Research Article

Isolation and Growth of N_2-Fixing Cyanobacteria from Organic Agricultural Areas in Sanamchaikate, Chachoeng-Sao Province, Thailand

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Abstract

Forty-five isolates of nitrogen-fixing cyanobacteria were obtained from organic soils at Chachoeng-Sao province. Of these, the most diverse genus belonged to *Nostoc* (31 isolates), followed by *Tolypothrix* (8 isolates), *Anabaena* (1 isolate), *Calothrix* (1 isolate), *Fischerella* (1 isolate), *Scytonema* (1 isolate) and 2 isolates were unknown. Five predominant isolates, including *Nostoc* sp. TUBT03, *Nostoc* sp. TUBT04, *Nostoc* sp. TUBT05, *Nostoc* sp. TUBT06 and *Tolypothrix* sp. TUBT31, were selected to study for growth in BG-11 liquid medium without nitrogen source (BG-11_0) at 25°C on a 120 rpm rotary shaker and 12: 12 light-dark cycles. Samples were taken every 5 days for 45 days and packed cell volume (PCV) and dry cell weight (DCW) were examined. Accoding to DCW, *Nostoc* sp. TUBT05 had the highest specific growth rate of 0.2952 d^{-1}, follow by *Nostoc* sp. TUBT04 (0.2424 d^{-1}), *Nostoc* sp. TUBT06 (0.2184 d^{-1}), *Nostoc* sp. TUBT03 (0.1968 d^{-1}) and *Tolypothrix* sp. TUBT031 (0.0528 d^{-1}), respectively. The highest PCV of 105.79 ml L^{-1} was shown by *Nostoc* sp. TUBT06, followed by *Nostoc* sp. TUBT03 (75.72 ml L^{-1}), *Nostoc* sp. TUBT05 (61.56 ml L^{-1}), *Nostoc* sp. TUBT04 (28.22 ml L^{-1}) and *Tolypothrix* sp. TUBT031 (21.69 ml L^{-1}), respectively. *Tolypothrix* sp. TUBT031 showed the highest DCW (2.02 g L^{-1}), follow by *Nostoc* sp. TUBT03 (1.02 g L^{-1}), *Nostoc* sp. TUBT06 (0.93g L^{-1}), *Nostoc* sp. TUBT04 (0.74 g L^{-1}) and *Nostoc* sp. TUBT05 (0.60g L^{-1}), respectively. All these 5 cyanobacterial isolates can grow well in BG-11_0. Therefore, they can be applied as biofertilizers. Further study on cyanobacterial biochemical substance production is needed.

Keywords: nitrogen-fixing cyanobacteria; specific growth rate; packed cell volume; dry cell weight.

1. Introduction

N_2 fixing cyanobacteria have been well known as a natural biofertilizer. Their role is to supply field fertility, especially as nitrogen supplier via a process of biological nitrogen fixation, converting dinitrogen from the atmosphere into plant usable forms [1]. N_2 fixing cyanobacteria have a potential to contribute to the productivity of a variety of agricultural crops. In addition, they have been reported as a biofertilizer to substitute part of the use of synthetic fertilizer that can decrease the quantity and the cost of synthetic fertilizers. Application of

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cyanobacterial isolates along with 1/3 N+P+K presented statistically comparable results as compared to an application of a full dose of chemical fertilizers in terms of wheat grain yields [2]. The use of cyanobacteria as biofertilizer allowed a 50% saving in the use of chemical fertilizer with similar results in terms of grain yield and quality in rice and peas [1, 3].

Besides, the N_2 fixing cyanobacteria offer an ecologically sound alternative for increasing productivity especially in organic agricultural areas. Moreover, inoculation with cyanobacteria in agricultural soil improves soil structure and fertility. Inoculated degraded soils with *Nostoc* resulted in soil N and C increase and enhance soil fertility and structure [4]. However, the introduction of cyanobacteria species that are unadapted to the edaphoclimatic condition, some brand of commercial biofertilizer, have any effect on crop productivity [1]. Therefore, to obtain the Thai organic indigenous N_2-fixing cyanobacteria applied in Thai agricultural areas, this work was aimed to isolate, culture and study the growth of the N_2-fixing cyanobacteria from organic rice fields in Chachoeng-Sao province.

