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The Influence of Dominant Bacteria from Various Lakes of East Java, Indonesia on *Chlorella* sp. Culture

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Abstract. Synergetic bacteria need to be explored which can accelerate the growth of microalgae which have potential as oil producer related to renewable energy. This research aimed to analyze the influence of the dominant bacteria from Ranu Pane (POD code), Ranu Grati (GOD code), Ranu Regulo (ROD code), and the recombinant dominant bacteria from all sampling areas (PGR code) on the growth of *Chlorella* sp. in co-culture. Also, the individual crosscheck was done by making co-interaction between the predominant bacteria from each and biofuel producing microalgae (*Chlorella* sp.). The co-culture data between the dominant bacteria species and *Chlorella* sp. showed that dominant species had different effects on the number of microalgae cells in the co-culture. Thus, co-culture could accelerate the growth of *Chlorella* sp, extend the log and stationary phases and enhance the environmental carrying capacity.

Keywords: Synergistic bacteria, dominant, co-culture, growth, microalgae

1. Introduction

Microalgae biomass is comparable to the biofuel produced. The lipid in microalgae consists of glycerol, saturated fatty acids or unsaturated fatty acids. The fat composition in each microalga is influenced by several factors, such as differences in nutrition, environment, and growth phase [1]. Various efforts have been made to exploit microalgae for bioenergy production processes. One of these efforts is with a co-culture by adding bacteria to algae culture. Indeed, in aquatic ecosystems, there is a specific co-interaction between bacteria and algae in various ways [2]. Microalgae provide oxygen, organic C, low material weight in the form of proteins, and carbohydrates for bacteria. On the other hand, bacteria provide inorganic substances, Carbon, Nitrogen, Phosphor, some nutrients, and metabolites (iron, hormone, EPS-flocculation) [3].

The relationship between bacteria and microalgae depends on species and environmental conditions [4]. For instance, there was a positive relationship between bacteria and *Chlorella* sp. [5]. This study proved that *P. diminuate* and *P. vesicularis* isolated from cultural algae could accelerate the growth of microalgae *Scenedesmus bicellularis* and *Chlorella* sp. Another study was to accelerate the growth of *Chlorella ellipsoidea* by adding (co-inoculant) bacteria isolated from long-standing *Chlorella* cultures. The results of the study proved that the growth of *Chlorella ellipsoidea* could be accelerated to 0.5-3 times [6]. Microalgae and bacteria in the lakes have high diversity and abundance



which are affected by the activities around the lake [7]. Ranu Grati, Ranu Pane, and Ranu Regulo were chosen as sampling locations since they have different activities around the environment which causes high microbial diversity. Importantly, the abundance of microalgae is directly proportional to the wealth of heterotrophic bacteria [8].

Microalgae biomass and lipid production can be increased by growing microalgae with a community of bacteria associated with microalgae [9]. The previous study in aquatic ecosystems showed specific interactions between algae and bacterial species [10]. Hence, through this study, co-culture of dominant bacteria from three lakes in East Java Province with *Chlorella* sp. was conducted to improve the fundamental interaction between both organisms. It is suggested that the co-culture can increase biomass and lipid levels of microalgae.

2. Methods

2.1. Bacterial Culture

In this laboratory work, co-culture was conducted between *Chlorella* sp. with the dominant bacteria from Ranu Pane (bacterial species with POD code), Ranu Regulo (bacterial species with ROD code), and Ranu Grati (species with GOD code). PGR is a co-culture treatment of microalgae with a mixture of dominant bacteria from three lakes. The dominant bacterial culture was obtained from our previous studies (the data are not shown). Bacteria and *Chlorella* sp. were then prepared for the co-cultured in nutrient broth media for 24 hours.

2.2. *Chlorella* sp. culture

Chlorella sp. was amplified by using Walne media for approximately five days until they reached the exponential phase. Microalgae in the exponential phase were used as a starter in co-culture with the dominant bacteria.

