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Full Length Article

Bacterial Symbionts Isolated from Mixed Microalgae Culture of Glagah Strains

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Abstract

The current study described the diversity of indigenous bacteria associated with microalgae consortium from Glagah Coast and its potential for biodiesel production through a batch culture system. Bacterial consortium associated with microalgae consortium from Glagah Coast was identified by cell morphology, gram staining and also biochemical using the BD BBL Crystal kit. They were identified as *Corynebacterium ulcerans*, *C. bovis*, *Bacillus cereus*, *B. megaterium*, *Pediococcus parvulus*, *Staphylococcus vitulinus.* The role of the bacteria was to support microalgae growth by supplying cobalamin, thiamine and biotin. Therefore, co-culture microalgae-bacteria consortium from Glagah Coast could be used as effective sustainable strategies to produce biodiesel derived from microalgae and also could reduce the cost of the production as the bacteria provide vitamin B for the microalgae.

Keywords: Bacteria; Microalgae; Consortium; Glagah coast; Vitamin B; Biodiesel

Introduction

World demand for energy was increasing. The third-generation biofuels from microalgae potential was as an alternative to meet the global energy demand (Hirsch *et al.*, 2005). The third-generation biofuels from microalgae is potential as an alternative to meet the global energy demand (Ghasemi *et al.*, 2012). Unlike plant-based raw materials for biodiesel production, microalgae do not require a large land area for cultivation, have high growth rates and lipid from microalgae are generally neutral lipid thus potentially as a substitute for diesel fuel (Danquah *et al.*, 2009).

Biodiesel production using a microalgal still has a limitation due to the low of biomass and lipid content. Research conducted by Suyono *et al.* (2016b) showed that lipid productivity of single culture *Chlorella zofingiensis* on pond raceway only reached 0.16 mg L^{-1} day^{-1}. However, lipid productivity of mixed culture from Glagah isolates can reach 4 times higher than single culture *C. zofingiensis* about 0.71 mg L^{-1} day^{-1}. An important role of bacteria on the growth of microalgae in the co-culture is the main source of vitamin because only prokaryotic organisms that can synthesize and supply of vitamin B (Kazamia *et al*., 2012). Vitamin B is an important factor in supporting the growth of microalgae. Microalgae consortium from Glagah Coast identified as *Cyclotella polymorpha, Cylindrospermopsis raciborskii, Golenkinia radiata, Syracosphaera pirus, Corethron criophilum, Cochliopodium vestitum* and *Chlamydomonas* sp. (Suyono *et al*., 2016a)

Cultivation super strains of indigenous microalgae-bacteria from Glagah Coast in producing biodiesel offers a range of economic and environmental benefits, as a sustainable strategy to produce biodiesel from microalgae, can reduce the cost of production (do not require supply of vitamin B) and as CO_2 emission mitigation strategies. The objective of this study was to identify bacteria associated with microalgae consortium from Glagah Coast.

Materials and Methods

Isolation of Bacteria

Bacteria associated with microalgae were isolated from microlagae culture of Glagah Coast which has been sub-cultured in medium BBM. The Bacteria were isolated by serial streaking in BBM agar medium without antibiotics. The plates were incubated at 25°C, under continuous light with light intensity 1000 Lux for 5–6 days. Pure isolates were stored in BBM agar slant for further studies (Chevanton *et al*., 2013).

Identification of Bacterial Isolates

Identification included color colony, cell morphology, and gram staining. Biochemical identification was also performed using the BD BBL Crystal kit contains 29 enzymatic and biochemical substrates and then the results

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were processed using the BBL Crystal Auto Reader. Identification was derived from a comparative analysis of the reaction pattern of the test isolates to data base BBL Crystal GP ID System.

Results

Bacteria Associated with Microalgae

There were six isolates and subsequently characterized morphologically, gram staining and biochemically. Based on Table 1, six isolates had varying characters of color, morphology (bacilli and cocci) and biochemical, but gram staining showed that all isolates were gram-positive. Biochemical test showed that 2 isolates were *Corynebacterium*, 2 isolates were *Bacillu*s, 1 isolate was *Staphylococcu*s and 1 isolate was *Pediococcus*. The characteristic the *Corynebacterium* was gram positif, nonsporing, nonmotile, not acid-fast, straight or slightly curved rods and often exhibited typical V-shaped, facultatively anaerobic to aerobic (Eggeling and Bott, 2005). *Pediococcus* was lactic acid bacteria, gram positive, cytochromes absent, catalase negative and facultative aerobic. *Staphylococcus* was gram-positive, catalase positive cocci, facultative anaerobic (Dworkin *et al*., 2006). *Bacillus* was distinguished by production of endospores, which were round, oval or cylindrical highly retractile structure formed within bacterial cells. *Bacillus* was gram positive (Slepecky and Hemphill, 2006).