2. Materials and Methods

2.1 N_2 fixing cyanobacteria isolation

Organic soil was randomly sampled from organic paddies in Sanamchaikate, Chachoeng-Sao province. N_2-fixing cyanobacteria were isolated by dilution plating and manual-isolation techniques. The former technique was done by weight 1 g soil into 9 ml sterile water, and the 10^{-5} dilution was spread onto BG-11_0 agar medium. The latter technique was done by hand-sorting cyanobacterial cell, washing in sterile water 5 times and culture in 96-well micro plates containing 150 µl of BG-11_0 liquid medium. All were incubated at 25°C under continuous light. Then, cyanobacteria colonies were randomly selected and cultured in BG-11_0. An axenic N_2 fixing cyanobacteria culture was obtained by a repeated dilution plating technique.

2.2 Growth of N_2 fixing cyanobacteria

The 5 predominant isolates including *Nostoc* sp. TUBT03, *Nostoc* sp. TUBT04, *Nostoc* sp. TUBT05, *Nostoc* sp. TUBT06 and *Tolypothrix* sp. TUBT31 were selected for growth study. Each isolation stock was prepared by homogenizing 1 ml of cyanobacteria cell using a tissue grinder then resuspending the cell in 50 ml BG-11_0. Then, 1 ml of cyanobacteria stock was added into a 125 ml Erlenmeyer flask containing 25 ml BG-11_0. All were incubated at 25°C on a 120 rpm rotary shaker and 12:12 light-dark cycles. Samples were taken every 5 days for 45 days and the packed cell volume (PCV) and dry cell weight (DCW) were examined [5]. There were 2 replications for each isolate. Then, the specific growth rate (µ) of 5 isolates was calculated using a Sigma plot program.

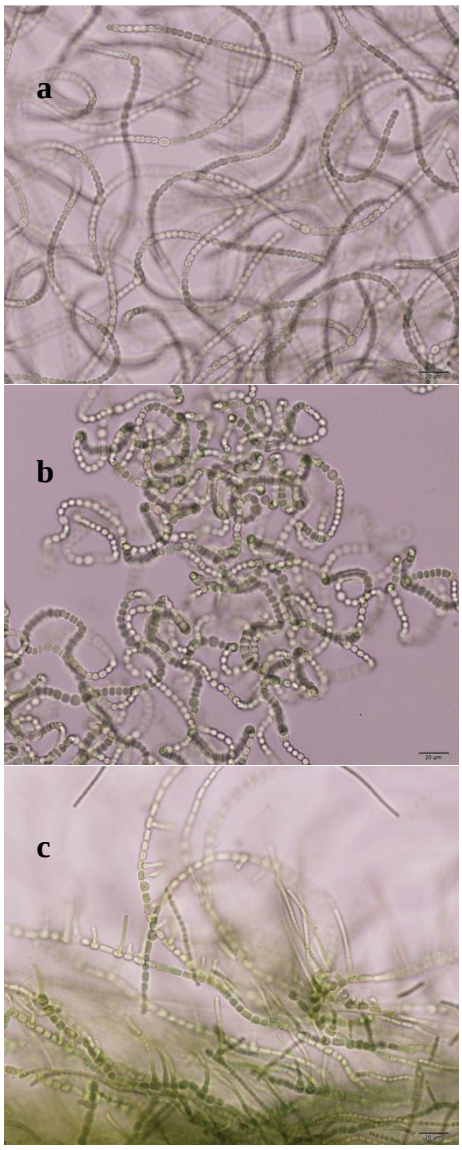
3. Results and Discussion

3.1 N_2 -fixing cyanobacteria Isolation

Forty-five isolates of nitrogen-fixing cyanobacteria were obtained from organic soils at Chachoeng-Sao province. Of these, the most diverse genus belonged to *Nostoc* (31 isolates) (Fig. 1a and 1b), followed by *Tolypothrix* (8 isolates), *Anabaena* (1 isolate), *Calothrix* (1 isolate), *Fischerella* (1 isolate) (Fig. 1c), *Scytonema* (1 isolate) (Fig. 1d). Two isolates were unknown. Most of them are filamentous heterocytous cyanobacteria. Heterocyst is a special cell providing anaerobic condition for nitrogenase enzyme [e.g. 6,7]. These special cells play a crucial role in nitrogen fixing, which bring cyanobacteria become a good candidate for a natural biofertilizer.

