

CHARACTERIZATION OF BIOFLOCCULANT-PRODUCING BACTERIA ISOLATED IN VIETNAM AND ITS USE FOR HARVESTING INDIGENOUS MICROALGAE

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ABSTRACT

Bioflocculation producing bacteria isolated from soil and sediment in Da Lat City, Lam Dong Province, Vietnam, were characterized. Four bacterial strains that had high flocculation activity were *Flavobacterium granuli* CL, *Hydrogenophaga pseudoflava* DA, *Alcaligenes cupidus* PT2, *Bacillus mucilaginosus* PT3, with the flocculation efficiency of 91.37%, 83.12%, 76.92%, and 75.81%, respectively at 22°C, pH 10, the initial algae concentration (optical density at 690 nm) of 1.25, and grown in media containing glucose as the carbon source. The bioflocculation efficiency was depended on the pH, carbon sources, the initial algae concentrations, bacteria strains, and the initial bioflocculant-producing bacteria concentrations. The temperature lightly affected the bioflocculation, no noticeable behavioral or activity changes were observed in mice that were orally administrated with four bacterial strains, and no treatment-related illness or death occurred after 72 hours. In general, the bioflocculation process is easy to operate, cost-effective, environment-friendly and therefore, it can be applied for industrial processing of microalgae.

Keywords: Algae concentrations, bio flocculation, bacteria, Lam Dong, Vietnam.

Citation: Le Thi Anh Tu, 2018. Characterization of bioflocculant-producing bacteria isolated in Vietnam and its use for harvesting indigenous microalgae. *Academia Journal of Biology*, 40(3): 52–64. <https://doi.org/10.15625/2615-9023/v40n3.10902>.

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Received 21 November 2017, accepted 30 September 2018

INTRODUCTION

Microalgae is known to have high growth rate, high lipids, carbohydrate and protein organisms. Microalgae can be grown on the land not suitable for agriculture. Algal biomass is considered as a source for biofuel, food, and chemical production. Algal biomass need to be harvested and dewatered before the biomass can be converted to fuel or adapted to other procedure. There are several methods to harvest microalgae, such as centrifugation, filtration, sedimentation, electrocoagulation, dissolved air floatation, chemical flocculation and bio-aggregation (Uduman et al., 2010). Biological agents such as chitosan, extracellular substances, or whole bacterial cells have been used to combine algae to form aggregates in biological flocculation (Sirin et al., 2012; Yuan et al., 2011; Pavoni et al., 1972).

Natural microbial flocculants have been

used to harvest microalgae because their high yield and eco-friendly procedure (Manheim & Nelson, 2013; Oh et al., 2001). Bioflocculation is an economic and environment-friendly dewatering method that is hastened with biomolecules from microbial cells (Jang et al., 2010). During the flocculation, the dispersed microalgal cells aggregate to form larger particles that are easy to precipitate. No energy consumption, thus less harvest cost, is one of the advantage of bio-aggregation. Powell & Hill (2013) reported that *Bacillus* sp. RP1137 can aggregate many algae that are suitable for biofuel production. The mechanism of aggregation was unknown. Extracellular polymeric substance of *Bacillus licheniformis* CGMCC 2876 including poly- γ -glutamic acid, γ -PGA hastened the bio-flocculation of microalgae (Chen et al., 2017; Ndikubwimana et al., 2014). Whole cells of bacteria also were used to aggregate the microalgae such as

Paenibacillus sp. AM49 and *Bacillus* sp. Gilbert (Nontembiso et al., 2011; Oh et al., 2001). Comparison of different harvesting methods showed that flocculation is the most promising alternative way (Schenek et al., 2008).

Bioflocculant is an extracellular polymer produced by microorganisms during their growth, and can form stable flocculation that compose of proteins, polysaccharide, glycoprotein, or poly-amino acid (Gao et al., 2006; Wu & Ye, 2007). Several bacteria have been reported to produce flocculants (Ndikubwimana et al., 2015; Ugbenyen et al., 2012; Suh et al., 1997). In the previous study, *Bacillus* sp. Strain RP1137 can rapidly aggregate several biofuel producing algae where charge neutralization occurs in both sea and fresh water (Powell & Hill, 2013). Ndikubwimana et al. (2014) reported that *Bacillus licheniformis* CGMCC 2876 broth with the active elements—poly γ glutamic acid (γ -PGA), can concentrate microalgae *Desmodesmus* sp. F51. *Bacillus licheniformis* CCRC 12826 was found to produce the extracellular biopolymer flocculants (Shih et al., 2001). *Bacillus firmus* isolated from soil samples produce a strongly acidic, polysaccharide flocculants (Salehizadeh & Shojaosadati, 2001). *Nocardia amarae* YK1 (Takeda et al., 1991) and *Paecilomyces* sp. (Takegi & Kadowaki., 1985) produce the protein flocculants. The flocculants produced by *Arcuadendron* sp. Ts-4 (Lee et al., 1995) and *Aratrobacter* sp. (Wang et al., 2012) are glycoprotein.

