

## SHORT COMMUNICATION

# Characterization of Bioflocculant Producing-Bacteria Isolated from Tapioca Waste Water

SURYANI\*, LAKSMI AMBARSARI, I MADE ARTIKA, HARTUTIK EKA SUSANTI

*Department of Biochemistry, Faculty of Mathematics and Natural Sciences  
Darmaga Campus, Bogor 16680, Indonesia*

Received June 7, 2010/Accepted December 15, 2011

**Bioflocculant producing-bacteria from tapioca waste water were characterized. Two bacterial isolates i.e. LT-5 and LT-6 isolates had high flocculation activity, with activity of 68.92 and 71.38% respectively. The flocculation activity of LT-5 isolate increased at pH 2.0-4.0 (acidic condition), however the activity of LT-6 increased at pH 6.0-8.0 (neutral). Addition of 0.05% of  $AlCl_3$  as cation was the most effective and had important role in flocculation activity. Based on the morphological properties, LT-5 isolate was identified as *Chromobacterium violaceum* and LT-6 isolate was identified as *Citrobacter koseri*.**

Key words: bioflocculant producing-bacteria, tapioca wastewater, flocculation activity, cation

### INTRODUCTION

Synthetic flocculant with high molecular weight (such as polyacrylamide derivatives) have been shown to be harmful to the environment and human health especially as neurotoxic and carcinogenic agents of acrylamide monomers and as source of unbiodegradable pollutants, despite it has effective flocculating performance and low cost. Because of the negative effect of synthetic flocculant, the used of biodegradable flocculant produced by microorganisms has been investigated in order to find the biodegradable flocculant in widely applications.

Bioflocculant is an extracellular polymer produced by microorganisms during their growth, resulting in the formation of stable aggregates of flocs and it has different composition such as protein flocculants, polysaccharide flocculants, glycoprotein flocculants and poly(amino acid) flocculant (Jie *et al.* 2006). Many studies have been reported on bioflocculants to replace synthetic flocculants which are industrially used. Bioflocculant may potentially be applied in drinking and wastewater treatment, downstream processing, and fermentation processes (Salehizadeh & Shojaosadati 2001). Kurane and Nohata (1997) found that bioflocculants have widebroad spectrum to be applied in industries. They found that bioflocculants produced by *Alcaligenes latus* can absorb water 1000 time of its weight and 5 time stronger than that of synthetic absorber polymers.

In recent years, a major emphasis has been laid on the search for novel bioflocculant producing-bacteria with

different composition and properties and several of them have been under investigated. The use of bioflocculants is very prospective in the future, therefore it is important to explore potential bioflocculant producing-bacteria with high activity bioflocculant-producing ability and improve the flocculating activity. In this study, the screening of a bioflocculant producing-bacteria and optimization of the flocculation activity were carried out.

### MATERIALS AND METHODS

**Isolation Bioflocculants Producing Bacteria.** Samples were taken from tapioca waste water at Sukaraja region, Bogor, West Java, Indonesia. Isolation of bacteria was done according to general method for microbe isolation by using NB medium. One ml of samples were cultivated in 9 ml of NB medium and incubated at 27 °C for 16 hours. The samples were serially diluted and 0.1 ml was plated onto petri dish. After 16 hours incubation, bacterial colonies were counted and grown in NB medium.

**Bioflocculants Production.** Pure bacterial isolates were inoculated into 30 ml of production medium. Composition of the medium was 10 g of glucose, 10 g of sucrose, 0.5 g of peptone, 0.5 g of yeast extract, 1 g of urea, 0.5 g of  $(NH_4)_2SO_4$ , 1.5 g  $KH_2PO_4$ , 4.5 g of  $K_2HPO_4$ , 0.2 g  $MgSO_4$ , and 0.1 g of NaCl in 1 l of sterile aquades and the pH was adjusted to 7.3 (Kurane *et al.* 1997). The cultures were incubated in rotary shaker at 27 °C with 120 rpm for 70 hours. The bacterial cultures were used for determination of flocculation activity.