2.3. Co-culture

Co-culture of 200 L-volume bottles consisted of 150 mL of Walne media, 25 mL of bacterial starter and 25 mL of starter *Chlorella* sp. Cultures with batch systems were maintained with continuous light, at the intensity of 100-200 $\mu\text{E sec}^{-1} \text{ m}^{-2}$ and the temperature of 25 °C [11]. The growth of microalgae was observed every two days by counting the number of *Chlorella* sp. cells by using hemocytometer.

2.4. Data Analysis

Principal component analysis (PCA) was also carried out to the relationship between dominant species of bacteria parameters and the relative growth of microalgae and bacteria in the co-culture. Multivariate analyses were done using Paleontological Statistics software (PAST, version 3.15).

3. Results and Discussion

In recent years, microalgae biotechnology began with the development of the potential of microalgae as biomass producers for biofuel production [12]. It is essential to produce microalgae in large quantities at low cost [13], and it can be done by co-culture between bacteria and microalgae [14]. The research results obtained showed that the co-culture between microalgae *Chlorella* sp. co-culture and bacteria had faster growth; the number of cells in all treatments was more than the control (Figure 1.). The dominant bacteria from various lakes have a positive correlation to the increase in microalgae cell biomass. Naturally, microorganisms usually benefit from the community and the consortium. There is an increasing interest in the use of co-culture to improve productivity and product diversity. The biomass, specific growth rate, and maximum productivity of *C.vulgaris* increased than co-culture with *Stenotrophomona smaltophilia*. The results indicated microalgal biomass and the quality of biodiesel could improve with the microalgae-bacteria co-culture system [15].

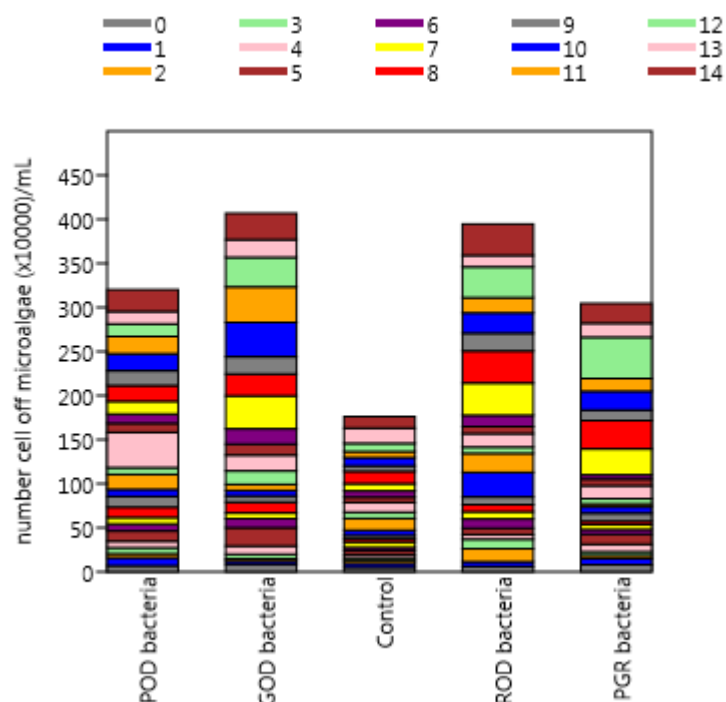


Figure 1. The number of microalgae cells in each treatment by co-culture with dominant bacteria