Many studies have been reported that bioflocculants can replace synthetic flocculants. Searching for novel bioflocculant-producing bacteria with different properties has been attracted more interest due to their biodegradability, benign nature and lack of secondary pollution. These economic and environment-friendly dewatering flocculants may potentially be applied in various fields such as wastewater treatment, downstream processing, fermentation processing and algae harvesting (Salehizadeh & Shojaosadati, 2001). Thus, it is important to investigate the potent

bioflocculant producing-bacteria that can produce the high activity flocculants. In this study, bioflocculant-producing bacteria from soil, water, and sediment sample from Vietnam were isolated, screened, and characterized. The bioflocculation activity is discussed in detail.

MATERIALS AND METHODS

Microalgal strain and cultivation conditions

The *Chlorella* sp. HP01/2B strain isolated from the Northern Vietnam was offered by Prof. Tuan Lam of Ecology laboratory, Da Lat University, Lam Dong, Vietnam. The growth medium BG11 was used to culture *Chlorella* sp. HP01/2b. The algae were cultured at room temperature for 5 days in a rotary shaker with 150 rpm under an aeration rate of 0.2 vvm with 2% CO₂ enriched airflow. The culture was illuminated 15/9h light/dark cycle with a light intensity of approximately 500 $\mu\text{mol}/\text{m}^2\cdot\text{s}$. The suspension was used to estimate the flocculating activity.

Isolation and screening of bioflocculants producing bacteria

Samples were collected from water and sediment of the Xuan Huong lake (belonging to the center stream system called the Cam Ly stream system), the Phuoc Thanh stream (belonging to the northern stream system of the city) and the Datanla stream (belonging to the southern stream system of the city, called the Da Tam stream system) in Da Lat City, Lam Dong, Vietnam. The samples collected from the Cam Ly, Phuoc Thanh and Datanla Stream systems were labeled with CL, PT and DA, respectively. The cells were isolated and cultured in the growth medium consisting of 40g glucose, 1g NH₄NO₃, 0.3g K₂HPO₄, 0.3g KHPO₄, 0.1g MgSO₄.7H₂O, 0.1g MnSO₄.4H₂O, 0.05g NaCl, 0.4g CaCO₃. The initial pH was adjusted to 6.8–7.2 with NaOH (1M) and HCl (0.5M) (Suh et al., 1997). All cultivation were done at 30°C. Pure cultures of bacteria were isolated by re-plating on the agar plates.

Screening process for bioflocculants-producing bacteria was carried out in the growth

medium. Bioflocculant producing bacteria were screened based on their morphology (the mucoid and ropy characteristics) (Gao et al., 2006; Zaki et al., 2011). The positive strains were grown in 50 mL medium in 250 mL flasks on a rotary shaker at 30°C for 48 hours. The culture broths were used to measure flocculation activities. Four strains with the high flocculating activities were collected to perform further test. These four strains were identified according to the Bergey's Manual of Systematic Bacteriology and the Bergey's Manual of Determinative Bacteriology.

Flocculation of microalgae culture

Algae biomass was used as the suspension material to determine flocculation activity. The broth of selected bacteria was used straight away as the bioflocculants without any further purification. In a 100 ml beaker, 50 ml of algae suspension was stirred at 200 rpm with a magnetic bar for 1 min. After stirring, the culture broth (2ml/L) was added to the algae suspension. The mixture then was mixed at room temperature (23–25°C) at 200 rpm for 1 min. The microalgal suspensions were left to settle at room temperature without agitation. The sample supernatants were taken from the half height of the clarified layer and were used to measure the optical density at wavelength of 690 nm (OD₆₉₀ nm) using a UV-vis spectrophotometer (Rundquist et al., 1996) which is defined as the absorption of visible radiation (adsorption peak of chlorophyll is at about 680 nm). The flocculation activity was calculated by the following equations (Foley et al., 2011; Surendhiran & Vijay, 2013).

$$FE = \left(1 - \frac{A}{B}\right) \times 100$$

FE: Flocculating efficiency

A: Optical density of the sample at 690 nm

B: Optical density of the control at 690 nm

Optimization of cultural parameters on bioflocculant activity

Several factors affecting on bioflocculant activity were studied including temperature, pH, the initial concentrations of algae, the initial concentrations of bacteria, and carbon sources.

The effect of temperature was determined at temperature range of 22 and 30°C. Effect of pH were done at 5, 7, 9, and 10 (pH of algal suspensions is 10). The study of algae concentrations on the bioflocculation was carried by varying the initial microalgae concentrations (OD₆₉₀ nm of 1.25, 2.5, and 5.0 at 690 nm). The effect of concentrations of bacteria was done over the dosage of bioflocculant solution range of 0.6, 0.3, and 0.15 mg/ml with the constant algae concentration (OD₆₉₀ = 1.25). The effect of different carbon sources on flocculating activity was conducted by replacing glucose in the medium by one of the following carbon sources (40 g/l): fructose and maltose.

