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Synergistic effect of co-culture of microalga and actinomycete in diluted chicken manure digestate for lipid production

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ABSTRACT

Microalgae have high potential as organisms that can be used for biodiesel production because they can accumulate high amounts of lipids in a similar manner to oleaginous plants. However, the economic feasibility of their lipid production is still in question. Possible methods of improving that feasibility involve a reduction in cultivation costs using effluents along with an increase in lipid production by co-cultivation with plant growth-supporting microorganisms. Previous studies have found that actinomycetes possess a potential as plant growth-promoters. Thus, this research study is aimed at investigating the effects of the co-cultivation of actinomycetes with microalgae on growth and lipid production in diluted chicken manure digestate. In this study, over 190 actinomycetes were screened for their ability to grow in the digestate, as well as for their plant growth promoting abilities. The actinomycete, *Nocardia bhagyanarayanae* I-27, could promote chlorophyll *a*, biomass and lipid contents in a co-culture with green microalga *Tetradesmus obliquus* AARL G022 in 25% diluted digestate. Microalgal major fatty acid methyl esters are a suitable substrate for biodiesel production. In addition, the essential Ω3 fatty acids, including α-linolenic acid and eicosapentaenoic acid, also increased in volume. Thus, the co-cultivation of plant growth promoting actinomycetes with microalgae in wastewater could be an alternative strategy used to increase biomass and lipid production.

1. Introduction

Microalgae are organisms possessing lipid production potential because they can accumulate high contents of lipids in a similar way to those found in other oil-bearing plants. Their lipids could be either applied as feedstock for biodiesel production or consumed as highly nutritional essential fatty acids depending on the lipid profiles [1]. However, the cost of these nutrients for microalgal cultivation is still high [2]. Thus, it is essential to find cheaper sources of nutrients which could promote biomass and lipid accumulation. The effluent acquired from livestock production facilities in the form of animal manure (swine and chicken manure), along with its anaerobic digestate, showed high nitrogen and phosphorous concentrations [3]. The amalgamation of wastewater nutrient removal and microalgal production has been acknowledged for capital restraint [3].

Previous studies have found that several microorganisms could promote the growth of plants through their plant growth regulators [4], such as bacteria [5], actinomycetes [6,7], and fungi [8]. In order to improve microalgal biomass production, the supplementation of plant growth regulators has been suggested. Plant growth regulators could successfully enhance microalgal growth rate [9], lipid production and hydrocarbon chain length [10]. Co-cultivation of plant growth promoting bacteria and fungi with microalgae has been found to facilitate both algal biomass and lipid production [11].

However, the application of actinomycetes to enhance microalgal production has not yet been fully investigated. Actinomycetes are high GC Gram-positive bacteria that generally inhabit soil and rhizosphere, and can colonize the internal tissues of plants without causing any damage or morphological changes in those plants [12]. They are a rich source of secondary metabolites with diverse biological activity and have shown potential in various beneficial applications, such as in antibiotics and as anticancer agents. They also contain notable amounts of various bioactive substances, along with vitamins and enzymes. In addition, some strains are able to provide a broader array of microalgae growth-promoting substances from general respiration end-products such as CO_2 and more specific compounds such as nutrients and indole-3-acetic acid (IAA) [13]. Some actinomycetes also produce siderophores and phosphate solubilizing substances [14,15]. These abilities will enhance the nutrient uptake of microalgae, especially in wastewater, in which the nutrients may be present in the form of

Abbreviations: PGP, plant growth promoting; DEM, digestate effluent medium

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macromolecules or insoluble substances [16,17]. Thus, cultivation of actinomycetes along with microalgae may promote the growth and lipid production of microalgae.

The microalga AARL G022 has recently been identified as a fast-growing strain that possesses a large colony and can be easily harvested. It contains high levels of lipids at up to 17% of the cell dry weight [18]. It can grow well in 25% chicken manure digestate and is tolerant toward contamination [19]. This microalga was morphologically identified as *Scenedesmus* sp. AARL G022 in the previous study; however, present molecular identification has revealed that this strain is *Acutodesmus obliquus* AARL G022. Recently *A. obliquus* (Turpin) Hegewald & Hanagata (2000) is regarded as a synonym of *Tetradesmus obliquus* (Turpin) M.J.Wynne (2015) [20]. Thus, this G022 strain is presently referred to as *Tetradesmus obliquus* AARL G022. The co-culture of microalgae with other bacteria in wastewater for lipid production has been reported [21], but the co-culture of microalga and actinomycete in wastewater has not yet been studied. This study aimed to screen actinomycetes that possess plant growth promoting abilities and can be grown in chicken manure digestate. In addition, the effect of co-cultivation of these actinomycetes with green microalga in chicken manure digestate was also investigated. The growth and lipid production of microalga was observed along with its lipid profile. Thus, this manuscript is the first to report on the co-cultivation of actinomycetes and green microalga in diluted wastewater. This co-cultivation process will promote growth and lipid production in cheap sources of nutrients, which may provide an opportunity to improve the economic feasibility of microlgal biofuel production.