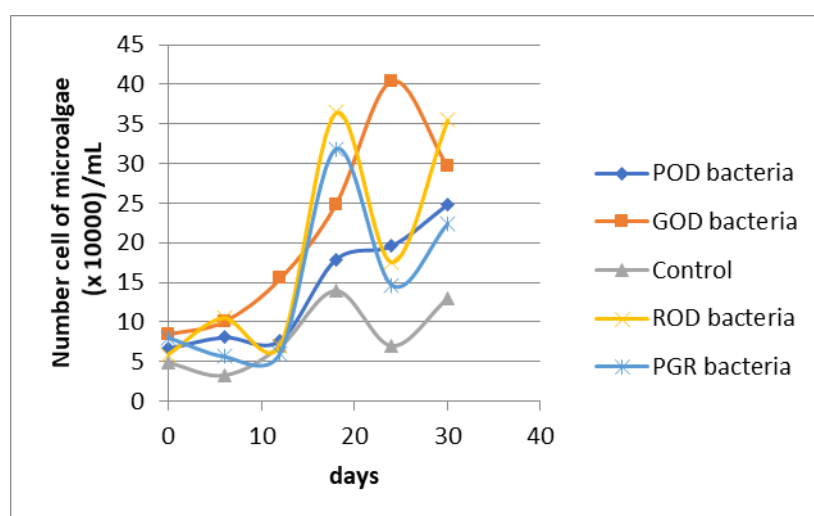


Figure 2. The growth of microalgae in co-culture for 30 days of observation.

The growth of microalgae started from the adaptation phase around 2nd-10th day. The exponential phase of the 12th-20th day and the stationary phase started on the 20th day until the 26th day then the death phase began on the 28th and 30th day. The log phase until the end of the stationary phase lasted in ten days (Figure 2). The results showed that the number of cells in co-culture was more comparable to monoculture treatment.

The data showed that the control was in one cluster (almost the same) with the addition of PGR bacteria. While the treatment co-culture with bacteria POD was further, the cluster and treatment with ROD bacterium was the farthest. The number of microalgae cells in the control treatment was the least, and the co-culture treatment with ROD bacteria (Ranu Regulo dominance) and the highest number of microalgae cells was co-culture with GOD dominant bacteria from Ranu Grati (Figure 3).

In nature, the abundance of microalgae is directly proportional to the plenty of bacteria. It is known that the interaction between microalgae and bacteria has the potential to increase algae biomass that produces desirable industrial compounds such as proteins, lipids, and carbohydrates. In the future, the symbiotic approach of bacteria and algae to produce more compounds and increase the number of each could use an ecological engineering approach [17]. Environmental engineering or synthetic ecology is a broad term used for artificial biomimetic systems that employ a multi-organism approach to present-day solutions [17]. In terms of increasing interest in applying co-culture, it is necessary to carefully and systematically consider several aspects if it will be applied in the industrial field [18].

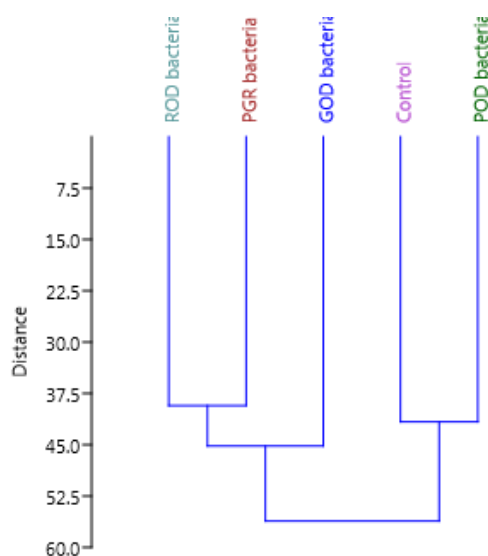


Figure 3. The potential clustering model of potential bacterial-microalgae co-culture

It was proven that the addition of bacteria to microalgae culture could increase the number of microalgae cells. The abundance of microalgae in the aquatic environment is directly proportional to the wealth of heterotrophic bacteria. There is a mutually beneficial symbiotic relationship between the two groups of microbes. The bacteria decompose organic material into inorganic substances that are ready to be used as microalgae nutrients, besides bacteria can also produce hormones and vitamins that are useful for the growth of microalgae. Meanwhile, microalgae provide organic substances that can be used as nutrients from bacteria. This symbiosis occurred in co-culture between microalgae and bacteria causing microalgae in the co-culture could grow faster, log phase to stationary end longer and higher environmental carrying capacity. The co-culture of *Chlorella* sp. with dominant bacteria from several lakes in East Java is quite novel. Hence, this study is the first report for future ecological engineering in green energy development by using certain potential bacteria to produce maximum biomass from microalgae.

4. Conclusion

Co-culture among the dominant bacteria from several lakes in East Java could accelerate the growth of *Chlorella* sp., extend the log and stationary phases, and enhance the environmental carrying capacity.

Acknowledgments

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