Acute toxicity test on mice

The acute toxicity of selected bacteria strains was tested in pathogen-free mice of 20g and acclimated for 7 days prior to starting the experiments (Auletta, 1995). During the acclimation and experimental periods, the mice were housed individually in stainless mesh cages in a room with controlled temperature (20.5–23.2°C) and a 12 hours light/dark cycle. The mice were fed rodent chow and filtered water. After 7 days of acclimation, the mice were assigned in to 16 groups (6 mice in each group), and were orally administrated with 1ml either selected bacterial strains (cell suspensions of 1.5×10^9 , 3.0×10^9 , and 6.0×10^9 cells/ml in 10% skim milk) or 10% skim milk only (control). Mice were fed orally using a sterile pipette three times a day for 72 hours. The animals' activity, behavior and general health status (death/alive) were observed twice daily. Activity (AS) was monitored once a day using a three-scale method: lazy, moving slowly; intermediate; and active moving or searching.

Statistical analysis

One-way analysis of variance (ANOVA) and t-test were performed using Excel 2011 statistical tools. A p-value < 0.05 was used as a criterion for significance level. ANOVA was used to determine the statistical difference of bioflocculation efficiency at different conditions (pH, temperature, the initial algae and carbon sources).

RESULTS AND DISCUSSION

Screening of biofloculant producing bacteria

A total of 21 bacterial isolates were obtained from the water and sediment samples collected from the stream systems around Da Lat city. Their colony appeared from white to yellow in color, ropy and mucous. Among 21 bacteria, four isolates of biofloculant-producing bacteria were able to aggregate the algal suspension efficiently. These selected strains were designated as CL (collected from water of the Xuan Huong lake belonging to Cam Ly stream system), DA (collected from Datanla stream belonging to Da Tam stream system), and PT2 and PT3 (collected from the sediment of Phuoc Thanh stream belonging to the Northern stream system of Da Lat city). The different behavior of four strains in flocculation

was observed. Large and dense flocs were obtained. Even the highest flocculation efficiency was observed in the CL strain, the largest aggregates were found in DA strain.

The growth curve of four promising strains are shown in Fig. 1. While two strains, DA and PT3 reached the stationary phase by 24 hours thereafter, CL reached its peak by 48 hours and PT2 required 72 hours. All four strains of bacteria were harvested at the stationary phase at 72 hours. Badireddy et al. (2010) reported that concentrations of the high molecular weight compounds such as protein and carbohydrates of extracellular polymeric substrates that promoted bioflocculation by increasing the polymeric bridging and flocculation between bacteria and algae increased during the stationary growth.

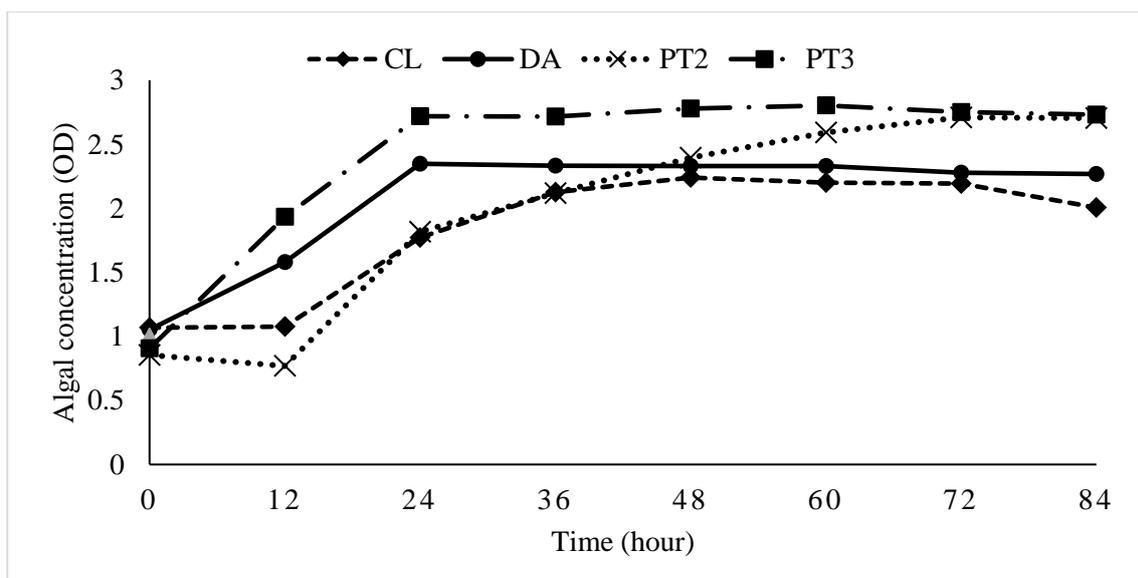


Figure 1. Growth curve of four selected strains of flocculant-producing bacteria. CL: *Flavobacterium granuli* CL, DA: *Hydrogenophaga pseudoflava* DA, PT2: *Alcaligenes cupidus* PT2, PT3: *Bacillus mucilaginosus* PT3

Identification of strains

The morphological characters and physiological/biochemical characteristics of isolated bacteria are listed in tables 1 and 2. The investigated bacteria CL, DA, PT2, PT3

resemble as: *Flavobacterium granuli* CL, *Hydrogenophaga pseudoflava* DA, *Alcaligenes cupidus* PT2, *Bacillus mucilaginosus* PT3, respectively, According to Bergey's Manual of Systematic Bacteriology (Vol. 2, 2nd Ed., Part B and C; Vol. 3, 2nd Ed.; Vol. 4, 2nd Ed.).

