibacillus* sp. AM49. *Biotechnol Lett* **23**, 1229-1234.
7. Institute of Microbiology, Chinese Academy of Sciences. Classical identification methods of bacteria. Beijing: Science Press, 1978.

8. Takeda M, Kurane R, Koizumi J, *et al*. (1991). A protein bioflocculant produced by *R. erythropolis*. *Agric Biol Chem* **55**, 2663-2664.
9. Yokoi H, Yoshida T, Hirose J, *et al*. (1998). Biopolymer flocculant produced by an *Pseudomonas* sp. *Biotechnol Lett* **12**, 511-514.
10. Suh H, Kwon G, Lee C, *et al*. (1997). Characterization of bioflocculant produced by *Bacillus* sp. DP-152. *J Ferment Bioeng* **84**(2), 108-112.
11. Salehizadeh H, Shojaosadati S A (2002). Isolation and characterization of a bioflocculant produced by *Bacillus firmus*. *Biotechnol Lett* **24**, 35-40.
12. Dermlim W, prasertsan P, Doelle H (1999). Screening and characterization of bioflocculant produced by isolated *Klebsiella* sp. *Appl Microbiol Biotechnol* **52**, 698-703.
13. Salehizadeh H, Vossoughi M, Alemzadeh I (2000). Some investigations on bioflocculant producing bacteria. *Biochem Eng* **5**, 39-44.
14. Zhang J, liu Z, Wang S, *et al*. (2002). Characterization of a bioflocculant produced by the marine myxobacterium *Nannocystis* sp. Nu-2. *Appl Microbiol Biotechnol* **59**, 517-522.
15. Deng S B, Bai R B, Hu X M, *et al*. (2003). Characteristics of a bioflocculant produced by *Bacillus mucilaginosus* and its use in starch wastewater treatment. *Appl Microbiol Biotechnol* **60**, 588-593.
16. Kwon G S, Moon S H, Hong S D, *et al*. (1996). A novel biopolymer flocculant produced by *Pestalotiopsis* sp. KCTC 8637P. *Biotechnol Lett* **18**, 1453-1464.
17. Kurane R, Takeda K, Suzuki T (1986). Screening for and characteristics of microbial flocculants. *Agric Biol Chem* **50**, 2301-2307.

(*Received May* 3, 2006 *Accepted March* 19, 2007)