**Determination of Flocculation Activity.** The flocculation activity was determined by optimization of

\*Corresponding author. Phone/Fax: +62-251-8423267,  
E-mail: ani3110@yahoo.com

coagulant and flocculation activity assay in kaolin suspension (Kurane *et al.* 1997). Optimization of coagulant was done by using  $\text{FeCl}_3$  with dosage range between 0.01-1.0% b/v. The solution was mixed gently at room temperature (27 °C) and left for standing in 5 minutes. The formation of visible aggregates was observed and measured its absorbance at wavelength of 550 nm. Flocculation activity assay was done in 80 ml of kaolin suspension (5.5 g/l) and mixed with the optimum of  $\text{FeCl}_3$  dosage solution and 1 ml of culture, then adjusted to 100 ml with sterile aquades. The solution was mixed gently at room temperature (27 °C) and left for standing in 5 minutes. The optical density (OD) was measured at 550 nm. Selection of bioflocculant producing-bacteria based on the highest activity of flocculation. Flocculation activity was calculated by the following equation:

$$\text{Flocculation activity} = \frac{A-B}{A} \times 100\%$$

where: A = OD<sub>550</sub> of reference, B = OD<sub>550</sub> of sample

**Optimization of Flocculation Activity.** Optimization of flocculation activity was conducted according to Kurane *et al.* 1997 with modification on cation addition, pH and temperature. Effect of cation addition were done in 80 ml of kaolin suspension (5.5 g/l), 1 ml of bacterial culture and several salt solution (0.05%) as cation sources i.e.  $\text{CaCl}_2$ ,  $\text{MgSO}_4$ ,  $\text{AlCl}_3$ ,  $\text{FeCl}_3$ ,  $\text{FeSO}_4$ , dan  $\text{ZnSO}_4$ . The effect of pH in kaolin suspension was determined at pH range of 2.0-9.0. The effect of pH on flocculation activity was done at temperature of 30, 40, 50, 60, and 70 °C. In this assay, the bacterial cultures were incubated for 30 minutes and the flocculation activity was determined as described previously.

**Bacterial Identification.** Identification of bioflocculant producing-bacteria was done for 2 selected isolates at Indonesian Research Center for Veterinary Sciences/BALITVET).

## RESULTS

**Flocculation Activity of Bioflocculants Producing-Bacteria.** Temperature and of sampling sites was 27.5 °C

and pH 7.0 respectively. Eight isolates of bioflocculants producing-bacteria were isolated from the waste water tapioca (Table 1). Production of bioflocculant was occurred at stationary phase after 70 hours incubation on a rotary shaker at 120 rpm (unpublished data). Addition of substrate in growth medium affected the bacterial growth and bioflocculant production.

**Flocculation Activity in Kaolin Suspension.** Based on optimization of  $\text{FeCl}_3$  dosage as coagulant, the optimum dosage was 0.05% b/v of  $\text{FeCl}_3$  which had the highest flocculation activity (55.47%) compared to other concentrations (Table 2). Flocculation activity increased up to 55.47% at 0.01-0.05%. There was no flocculation activity observed at 1% of  $\text{FeCl}_3$ . The ability of each isolate showed different flocculation activity (Table 1). The highest activity of flocculation activity was shown by LT-5 and LT-6 isolates with the flocculation activity up to 68.92 and 71.38%, respectively. Therefore, LT-5 and LT-6 isolates were selected for further investigation (optimization of flocculation activity).

**Optimum Condition of Flocculation Activity.** Bacterial isolate of LT-5 gave optimum activity when  $\text{AlCl}_3$  was used as cation with activity of 30.76%, whereas LT-6 isolate gave the optimum activity when  $\text{AlCl}_3$  and  $\text{FeCl}_3$  were used as cation with activity of 62.29 and 54.12%, respectively (Figure 1). Cation addition gives the effect significantly on flocculation activity for all isolates in kaolin suspension. Optimum flocculation activity was found on addition of  $\text{AlCl}_3$  0.03%.