Table 1. A physiobiochemical characteristics of selected bacterial strains

Physiobiochemical test	Strains			
	CL	DA	PT2	PT3
Colony color	White to yellowish	Yellowish	White	White
Colony form	circle	circle	circle	circle
Colony elevation	Convex	convex	convex	Crateriform
Colony margin	entire	entire	entire	entire
Shape	Rod	Spherical	Rod	Rod
Size (µm)	2 × 0.75	5 × 0.4	1.8×0.7	3 × 1
Spore	-		-	+
Gram's reaction	-	-	-	-
Motility	-	+	+	-
Growth aeration	Aerobic	Facultative anaerobic	Facultative anaerobic	Aerobic
Oxidase	+	+	-	-
Catalase test	+	+	+	+
Glucose fermentation	+	+	+	-
Oxidative/fermentation test	O	F/O	F/O	O
Casein hydrolysis	-	+	-	-
Starch hydrolysis	-	-	-	+
Gelatin hydrolysis	-	+	-	-
Indole test	-	-	-	-
Voges-Proskauer test	+	+	-	-
Ammonia production	-	+	+	-
H ₂ S production	-	-	-	-
Nitrate reduction	+	+	+	-
Ashby's medium	+	-	-	*
Mineral salt medium	++	+++	+	++++
Temperature 22°C	+	++	+++	+++
Temperature 30°C	+++	++++	++++	+++
Temperature 37°C	++++	+++	+++	++++
Temperature 44°C	+	+	-	+++
Salt tolerance 2.5% NaCl	*	*	+	*
Salt tolerance 6.5% NaCl	*	-	*	*

*: weak, +: positive, -: negative, F: fermentation, O: Oxidase, CL: *Flavobacterium granuli* CL, DA: *Hydrogenophaga pseudoflava* DA, PT2: *Alcaligenes cupidus* PT2, PT3: *Bacillus mucilaginosus* PT3

Table 2. Carbohydrate fermentation

Strains	Carbonhydrate fermentation	Carbonhydrate							
		Glu	Gal	Lac	Ara	Sac	Fru	Man	Gly
CL	Acid (fermentation)	-	+	-	+	+	+	-	+
	Gas production	-	-	-	-	-	-	-	-
	Fermenter	+	+	-	+	+	+	+	+
DA	Acid (fermentation)	+	+	+	+	+	+	+	+
	Gas production	+	+	+	+	+	+	+	-
	Fermenter	+	+	+	+	+	+	+	+
PT2	Acid (fermentation)	+	+	+	+	+	+	+	+
	Gas production	+	+	+	-	+	+	+	-
	Fermenter	+	+	+	+	+	+	+	+
PT3	Acid (fermentation)	+	+	+	+	+	+	+	+
	Gas production	-	-	-	-	-	-	-	-
	Fermenter	+	+	+	+	+	+	+	+

Glu: Glucose, Gal: Galactose, Lac: Lactose, Ara: Arabinose, Sac: Succrose, Fru: Fructose, Man: Manitol, Gly: Glyceryl, +: positive, -: negative, CL: *Flavobacterium granuli* CL, DA: *Hydrogenophaga pseudoflava* DA, PT2: *Alcaligenes cupidus* PT2, PT3: *Bacillus mucilaginosus* PT3

Effects of initial pH and temperature on bioflocculation efficiency

The bioflocculation process is markedly affected by the pH of the culture. Different initial pH of the culture significantly affected the bioflocculation activity. Generally, the flocculation efficiency increased with the increase of pH values from 5 to 10 (Fig. 2). The maximum efficiency was observed at pH 10. The pH range for flocculation activity of the bacterial strains examined in this study were much wider than *Bacillus subtilis* PY-90

(pH rang 3–5) (Yokoi et al., 1996) or *Paecilomyces* sp. I-1 (pH range 4–8) (Takagi & Kadowaki, 1985). The flocculation activity of *B. licheniformis* CGMCC 2876 against green algae *Desmodesmus* sp. F51 was reduced sharply in the first 3 hours at pH 10 compared with the lower pH ranges of 2.5, 3 and 4. Meanwhile, Zheng et al. (2012) investigated that, the pH range from 6.5 to 8.5 offered the great flocculation activity (up to 81%) for *C. vulgaris* when using the similar flocculant, γ -PGA.