From this study, the most diverse genus of N_2 fixing cyanobacteria was *Nostoc* and

followed by *Tolypothix*. All of genera we report here have been recorded from many parts of Thailand [8]. The two genera, *Nostoc* and *Tolypothix* have been well documented that they are well known use as biofertilizers [9]. In particular, *Nostoc* spp. has been used as a biofertilizer for a variety of crops such as rice [1, 10], pea [3] and wheat [2].



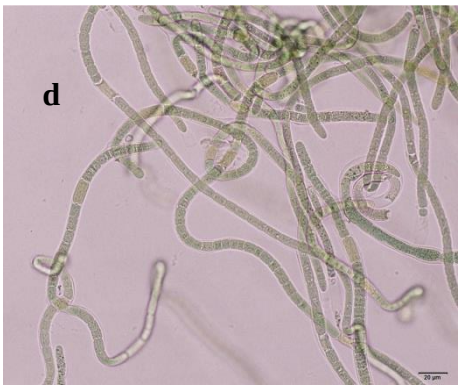


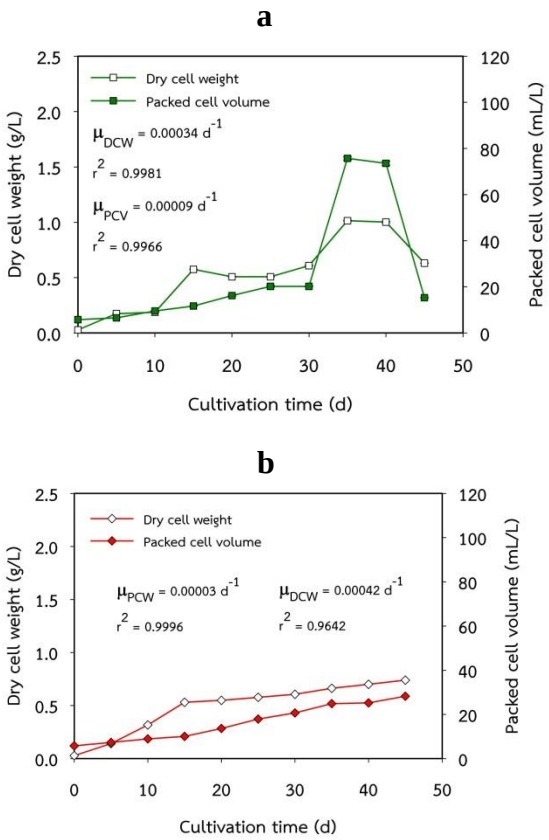
Fig.1. Some N_2-fixing cyanobacteria isolated from soil collected from organic agricultural areas in Chachoeng-Sao Province (a: *Nostoc* sp. TUBT04, b: *Nostoc* sp. TUBT05, c: *Fischellara* sp. TUBT043 and d: *Scytonema* sp. TUBT044).

3.2 N_2-fixing cyanobacteria growth

The five predominant isolates including *Nostoc* sp. TUBT03, *Nostoc* sp. TUBT04, *Nostoc* sp. TUBT05, *Nostoc* sp. TUBT06 and *Tolypothrix* sp. TUBT31 were selected to study for growth in $BG\text{-}11_0$ medium. Their growth curves were shown in Fig. 2. With respect to DCW, *Nostoc* sp. TUBT05 had the highest specific growth rate of 0.2952 d^{-1}, followed by *Nostoc* sp. TUBT04 (0.2424 d^{-1}), *Nostoc* sp. TUBT06 (0.2184 d^{-1}), *Nostoc* sp. TUBT03 (0.1968 d^{-1}) and *Tolypothrix* sp. TUBT031 (0.0528 d^{-1}), respectively. The highest PCV of 105.79 ml L^{-1} was shown by *Nostoc* sp. TUBT06, followed by *Nostoc* sp. TUBT03 (75.72 ml L^{-1}), *Nostoc* sp. TUBT05 (61.56 ml L^{-1}), *Nostoc* sp. TUBT04 (28.22 ml L^{-1}) and *Tolypothrix* sp. TUBT031 (21.69 ml L^{-1}), respectively. *Tolypothrix* sp. TUBT031 showed the highest DCW (2.02 g L^{-1}), followed by *Nostoc* sp. TUBT03 (1.02 g L^{-1}), *Nostoc* sp. TUBT06 (0.93g L^{-1}), *Nostoc* sp. TUBT04 (0.74 g L^{-1}) and *Nostoc* sp. TUBT05 (0.60g L^{-1}), respectively.