The result showed that pH had significant effect on flocculation activity. Each isolate had different optimum pH for flocculation activity (Figure 2). Flocculation activity of LT-5 isolate was increased at pH 2.0 up to 4.0 (acidic condition) with flocculation activity up to 40.47%. However, LT-6 isolate had optimum pH in the range of 6.0-8.0 (neutral), and the highest flocculation activity was at pH 6.0 (42.43%).

Temperature effect on flocculation activity showed that LT-5 isolate had optimum temperature at 40 °C with flocculation activity up to 73.11%, whereas LT-6 isolate

Table 1. Morphological colony and their flocculation activity of bacterial isolates isolated from tapioca waste water

Isolate	Color/shape	Rate of flocculation formation	Flocculation activity (%)
LT-1	White/round plate	+	47.07
LT-2	Yellow/round	++	62.77
LT-4	Yellow/rooty	+	58.46
LT-5	Blue/rooty	+++	68.92
LT-6	Yellow greenish	+++	71.38
LT-9	Green	++	61.23
LT-11	Light yellow	-	13.54
LT-12	White/round thick	+	50.46

- = none, + = low, ++ = medium, +++ = high.

Table 2. Optimization of  $\text{FeCl}_3$  dosage as coagulant

Dosage of $\text{FeCl}_3$ (%)	Rate of floc formation	Optical density of supernatant	Flocculation activity (%)
0.010	++	0.203	30.48
0.025	+++	0.155	46.92
0.050	+++	0.130	55.47
0.100	+	0.205	29.79
1.000	+	0.363	-24.70

+ = low, ++ = medium, +++ = high.

had optimum temperature at 30 °C with flocculation activity up to 75.94% (Figure 3). Flocculation activity for LT-6 isolate was decreased slightly at 40 °C compared with at 30 °C. Flocculation activity at 40 °C was 73.58% and this flocculation activity was the same value with LT-5 isolate.

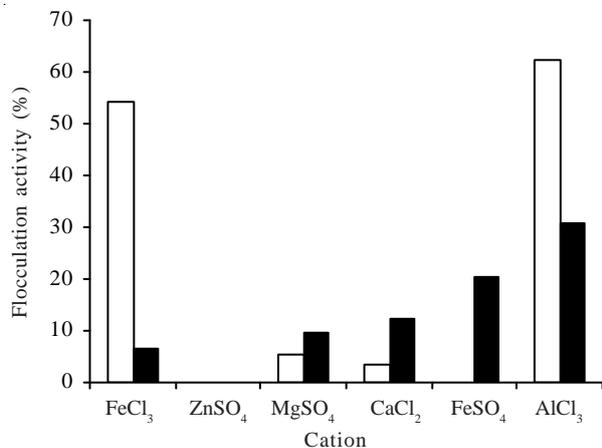


Figure 1. Effect of cation addition on flocculation activity of bioflocculant from LT-5 and LT-6 isolates. □: LT-5, ■: LT-6.

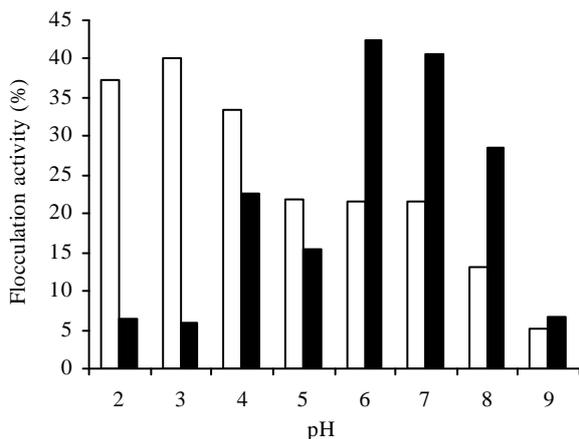


Figure 2. Effect of pH on flocculation activity of bioflocculant from LT-5 and LT-6 isolates. □: LT-5, ■: LT-6.