Discussion

Most microalgae auxotroph required vitamin B from an external source, such as from bacteria due to the concentration of vitamin B in the environment was very low. Symbiotic relationship between microalgae with bacteria was very important. Bacteria synthesized and transferred most of the vitamin into the cells of microalgae. Instead, the products of photosynthesis microalgae were used by bacteria (Croft *et al*., 2006).

Archaea and bacteria had ability to produce vitamin B (cobalamin, thiamine and biotin) (Martens *et al*., 2002; Rodionov *et al.*, 2003; LeBlanc *et al.*, 2011). Vitamin B was needed for the growth of some bacteria and archaea (Roth *et al*., 1996), as well as approximately 75% of all microalgae (Croft *et al*., 2005). The ability of bacteria to produce vitamin B could be utilized in supplying vitamins B for the growth of microalgae through the symbiotic interaction between bacteria with microalgae.

Among the species of bacteria that produced vitamin B were *Aerobacter, Aeromonas, Agrobacterium, Alcaligenes, Azotobacter, Bacillus, Brevibacterium, Clostridium, Enterobacter, Escherichia, Corynebacterium, Flavobacterium, Micromonospora, Mycobacterium, Arthrobacter, Norcardia, Propionibacterium, Rhodopseudomonas, Protaminobacter, Proteus, Pseudomonas, Rhizobium, Salmonella, Serratia, Streptomyces Streptococcus* and *Xanthomonas* (Perlman, 1959; Sugita *et al*., 1992; Martens *et al*., 2002; Du *et al*., 2011), *Halomonas* (Croft *et al*., 2006), *Roseobacter* (Wagner-Döbler *et al.,* 2010), Lactic acid bacteria (Leblanc *et al*., 2011).

Bacteria associated with microalgae consortium from Glagah Coast consisted of genera: *Corynebacterium, Bacillus, Pediococcus* and *Staphylococcus* (Table 1). Those genera had potency to support microalgae growth by supplying availability of cobalamin, thiamine and biotin. *Staphylococcus vitulinus* also had ability to convert nitrate to nitrite. According to Gilet *et al*. (2011), *Staphylococcus vitulinus* was optionally in association with lactic acid bacteria such as *Lactococcus* or Pedioccoccus. Lactic acid bacteria could increase the nitrate reductase activity (NRA) of *Staphylococcus vitulinus.*

Co-culture, which consisted of several types of microalgae and bacteria potentially could optimize the growth of culture (Kazamia *et al*., 2012). This could be achieved because of the symbiotic relationship between microalgae and bacteria. The bacteria could degrade complex compound, play a role in the process of nitrification/denitrification, reduce the oxygen pressure, provide vitamins and growth factors for promoting the growth of microalgae. Meanwhile, microalgae could support the growth of bacteria by producing O_2, provide nutrients from decomposing of microalgae cells and act as a secondary habitat to protect bacteria from unfavorable conditions (Mouget *et al.,* 1995; Subashchandrabose *et al*., 2011; Lakaniemi *et al.*, 2012; Nascimento *et al*., 2013; Unnithan *et al*., 2013).

A study conducted by Suyono *et al*. (2016b) showed the productivity of lipid in Glagah mixed culture was higher than single culture *C. zofingiensis*. Single culture lipid productivity of *C. zofingiensis* in raceway pond was 0.16 mg L^{-1} day^{-1}. Meanwhile, lipid productivity Glagah mixed cultures could achieve four times higher than single culture *C. zofingiensis*, accounted for 0.71 mg L^{-1} day^{-1}. In addition, fatty acids from microalgae cells Glagah mixed culture containing saturated fatty acids (SFA) was quite high. According to Dubois *et al.* (1956) the SFA had a higher cetane number value than MUFA and PUFA, so SFA was more effective as a source of biodiesel. It was addressed that Glagah mixed culture was effective as a source of biodiesel saturated fatty acids (SFA) was quite high.

Conclusion

Bacterial association with microalgae consortium from Glagah Coast consisted of *Corynebacterium, Bacillus, Pediococcus* and *Staphylococcus,* which had potency to support microalgae growth by supplying availability of vitamin B (cobalamin, thiamine and biotin). Co-culture microalgae-bacteria from Glagah Coast could be used as an effective sustainable strategy to produce biodiesel derived

Table 1: Characterization results of isolates of bacteria associated with microalgae

Isolates	Picture	Color	Cell Morphology	Gram	Species
G9B1		Orange whitish	Bacilli	+	*Corynebacterium ulcerans*
G10A		Orange	Bacilli	+	*Corynebacterium bovis*
G9B2		White milk	Bacilli	+	*Bacillus cereus*
G9C2		Brownish white	Bacilli	+	*Bacillus megaterium*
G9E2		Yellow	Coccus	+	*Pediococcus parvulus*
G9A1		Cream	Coccus	+	*Staphylococcus vitulinus*

from microalgae. In addition, co-culture techniques microalgae-bacteria could reduce the cost of production (not require vitamin B).

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