STUDY GROWTH CURVE AND SOME FACTORS THAT EFFECT TO RED PIGMENT PRODUCING OF RED YEASTS.

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ABSTARCT

The three isolates of Red yeast, isolate B, isolate G and isolate Hwere screen from soil that collected in Dusit province, Bangkok, Thailand. This study was direct to study some optimal culture condition for red pigment production by Red yeasts isolate B, G and H under liquid medium in 500 ml Durham bottle culture. The growth curve of three isolates were study. The effect of four carbon sources, glucose, sucrose, glycerol and fructose and aeration by shaking at 150 rpm and 190 rpm on red pigment production by Red yeasts were test. The result found that aeration by shaking at 190 rpm, all of three isolates reached to stationary phase faster than aeration by shaking at 150 rpm. Aeration by shaking at 190 rpm all of three isolates reached to stationary phase at 48 hrs and aeration by shaking at 150 rpm all of three isolates reached to stationary phase at 72 hrs. For the effect of carbon sources and aeration on red pigment production, glucose 10 grams per liter was the best carbon source follow by sucrose, glycerol and fructose respectively and aeration by shaking at 150 rpm was the best condition for all the three isolates.

Keywords: Red yeast, Red pigment, Optimal culture conditions

INTRODUCTION

Carotenoids are very interesting natural lipid-soluble pigments displaying red, orange and yellow colors that exist in wide variety of plants and microorganisms [1], [2], [3]. Carotenoids play an importance role as vitamin A precursors in animals, against activated oxygen and increase of invitro antibody production [2]. The industrial demand for carotenoid pigments, such as □-carotene and astaxanthin, is increasing due to the wide variety of applications as food coloring agents, e.g., margarine, soft drinks, and baked goods, as precursors of Vitamin A (pro-Vitamin A) in food and animal feed, as additives to cosmetics and multivitamin preparations, and in the last decade as antioxidants to reduce cellular or tissue damage [4]. Carotenoids are produced primarily by filamentous fungi and yeasts and by some species of bacteria, algae and lichens [5]. The commercial carotenoids are obtained by extraction from vegetable and chemical synthesis [6]. Microbial carotenoids are very interesting in recent years. The main reason for the interest in using microorganisms to produce carotenoids that can otherwise be isolated from plants and animals or synthesized is not difficult in increasing production by environmental and genetic manipulation [7]. Although there are several microorganisms that produce carotenoids, only a few of them are interested by biotechnological. For these reasons, the isolation and screening of microorganisms, which are produce worth carotenoids such as astaxanthin, torulene, lutein and β–carotene must be study [8].

OBJECTIVES

1. To study growth curve of red pigment producing yeasts 3 isolates, isolate B, isolate G and isolate H.
2. To study some factors that effect to red pigment production of the 3 red yeasts.

METHODOLOGY

Microorganism

The three red yeast isolates, isolate B, isolate G and isolate H were screen from soil that collected in Dusit province, Bangkok, Thailand.

Determination of yeast growth curve

This study was carried out by determining the optical density (OD) of cell yeast in YM broth 250 ml in 500 ml durham bottle using spectrophotometer (Thermo Spectronic Genesys 20). Cell culture broth OD 0.1 was incubated on orbital shaker at 190 or 150 rpm at 30 ^{O}C in triplicate. The absorbance of the culture were determine at 1 day intervals for 10 days starting at day zero (0 Day). The wavelength of spectrophotometer was set to 600 nm and blanked with a cuvett containing 1 ml sterile YM broth.

Determination of carotenoid pigment

For extract carotenoid, 3 ml of sample were taken from each durham bottle at 1 day intervals for 10 days. Cells were harvested by centrifugation at 5000 rpm for 15 min at 4^{O}C, the cell pellet was washed twice with sterile deionized water resuspension in 3 ml of 90 % aceton incubated in the dark at 4^{O}C for 2 days. The mixer was subjected to 5 ultrasonic cycles at 40 kHz for 10 min. Centrifugation was performed at 5000 rpm for 15 min at 4^{O}C to remove the biomass from the extracted carotenoids. The carotenoids-containing supernatant was analyzed by spectrophotometer the wavelength of spectrophotometer was set to 455 nm [2].