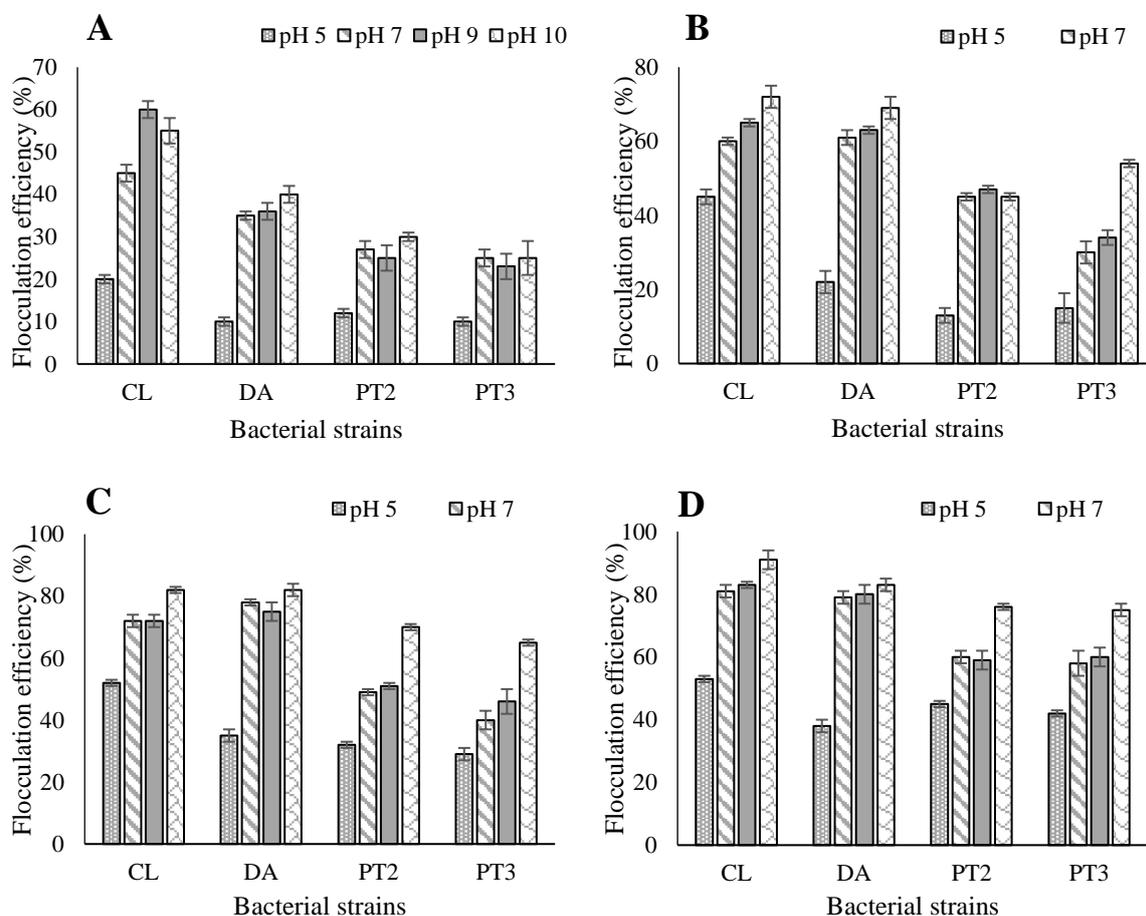


Figure 2. Effect of pH on bioflocculation activity of four selected strains. A: After 1 hour, B: after 2 hours, C: after 3 hours, D: after 4 hours, CL: *Flavobacterium granuli* CL, DA: *Hydrogenophaga pseudoflava* DA, PT2: *Alcaligenes cupidus* PT2, PT3: *Bacillus mucilaginosus* PT3

Spilling et al. (2011) reported that autoflocculation of microalgae occurs when pH exceeded above 10 under the presence of phosphate. In this study, pH 10 is the pH value

of the algae broth 5 days after culture, due to the limited amount of phosphate in the suspension. The combination of bacterial strains and algae may be related to this difference. pH 10 is the

pH of algae suspension without adjustment. Thus, this pH was applied for further experiments, except for the CL strain, of which flocculation activity was not profoundly affected pH at 2, 3 and 4 hours of culture at the pH range of 7–9. It was stated that the bacterial broth may contain variety of metabolites that have been influenced by changing pH values (Ntsangani et al., 2017). The bridging efficiency of flocculants may be affected by different electric states which is influenced by environmental pH values.

Although statistically not significant, the

flocculation rate increased slightly with the increase of reaction temperature (Fig. 3). This result agrees with most of the previous studies. Flocculation activity of *Bacillus* sp. RP1137 added to algae suspension was stable at the temperature range of 10–40°C (Powell & Hill, 2013). Zaki et al. (2011) also reported that the bioflocculations were not affected by the temperature at the range of 5–70°C. The slight increase of aggregation formation might be the results of the increase of diffusion and suspended particle collision frequency with the increase of temperature (Gong et al., 2008; Lu et al., 2005).