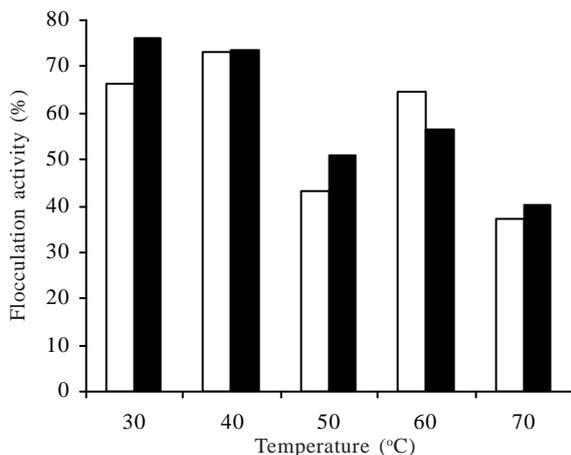


Figure 3. Effect of temperature on flocculation activity of bioflocculant from LT-5 and LT-6 isolates. □: LT-5, ■: LT-6.

**Bacterial Identification.** Identification result showed that LT-5 isolate was *Chromobacterium violaceum* and LT-6 isolate was *Citrobacter koseri*.

**DISCUSSION**

Characterization of bioflocculant producing-bacteria was carried out from the waste water tapioca. The wastewater tapioca contained soluble starch and other organic compounds as carbon sources. This condition can promote the growth of bioflocculant producing-bacteria. The starch can be modified into a flocculant through chemical reaction (Khalil & Aly 2001). Through the action of bacteria, starch can be easily changed into an effective polysaccharide or glycoprotein bioflocculants. Therefore, the bioflocculant producing-bacteria from the waste tapioca can grow easily. Yue (2006) found on *Klebsiella* sp. that high flocculating activity of the culture containing soluble starch mainly came from soluble starch which is also a polysaccharide. Soluble starch was the most suitable agent for bioflocculant, the flocculating rate of 0.5 ml of the sterile medium containing soluble starch was 70.1%. Zhang *et al.* 2002 found that the optimum carbon source for *Sorangium cellulosum* was soluble starch and 14.8 g l<sup>-1</sup> polysaccharide bioflocculant can be produced in the medium containing 30 g l<sup>-1</sup> soluble starch.

Production of bioflocculant has been carried out on medium containing glucose and sucrose as the main carbon source. Both of these sugars appeared favorable for cell growth as well as for bioflocculant production. The combination of urea and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> as inorganic of nitrogen sources enhanced the cell activity to produce bioflocculant. Production of bioflocculant was carried out for 70 hours at 120 rpm. Jie *et al.* 2006 reported that cells of *Vagococcus* sp. W13 grew rapidly in the first 60 hours of cultivation, and then leveled off. Production of bioflocculant occurs at the early of exponential phase then it is increased up to the optimum condition before it is decreased (Sumarno 2000) causing the bioflocculants were changed into others products by some enzymes of bacteria. Kurane *et al.* (1986) observed that the maximum bioflocculant formation was in the early stationary phase, and no rapid decrease of flocculating activity was observed in late stationary phase.

Flocculation activity was affected by cation, pH, and temperature. The concentration of FeCl<sub>3</sub> as coagulant was determined in order to obtain the optimum concentration for flocculation activity assay, since for each assay there is no specific coagulant and its concentration (Parwono 1998). Flocculation activity was different for each isolate. The formation of floc is due to the binding of bioflocculants and FeCl<sub>3</sub> 0.05% to form colloid. Flocculation process produces bigger particles and accelerates precipitation. Bigger form of aggregate will affect formation of flocculated kaolin clay.