From the result, PCV in *Nostoc* spp. was higher than *Tolypothrix* sp. while DCW in *Tolypothrix* sp. was greater than *Nostoc* spp.. Thick capsular polysaccharides enveloped *Nostoc* spp. while only a small

amount was found for *Tolypotrhix* sp.. Genus *Nostoc*, moreover, has been reported to produce a large amount of extracellular polysaccharides [11], which may cause a large pack cell volume in *Nostoc* sp.. Exocellular polysaccharides are well known as a soil conditioner that improves aggregate stability in soil [4, 12]. Therefore, *Nostoc* is not only a good candidate species for biofertilizer production but also a good applicant for a soil conditioner. Additionally, some of N_2 fixing cyanobacteria can produce indole-3-acetic acid (IAA), which promotes plant growth [13, 14]. According to previously reports, their ability in nitrogen fixation and exopolysaccharide and phytohormone production make cyanobacteria as a good source for biofertilizer.



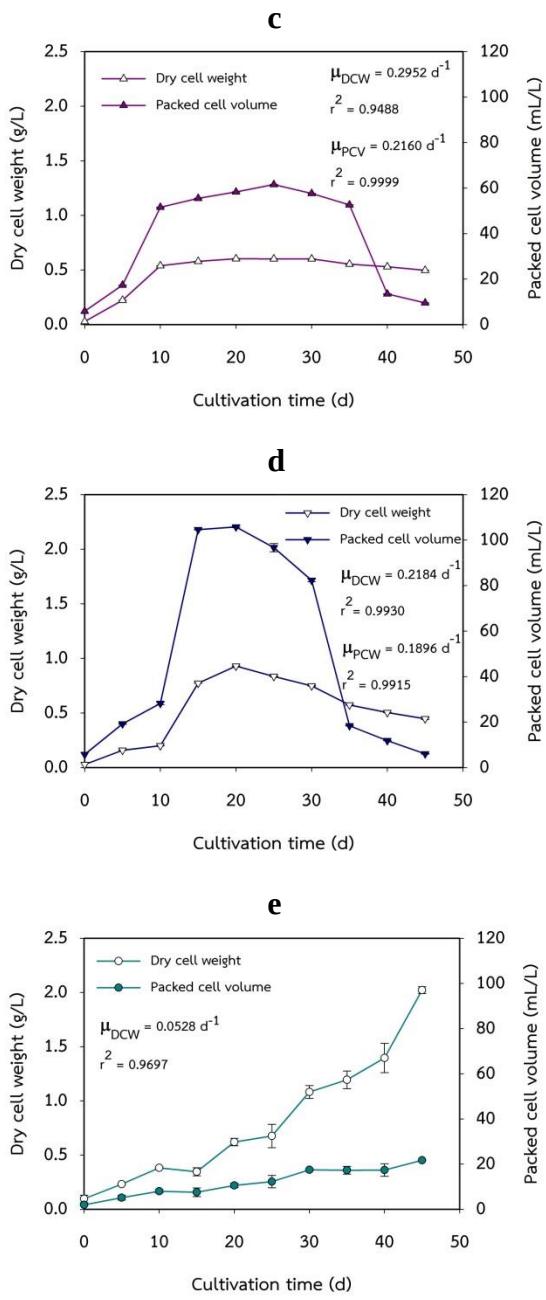


Fig.2. Growth curve of five N_2-fixing cyanobacteria grown in BG-11N_0 for 45 days (a: *Nostoc* sp. TUBT03, b: *Nostoc* sp. TUBT04, c: *Nostoc* sp. TUBT05, d: *Nostoc* sp. TUBT06 and e: *Tolypothrix* sp. TUBT031; transparent symbols represented DCW and opaque symbols represented PCV).

4. Conclusion

Among these 5 cyanobacterial isolates, *Nostoc* spp. and *Tolypothrix* sp. can grow well in the experimental condition used with no nitrogen sources. Therefore, they show potential for biological nitrogen fixation, thus allowing them to be used as biofertilizers in agricultural areas. For a practical application of cyanobacteria fertilizer in the field, however, further studies mass culture, metabolite production and the effect of metabolic compounds on plant growth will be needed.

5. References

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