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Determining the potential indigenous red-yeasts producing β-carotene and their phylogenetic relationship

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Abstract

Red-yeasts are pigmented yeast species that could synthesize carotenoids for food supplements and pharmaceutical purposes. However, this group contains a lot of species which need to be explored thoroughly. The objective of this study was to view the *β-carotene* production of three indigenous red-yeasts by modifying glucose content in the growth medium and verifying their phylogenetic relationship. The glucose content modification in growth media influenced the *β-carotene* production of each species. *Rhodosporidiobolus ruineniae* (InaCCY1638/Y15Eg075) and *Rhodosporidiobolus poonsokiae* (InaCCY1606/Y15Kr068) produced higher *β-carotene* than *Rhodotorula paludigena* (InaCCY1527/Y15Eg004). These *Rhodosporidiobolus* species were able to produce higher *β-carotene* from 0.5 to 2% glucose content while *Rhodotorula* was low production in 2% glucose content. The higher producers by *Rhodosporidiobolus* species were clustered to Ruineniae clade and could be a potential clade for higher *β-carotene* production. Based on this result, using a high carotenoid producer of red-yeasts from indigenous strains is potential to be developed for *β-carotene* bioindustry in the future.

Keywords: *β-carotene*, glucose content, phylogenetic tree, red-yeasts

Introduction

β-carotene, one type of carotenoids, is well known as a natural pigment which can be easily recognized by the yellow, red, and orange colours of the fruits or vegetables. The *β-carotene* from the microbial product is the highest carotenoid product sold in the market (Demain and Martens 2016). This compound is important for the biological processes on photoprotection, photomorphogenesis, and hormone precursors (Nisar et al. 2015); antioxidants (Demain and Martens 2016); genes and metabolites regulation (Bertram 1999; Giovannoni 2004; Yuan et al. 2015; Alcaíno, Baeza, and Cifuentes 2016).

Nowadays, carotenoids have been widely used for various purposes in food, pharmaceutical, cosmetics, and chemistry industry. Their characteristics which are associated with provitamin A, antioxidant, anti-cancer, and nutraceutical or food enhancer are beneficial for our life (Nisar et al. 2015; Saini and Keum 2017; Yuan et al. 2015; Demain and Martens 2016). For example, in the previous study, the use of the carotenoid supplement can decrease the health risks including cardiovascular diseases (CVDs), eye diseases, and cancers (Langi et al. 2018; Mussagy et al. 2019).

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Obtaining producers and sources of carotenoids are quite challenging in order to fulfill industrial demand. This situation leads to conducting research on natural carotenoid sources using the microbes (Mussagy et al. 2019; Saini and Keum 2017). By the bioprocess, the microbes can ensure the continuity of the production (Langi et al. 2018; Mussagy et al. 2019). For instance, there are groups of red-yeasts is potential to develop as a promising producer (Buzzini et al. 2007). However, each species has different characteristics which should be gained with a suitable process in order to produce the best carotenoid yield.

The red-yeasts are a group of yeasts that can produce carotenoids especially *β*-carotene. They contain red-pigmented species distributed mostly in Sporidiobolales of Basidiomycota and belong to *Rhodotorula, Rhodosporidium, Sporobolomyces*, and *Sporidiobolus* genera (Buzzini et al. 2007). Some of that species have been evaluated, but knowing in wider species is important to complete the red-yeasts group potency on producing *β*-carotene. However, much work is still needed to find the most potential strain and to verify the phylogenetic relationship. Improving the growth of media and conditional factors during the incubation process is the key to gain the best production strategy. This study focuses on the use of red-yeasts from InaCC collection in order to find out the best media composition, especially glucose for *β*-carotene production.

Materials and methods

Yeast strains and growth condition

In order to obtain a potential β-carotene producer, three indigenous red-yeasts from Indonesian Culture Collection (InaCC; http://inacc.biologi.lipi.go.id/) were selected and the strains were grown in media containing different glucose concentration. The strains belonged to *Rhodotorula paludigena* (InaCCY1527), *Rhodosporidiobolus ruineniae* (InaCCY1638), and *Sporobolomyces* (*Rhodosporidiobolus*) *poonsokiae* (InaCCY1606). The red-yeasts from glycerol stock were pre-cultured onto potato dextrose agar (PDA) for 2 days and then one full loop of cell colonies was transferred into 100 mL starter medium with potato dextrose broth (PDB) for 1-2 days. 100 µL of starter medium were transferred to the test media which contained 0.5%, 1%, and 2% of glucose (Merck 1.08337). In addition of the carbon sources, each medium was completed by 2 g/L yeast extract (Oxoid LP0021), 2 g/L malt extract (Bacto 218630), 1 g/L $(NH_4)_2SO_4$ (Merck 1.01217), 1 g/L KH_2PO_4 (Merck 1.04873), 0.25 g/L $MgSO_4.7H_2O$ (Merck 1.05886), pH 6.5 - 7 (Aksu and Eren 2005). The cultures were grown for 7 days at room temperature in a 120 rpm shaker incubator. The growth of red yeasts was measured by harvesting the culture broth for the dry mass calculation at the end of the culture period.