Flocculation activity assay requires the cation as coagulant. Both LT-5 and LT-6 isolates gave the highest flocculation activity by adding FeCl<sub>3</sub> and AlCl<sub>3</sub> as

coagulant. It is assumed that  $\text{Fe}^{3+}$  ion stimulate flocculation by neutralization and stabilization of residual negative charges of carboxyl groups of uronic acid in acidic polysaccharides, forming bridges which bind kaolin particles to each other (Yokoi 1998). The positive charges of  $\text{FeCl}_3$  will uptake the negative charges of particles. This condition will cause the interaction between colloid and formed larger floc, it is usually called coagulation process.  $\text{FeCl}_3$  as a cation source was used in this assay.  $\text{FeCl}_3$  has trivalent and big positive charge. Therefore it has ability to replace negative charge in the colloid and reduce the barrier energy. Cation has important role to stimulate coagulation process in kaolin suspension. The addition of cation to the reaction mixture was necessary to induce effectively flocculation by forming complexes of polysaccharides and kaolin clay mediated by a cation (Kurane & Matsuyama 1994). The synergistic effects of trivalent cations were stronger than that of bivalent cations, with  $\text{Al}^{3+}$  being the most effective cation (Toeda 1991). Addition of cation gave significant effect to the reaction mixture on flocculation activity of LT-5 and LT-6 isolates in kaolin suspension. Optimum of flocculation activity was reached by adding 0.05% of  $\text{AlCl}_3$ . The results proved that bioflocculant has ability to form agregation with alumunium and increase flocculation effectivity.

The pH reaction is known to be a key factor influencing flocculating activity (Yokoi *et al.* 1996). This study showed that there was a significant effect of pH on flocculation activity (Figure 2). Different response of flocculation activity on LT-5 and LT-6 isolates to various pH described the difference of isoelectric point for each isolate. Isoelectric point is requirements for flocculation and coagulation process. Zhang *et al.* (2002) found that flocculation activity of crude exopolysaccharide from *S. cellulosum* was correlated to the pH. Bioflocculant p-KG03 was reported to be active at acidic conditions (pH 3-6) (Yim *et al.* 2007), while bioflocculant of *Nannocystis* sp. NU-2 was active in alkaline conditions (pH 12-14) in the presence of 30 mg/l  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (Zhang *et al.* 2002). According to Jie *et al.* (2007) either using broth culture of *Vagococcus* sp. W13 or purified flocculant, the flocculating activity was high and stable at pH 7.0-10.0. The LT-5 and LT-6 isolates showed flocculation activity at pH 2.0-4.0 (40.47%) and pH 6.0-8.0 (42.43%), respectively. It is showed that each isolates has different optimum pH for flocculation. Most reports indicated that pH affected the flocculating efficiency of bioflocculant. According to Kurane *et al.* (1994), little flocculation occurred in the pH range above 8.0. It suggested that the property of LT-5 and LT-6 isolates may facilitate its application in various pH conditions of waste-water environments.

Optimum temperature is needed to obtain the maximum flocculation activity. Based on this study, the increasing of temperature affected to decreasing of flocculation activity with average about 34% for LT-5 isolate and 33% for LT-6 isolate. However, flocculation acivity for both isolates was still observed at 50, 60, and 70 °C after 30 minutes incubation. Average of flocculation activity of LT-5 and LT-6 isolates was 48 and 49% respectively. Based on the data, it can be concluded that LT-5 and LT-6

isolates had optimum temperature for flocculation activity at 40 °C.

## ACKNOWLEDGEMENT

We thank to Directorate General of Higher Education for this research funding. We would like to acknowledge Department of Biochemistry, Faculty Mathematics and Natural Sciences, Bogor Agricultural University for providing the laboratory facilities, and Indonesian Research Center for Veterinary Sciences/BALITVET for bacterial identification assistance.