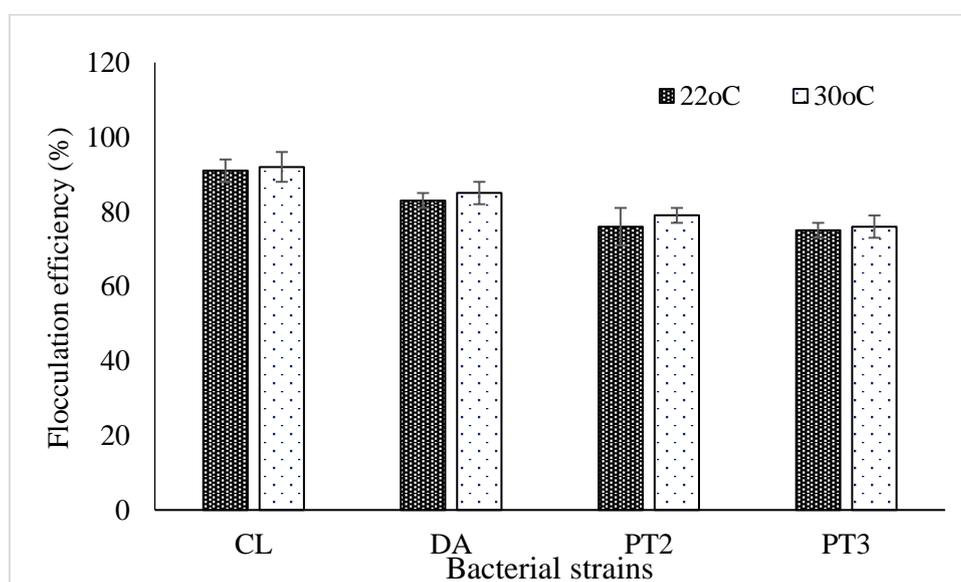


Figure 3. Effect of temperature on bioflocculation activity of four selected bacterial strains. CL: *Flavobacterium granuli* CL, DA: *Hydrogenophaga pseudoflava* DA, PT2: *Alcaligenes cupidus* PT2, PT3: *Bacillus mucilaginosus* PT3

Effect of the initial algae concentrations

In this study, the initial algae concentrations affected the bioflocculation activity. As shown in Fig. 4, the flocculation activity decreased along with the increase of algae concentration. All four bacterial strains showed the greatest flocculation efficiency at the initial algae concentration of 1.25 (OD₆₉₀). At pH10, 22°C with the initial algae concentration of 5 (OD₆₉₀), the flocculation efficiency of CL, DA, PT2 and PT3 were 45.24%, 49.56%, 24.72% and 27.61%. These flocculation efficiency was far less than that observed at the initial algae concentration of 1.25 (OD₆₉₀). Zheng et al. (2012) reported the similar results when they

used γ -PGA produced by *B. subtilis* to flocculate *C. vulgaris*. In contrast, Ndikubwiman et al. (2014) observed the contrary trend during their study on the flocculation efficiency of *Desmodium* sp. F51 by *B. licheniformis* CGMCC 2876.

The high concentration of microalgae possess high cell mass per unit volume of culture, which led to greater potential for the interaction between algal cells and flocculant. The flocculation was formed by attaching algae cells on flocculant. The concentrations of algae is related to the surface area available of flocculant. Surface charge also influences the flocculation. Thus, the optimum ratio of

algae cell and flocculant may vary depending on the combination of algae and flocculant. Observed differences of our data from

previous studies can be attributed to differences in flocculants, algal cells, and the density.

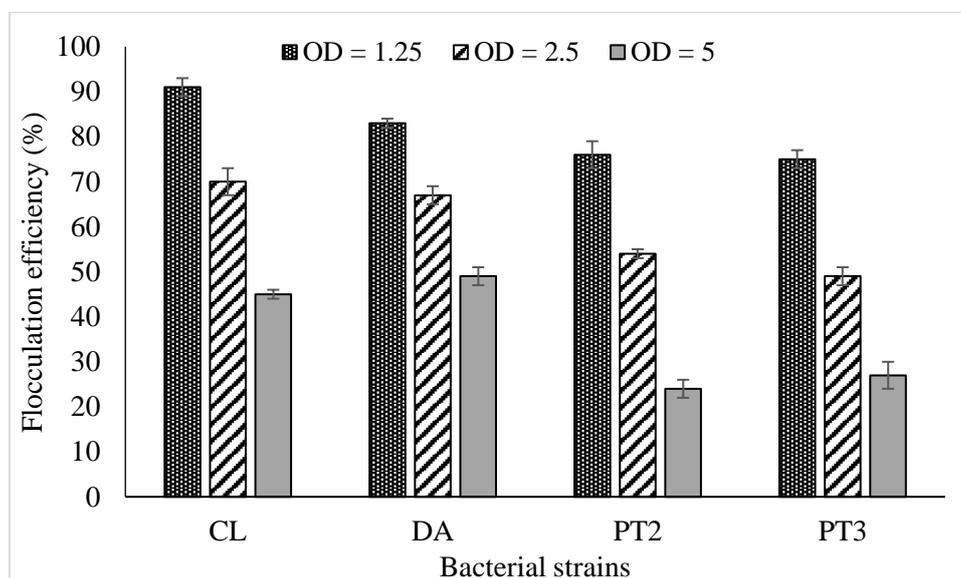


Figure 4. Effect of the initial algae concentrations on bioflocculation activity of four selected bacterial strains. CL: *Flavobacterium granuli* CL, DA: *Hydrogenophaga pseudoflava* DA, PT2: *Alcaligenes cupidus* PT2, PT3: *Bacillus mucilaginosus* PT3