Effect of carbonsource

Cabon sources were veried with glucose, sucrose, fructose and glycerol. These carbons sources were used at a final concentration of 1% in YM broth 250 ml in 500 ml durham bottle. The cultures were incubated on orbital shaker at 30 ^{O} C and the experimental was done in triplicate [7]. Determination of carotenoid pigment were determined from cell extract at 1 day intervals for 10 days starting at day zero (0 Day). The wavelength of spectrophotometer was set to 455 nm.

RESULT AND DISCUSSION

Effect of aeration on growth and red pigment production

The 3 isolates of yeast isolate B, isolate G and isolate H, were screen from soil that collected in Dusit province, Bangkok, Thailand. The result of growth curve of 3 yeast isolates that incubated on orbital shaker at 190 or 150 rpm at 30 ^{O}C in triplicate, were determined the culture broth by optical density that measurement at 600 nm (figure 1 and 3). From the results, the 3 isolates of yeast culture that incubated on orbital shaker at 190 rpm reach to stationary phase faster than incubated on orbital shaker at 150 rpm, aeration by shaking at 190 rpm all of three isolates reached to stationary phase at 48 hrs and aeration by shaking at 150 rpm all of three isolates reached to stationary phase at 72 hrs. The result of red pigment production (figure 2 and 4) determined by optical density measurement from cell extract at 455 nm. From the results, all of the 3 isolates of yeast culture that incubated on orbital shaker at 190 rpm produced red pigment less than incubated on orbital shaker at 150

rpm, the highest optical density was measure at day 6 in both conditions these result suggest that red pigment production of the 3 yeast isolates was associate with the growth and red pigment was produced more when growth was slower.

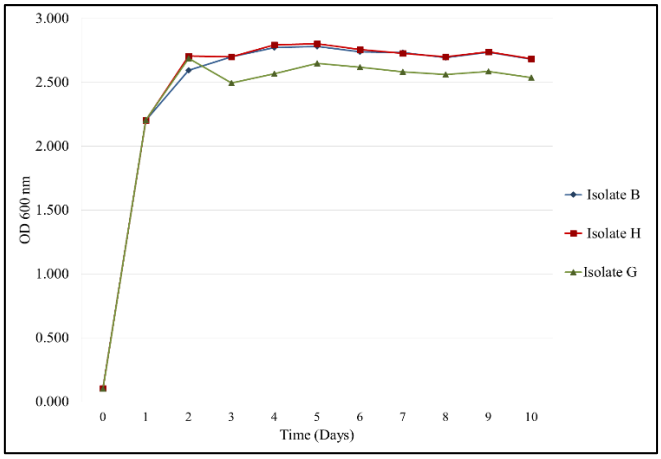

Figure 1. The growth curve of three yeast isolate, Isolate B, Isolate H and Isolate G obtained by optical density at 600 nm. The incubation conditions were shake at 190 rpm at 30 ^{O}C and glucose as carbon source.

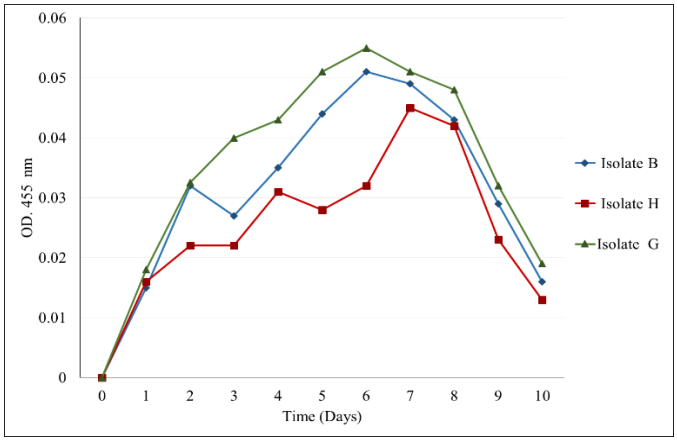

Figure 2. Effect of aeration by orbital shaking at 190 rpm and glucose as carbon source on pigment production by red yeast isolate B, isolate G and isolate H at 30 ^{O}C.