## REFERENCES

- Jie G, Hua-ying B, Ming-Xiu X, Yuan-xia, L, Qian L, Yan-fen Z. 2006. Characterization of a bioflocculant from a newly isolated *Vagococcus* sp. W31. *J Zhejiang Univ Sci* 7:186-192. <http://dx.doi.org/10.1631/jzus.2006.B0186>
- Khalil MI, Aly AA. 2001. Preparation and evaluation of some cationic starch derivatives as flocculants. *J Starch* 54:132-139. [http://dx.doi.org/10.1002/1521-379X\(200204\)54:3/4<132::AID-STAR132>3.0.CO;2-E](http://dx.doi.org/10.1002/1521-379X(200204)54:3/4<132::AID-STAR132>3.0.CO;2-E)
- Kurane R, Matsuyama H. 1994. Production of a bioflocculant by mixed culture. *Biosci, Biotechnol, Biochem* 58:1589-1594. <http://dx.doi.org/10.1271/bbb.58.1589>
- Kurane R, Nohata. 1986. Culture condition for production of microbial flocculant by *Rhodococcus erythropolis*. *Agric Biol Chem* 50:2309-2313. <http://dx.doi.org/10.1271/bbb1961.50.2309>
- Kurane R, Nohata Y. 1997. Complete defined medium for large-scale production of polysaccharide bioabsorbent from *Alcaligenes latus* B-16. *Agric Biol Chem* 83:116-117.
- Parwono A. 1998. Uji kemampuan flokulasi dan kajian aktifitas flokulasi isolat bakteri potensial penghasil bioflokulan pada suspensi kaolin. Bogor: Fakultas Teknologi Pertanian, Institut Pertanian Bogor.
- Salehizadeh H, Shojaosadati SA. 2001. Extracellular biopolymeric flocculants: recent trends and biotechnology importance. *Biotechnol Adv* 19:371-385. [http://dx.doi.org/10.1016/S0734-9750\(01\)00071-4](http://dx.doi.org/10.1016/S0734-9750(01)00071-4)
- Sumarno L. 2000. Studi Kinetika Pertumbuhan *Alcaligenes latus* pada Produksi Bioflokulan Menggunakan Medium Hidrolisat Pati Ubi jalar [Tesis]. Bogor: Program Pasca Sarjana, Institut Pertanian Bogor.
- Toeda K, Kurane R. 1991. Microbial flocculant from *Alkaligenes cupidus* KT201. *Agric Biol Chem* 55:2793-2799. <http://dx.doi.org/10.1271/bbb1961.55.2793>
- Yim JH, Kim SJ, Ahn SH, Lee HK. 2007. Characterization of a novel bioflocculant, p-KG03, from a marine dinoflagellate, *Gyrodinium impudicum* KG03. *Bioresour Technol* 98:361-367. <http://dx.doi.org/10.1016/j.biortech.2005.12.021>
- Yokoi H. 1998. Biopolymer flocculant produced by an *Pseudomonas* sp. *Biotech Tech* 12:511-514. <http://dx.doi.org/10.1023/A:1008843228743>
- Yokoi H, Arima T, Hirose J, Hayashi S, Takasaki Y. 1996. Flocculation properties of poly (gamma-glutamic acid) produced by *Bacillus subtilis*. *J Ferment Bioeng* 82:84-87. [http://dx.doi.org/10.1016/0922-338X\(96\)89461-X](http://dx.doi.org/10.1016/0922-338X(96)89461-X)
- Yue L, Chunling MA, Zhenming CHI. 2006. Bioflocculant produced by *Klebsiella* sp. MYC and its application in the treatment of oil-field produced water. *J Ocean Univ China* 5:333-338. <http://dx.doi.org/10.1007/s11802-006-0025-y>
- Zhang J, Liu Z, Wang S, Jiang P. 2002. Characterization of a bioflocculant produced by the marine *Myxobacterium Nannocystis* sp. NU-2. *Appl Microbiol Biotechnol* 59:517-522. <http://dx.doi.org/10.1007/s00253-002-1023-7>
- Zhang J, Wang R, Jiang P, Liu Z. 2002. Production of an exopolysaccharide bioflocculant by *Sorangium cellulorum*. *Letters in Appl Microb* 34:178-181. <http://dx.doi.org/10.1046/j.1472-765x.2002.01068.x>