2. Methods

2.1. Microorganisms and culture conditions

2.1.1. Actinomycetes

One hundred-ninety actinomycetes were obtained from the Sustainable Development of Biological Resources Lab or (SDBR), Department of Biology, Faculty of Science, Chiang Mai University. The selected isolates were screened for their growth on 25% effluent medium agar and plant growth promoting (PGP) traits. All strains were maintained on International Streptomyces Project 2 medium agar (ISP2) for use in further studies. The selected actinomycetes were molecularly identified and the genomic DNA was extracted using the FavoPrepTM Tissue Genomic DNA Extraction Mini Sample Kit (Taiwan), as was described by the manufacturer. DNA was amplified using the primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) [6]. The nucleotide sequences of the obtained 16S rDNA were subjected to BLAST analysis with the NCBI database and submitted to GenBank.

2.1.2. Microalga

Green microalga *T. obliquus* AARL G022 was obtained from the Applied Algal Research Laboratory (AARL), Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand. It was maintained in Jaworski's medium (JM) in 250 mL Erlenmeyer flasks under continuous illumination of 10.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a fluorescent lamp. It was shaken at 125 rpm and then kept at 25 °C until being used.

2.2. Preparation and characteristics of 25% digestate effluent medium (DEM)

Digestate effluent samples were collected from a biogas plant at a chicken farm in Lamphun Province, Thailand. 25% DEM was prepared by dilution of the effluent with dechlorinated tap water and then sterilized by being autoclaved at 121 °C for 15 min. This is the optimal concentration for cultivation of microalga *T. obliquus* AARL G022 [19]. After sterilization, the physical and chemical properties of the triplicate samples of 25% DEM were analyzed in accordance with the methods of the American Public Health Association (APHA) [22]. The physical factors of the 25% DEM included the following: pH value at 7.1 ± 0.0, nitrate-nitrogen (NO_3^--N) content at 26.0 ± 7.5 $mg \cdot L^{-1}$, soluble phosphate phosphorus (PO_4^{3-}-P) content at 34.8 ± 8.5 $mg \cdot L^{-1}$, ammonium-nitrogen (NH_4^+-N) content at 90.9 ± 13.6 $mg \cdot L^{-1}$ and chemical oxygen demand (COD) at 203.0 ± 12.3 $mg \cdot L^{-1}$.

2.3. Screening for effluent growth and plant growth promoting actinomycetes

2.3.1. Preparation of actinomycete isolates for the screening of plant growth promotion and other properties

The one hundred-ninety actinomycetes used in this study were cultivated on ISP2 agar and incubated at room temperature for 7 days to achieve good sporulation. The specimens were then stored at 4 °C in a refrigerator for use in further studies.

2.3.2. Growth on 25% digestate effluent medium (DEM) agar

Actinomycetes were screened for their growth on 25% DEM agar containing 25% digestate effluent and 1.5% agar. Each actinomycete isolate was streak-inoculated onto a plate, divided into equal sectors and incubated at 30 °C for 7 days. The growth on the plate (Fig. 1) was scored as (++) for well-growth, if the growth was observed over half of the streak; (+) moderate-growth, if the growth was observed less than half of the streak; and (−) non-growth if the growth was not observed.

2.3.3. Indole-3-acetic acid (IAA) production

The production of IAA was determined according to the method of Bano and Musarrat [23]. The isolates that grew well in 25% DEM agar were inoculated in 5 mL of ISP2 medium containing 0.2% of L-tryptophan and incubated at 30 °C with shaking at 125 rpm for 7 days. Cultures were centrifuged at 11,000 rpm for 15 min. One milliliter of the supernatant was mixed with 2 mL of Salkowski reagent (50 mL of 35% $HClO_4$, 1 mL of 0.5 M $FeCl_3$) and incubated for 20 min at ambient temperatures. The development of a pink color indicated indole production. Optical density (OD) was recorded at 530 nm using a spectrophotometer (Thermo spectromic, UV-VIS Spectrophotometer-G20). The IAA concentration was determined using the calibration curve of

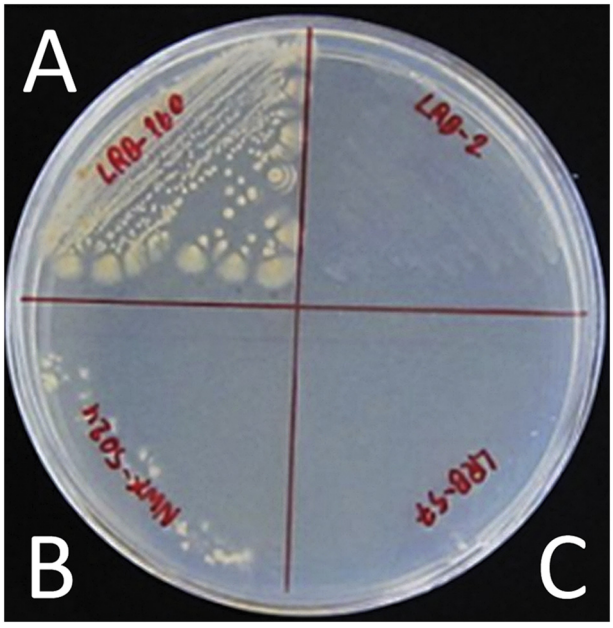

Fig. 1. Growth of actinomycetes on 25% digestate effluent medium (DEM) agar, (++) well-growth (A); (+) moderate-growth (B); and (−) non-growth (C)