Effects of the dose of bioflocculant

To determine the effects of the dose of bioflocculant, the algae biomass was kept constant ($OD_{690} = 1.25$) and the range of dosage (0.6, 0.3 and 0.15 mg/ml) of bioflocculant. The influence of the dose of bioflocculant on the bioflocculation activity was shown in Fig. 5. The flocculation activity increased in proportion of the dose of the flocculants, although there was no significant difference between the flocculant dose of 0.3 and 0.6 ml/ml. The magnitude of flocculation activity among the four strains was similar at different temperature and pH. The CL strain had a good flocculating capability and could achieve a flocculating rate up to 91.37% for algae suspension at the dosage of 0.6 ml/ml. Comparing to previous reports (Agunbiade et al., 2010; Liu & Cheng, 2010; Ugbenyen & Okoh, 2014) the flocculation rates produced by these bacteria were effectively. The flocculation efficiency of DA, PT2 and PT3 were 83.12%, 76.92%, and 75.81%, respectively, at the same condition as CL. Comparing with one inorganic compound,

aluminium sulfate that is used to harvest algae, the efficiency of three bacterial strains including DA, PT2, and PT3 are remarkable. Aragón et al. (1991) reported that a harvesting efficacy for *Scenedesmus acutus* species and *Chlorella vulgaris* were 80% and 20%, respectively, using aluminium sulfate (30–50 mg/L at a pH of 6–6.5).

Effect of different carbon sources on bioflocculation activity

The flocculation ability highly is depending on the carbon source of bacterial growth media. In this study, we compared the effect of glucose, maltose and fructose on the expression of bioflocculation activity (Fig. 6). The results show that glucose is the most preferable carbon source for all four strains, followed by maltose and fructose. It was reported that glucose was the most preferred carbon for bioflocculation production by *Bacillus* sp. AEMREG7 (Okaiyeto et al., 2016), *Chryseobacterium daeguense* W6, *Klebsiella* sp. PB12, *Axotobacter* sp. SSB81 (Mandal et al., 2013; Liu et al., 2010; Gauri et al., 2009). While

Aspergillus flavus reached optimal condition in the medium containing sucrose (Aljuboori et al., 2013), *Streptococcus phocae* PI80 expressed best flocculation production with lactose (Kanmani et al., 2011). When fructose was used

as a carbon source, bioflocculation production in the medium was highest in PT3 (35%). In contrast, when maltose was used as the carbon source, CL expressed the greatest flocculation efficiency (50%).

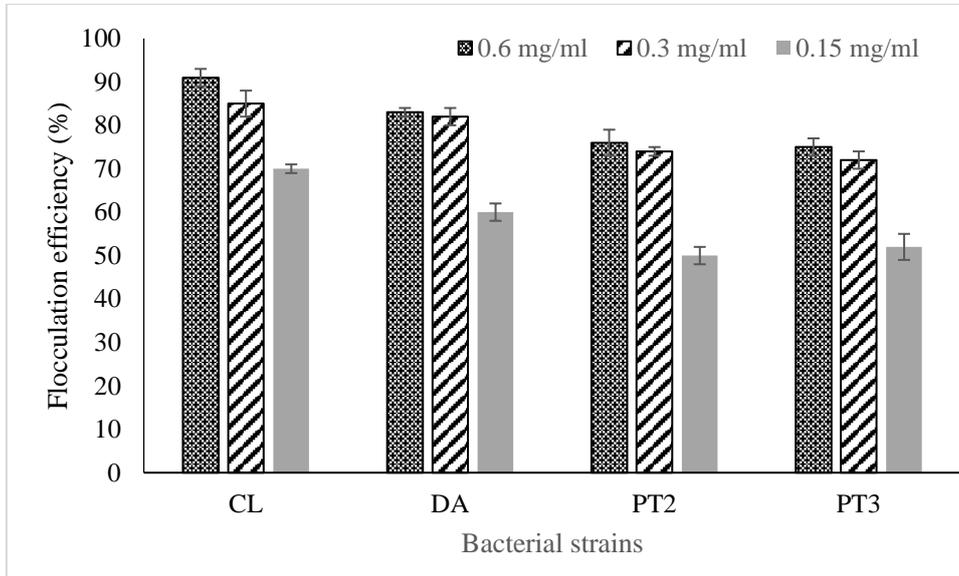


Figure 5. Effect of bioflocculant dosage on bioflocculation activity of four selected bacterial strains. CL: *Flavobacterium granuli* CL, DA: *Hydrogenophaga pseudoflava* DA, PT2: *Alcaligenes cupidus* PT2, PT3: *Bacillus mucilaginosus* PT3