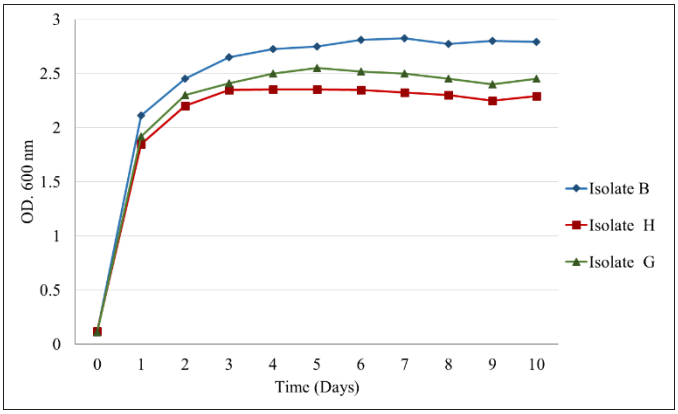

Figure 3. The growth curve of three yeast isolate, Isolate B, Isolate H and Isolate G obtained by optical density at 600 nm. The incubation conditions were shake at 150 rpm at 30 ^{O}C and glucose as carbon source.

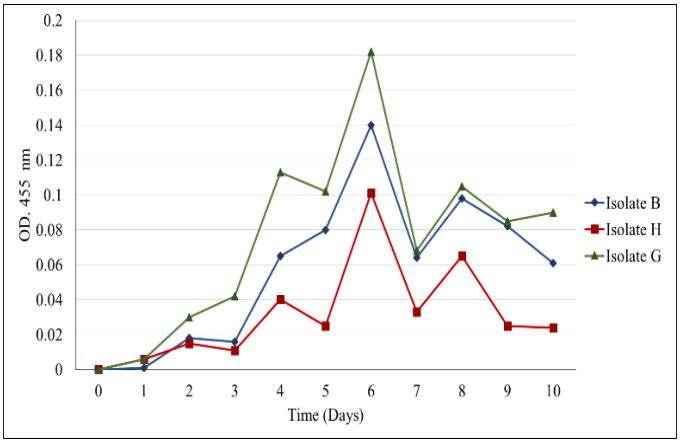

Figure 4. Effect of aeration by orbital shaking at 150 rpm and glucose as carbon source on pigment production by red yeast isolate B, isolate G and isolate H at 30 ^{O}C.

Effect of carbon sources on red pigment production

This study investigated the influence of several carbon sources, glucose, sucrose, fructose and glycerol on red pigment production of the 3 red yeast isolates under culture conditions, incubated on orbital shaker at 150 rpm at 30 ^{O}C. The results found that glucose was the most suitable carbon source for red pigment production for all 3 yeast isolates (figure 2, 4, 5, 6 and 7) follow by sucrose the highest optical density was measure at day 6 in both. The other two carbon sources, fructose and glycerol were not suitable carbon source for red pigment production for all 3 yeast isolates. These results were in accordance with study of Yanchen Zhao .et., al. in 2019 [2]. The report described that glucose can easily be assimilated in metabolic pathway for biosynthesis of carotenoids. From the results (figure 2, 4, 5, 6 and 7) the maximum yield of red pigment that measured from cell extract by optical density at OD 455 nm was found for isolate G follow by isolate B and isolate H respectively.