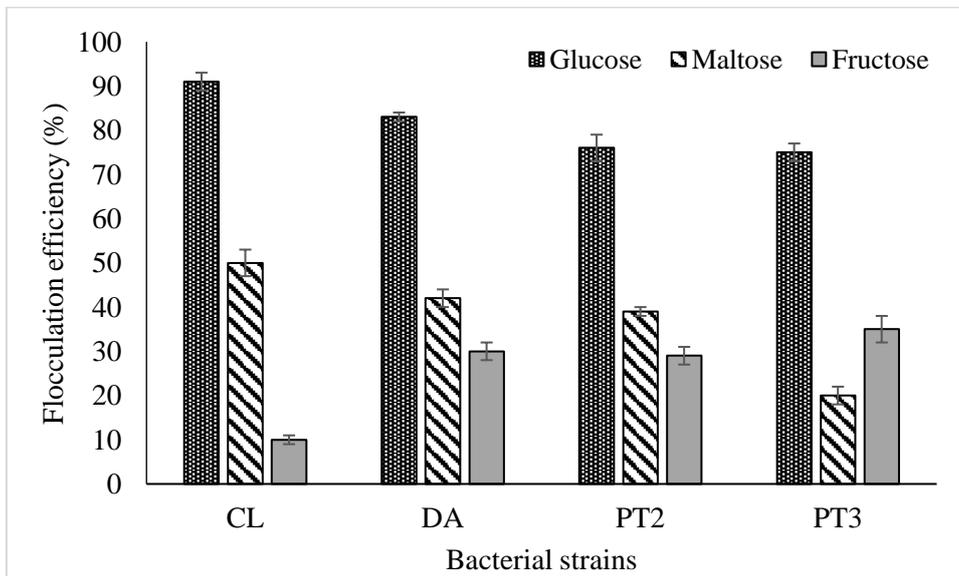


Figure 6. Effect of carbon sources on bioflocculation activity of four selected bacterial strains. CL: *Flavobacterium granuli* CL, DA: *Hydrogenophaga pseudoflava* DA, PT2: *Alcaligenes cupidus* PT2, PT3: *Bacillus mucilaginosus* PT3

Acute toxicity to mice

Throughout the experiment period, no noticeable behavioral or activity changes were observed in mice fed with flocculant-producing

bacteria, and no treatment-related illness or death occurred by 72 hours (Table 3). No death was observed even at the highest dose of 6.0×10^9 bacterial cells/ml in 10% skim milk at 24, 48, and 72 hours testing.

Table 3. Number of mice after acute toxicity test. CL: *Flavobacterium granuli* CL, DA: *Hydrogenophaga pseudoflava* DA, PT2: *Alcaligenes cupidus* PT2, PT3: *Bacillus mucilaginosus* PT3; AS: activity, Inter: Intermediate, 0h: at the beginning, 72h: 72 hours

CFU(cell/ml)	Control (10% skim milk)			Strains											
				CL			DA			PT2			PT3		
	0h	72h	AS	0h	72h	AS	0h	72h	AS	0h	72h	AS	0h	72h	AS
1.5×10^9	6	6	Inter	6	6	Inter	6	6	Inter	6	6	Inter	6	6	Inter
3.0×10^9	6	6	Inter	6	6	Inter	6	6	Inter	6	6	Inter	6	6	Inter
6.0×10^9	6	6	Inter	6	6	Inter	6	6	Inter	6	6	Inter	6	6	Inter

CONCLUSION

Four bioflocculant-producing bacteria, *Flavobacterium granuli* CL, *Hydrogenophaga pseudoflava* DA, *Alcaligenes cupidus* PT2, and *Bacillus mucilaginosus* PT3, were isolated from the stream systems of Da Lat city, Lam Dong, Vietnam. The highest flocculation of CL, DA, PT2 and PT3 incubated in medium containing glucose as the carbon source were 91.37%, 83.12%, 76.92%, and 75.81%, respectively at 22°C, pH10, with the initial algae concentration of 1.25 (OD 690nm). Bioflocculation using bacteria is a potential methods to harvest algae biomass. The pH, the initial concentrations of algae, the initial concentrations of bacteria, the carbon sources influenced the bioflocculation activity. The temperature had little influence on the flocculation activity. No noticeable behavioral or activity changes were observed in mice fed with all four flocculant-producing bacteria at the dose up to 6.0×10^9 bacterial cells/ml in 10% skim milk for 72 hours. This study suggests that bioflocculation is effective and can be applied at the industrial scale. The further understanding of the mechanism of bioflocculation of these four strains, especially *Flavobacterium granuli* CL, are necessary.

Acknowledgements: We thank Dr. Lam Ngoc Tuan for providing algal strain. This work was supported by grant of Da Lat University, Lam Dong province, Vietnam.

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