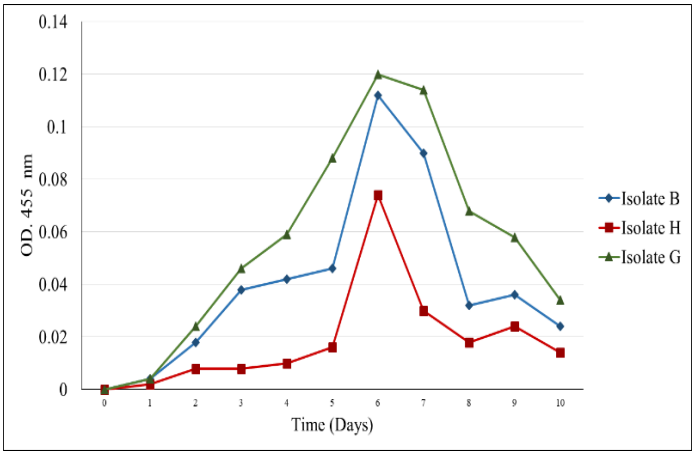

Figure 5. Effect of aeration by orbital shaking at 150 rpm and sucrose as carbon source on pigment production by red yeast isolate B, isolate G and isolate H at 30 ^{O}C.

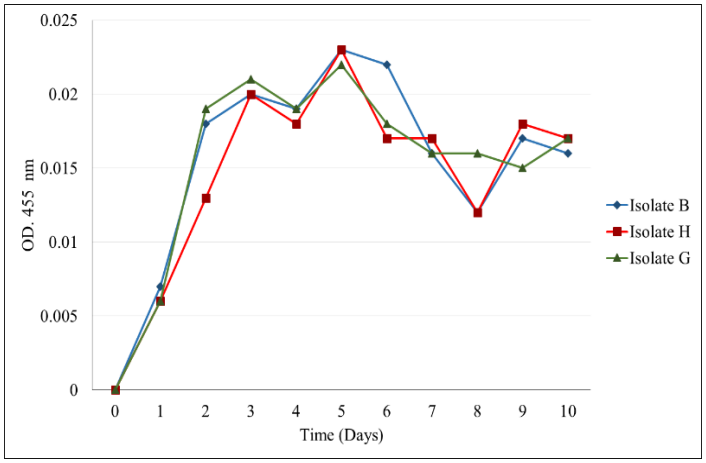

Figure 6. Effect of aeration by orbital shaking at 150 rpm and fructose as carbon source on pigment production by red yeast isolate B, isolate G and isolate H at 30 ^{O}C.

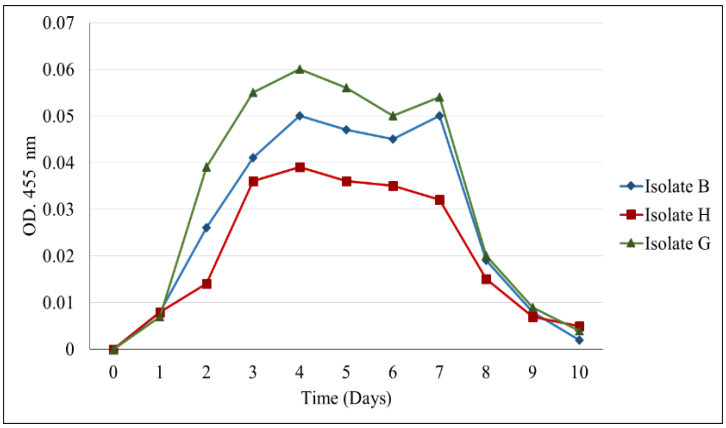

Figure7. Effect of aeration by orbital shaking at 150 rpm and glycerol as carbon source on pigment production by red yeast isolate B, isolate G and isolate H at 30 ^{O}C.

CONCLUSION AND FUTURE WORK

The 3 red yeast isolates, isolate B, isolate G and isolate H were screen from soil that collected in Dusit province, Bangkok, Thailand. Isolate G was considered as the best red pigment production and glucose was the most suitable carbon source for red pigment production under condition that incubated on orbital shaker at 150 rpm at 30^{O}C. The next study is optimization of physiological conditions with the purpose to promote red pigment production from yeast Isolate G.

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