Carotenoid extraction and analysis

The β-carotene extraction was conducted following the method as described by Perrier, Dubreucq, and Galzy (1995) and Kim et al. (2009). Approximately 5 mL of culture broth were collected from each test media. The cells were harvested by centrifuging at 13,000 rpm (Kitman T-24, Tomy) and removed the supernatant. The pellet was rinsed twice with sterilized water. The pellet then air-dried before added with 500 mL of 80% acetone. The acetone mix and transfer to the new tube with bead 1 mm diameter then crushed the cell by using a bead beater (Microsmash MS-100, Tomy). Furthermore, after crushed, it was centrifuged at 13,000 rpm then used for analysis. The β-carotene content was determined spectrophotometrically (Varioskan Flash, Thermo Scientific) by the Nagata et al. (2007) protocol. Separately, three tubes were dried out for dry-mass analysis.

Phylogenetic tree construction

The phylogenic relation of red-yeasts was performed by molecular identification of the D1/D2 domain of 26S ribosomal DNA, following the methods as described by Sumerta and Kanti (2018). After conducting homology analysis in BLASTn, all retrieved sequences and related type strains were aligned by MAFFT (Katoh and Standley 2013). Furthermore, the aligned sequences were analyzed by MEGAX software (Kumar et al. 2018). The phylogenetic tree was constructed by Neighbor-Joining method, 1000 bootstraps evolutionary distance, Kimura 2-parameter model with complete deletion, and *Spencerozyma crocea* CBS 2029 as an outgroup.

Results

The red yeasts were cultured in the solid medium before inoculated into supplemented glucose broth medium and the results were measured as *β*-carotene concentration (mg/L) and dry cell mass (mg). The appearance of these yeasts in culture media is orange to red (Fig. 1). Through the cultivation periods, this carotenoid value was obtained by cell extraction in acetone solvent and measured spectrophotometrically. On the plate, the colony seems similar in colour appearance while the amount of *β*-carotene production was varied during the broth cultivation (Fig. 2).

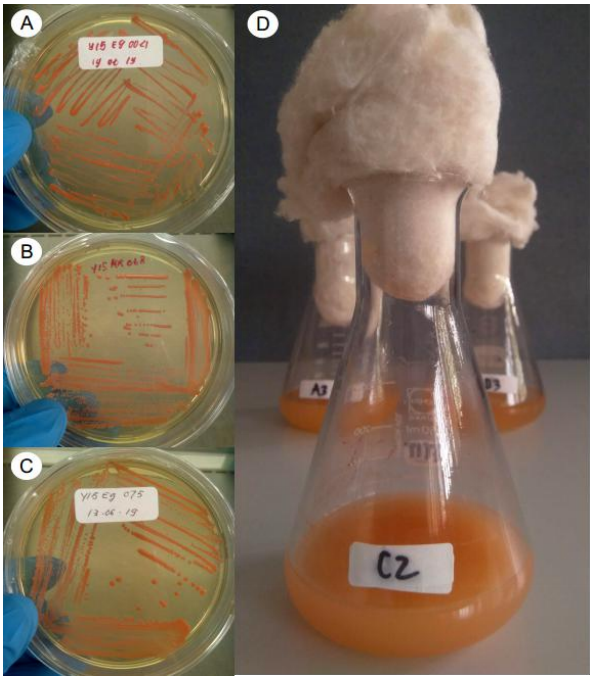

Figure 1. The red-yeasts appearance in culture media. A. *Rhodotorula paludigena* (InaCCY1527/Y15Eg004); B. *Sporobolomyces (Rhodosporodiobolus) poonsokiae* (InaCCY1606/Y15Kr068); C. *Rhodosporidiobolus ruineniae* (InaCCY1638/Y15Eg075); D. Cultured red-yeasts in broth medium.

All strains produced different amounts of *β-carotene* (Fig. 2A). Generally, all strains produced the lowest concentration of this carotenoid in 2% glucose of growth medium. The InaCCY 1527 strain produced 19.9±0.19 mg/L which was the lowest production in 2% glucose. While this carotenoid was obtained at 64.51±6.99 mg/L and 56.77±5.33 mg/L in respective 1% and 0.5% of that media, this strain remained the lower production among others. Meanwhile, the InaCCY 1606 strain increased the production by 68.65±13.06 mg/L than the previous strain in 2% glucose. It also produced 83.37±11.89 mg/L and 71.24±10.79

mg/L in 0.5% and 1% glucose supplemented media. Likewise, InaCCY 1638 strain also yielded a high amount *β*-carotene which tripled at 82.95±2.55 mg/L compared with InaCCY 1527 in a 2% glucose supplemented medium. In a 1% medium, this carotene was the highest at 93.01±13.13 mg/L while in 0.5% glucose, the InaCCY 1638 was produced approximately the same amount as InaCCY 1606 at 83.46±10.01 mg/L. Meanwhile, the dry mass was obtained to monitor the growth of the red yeast and the *β*-carotene production (Fig. 2B). However, there was no correlation between the biomass growth and *β*-carotene production since the *β*-carotene production was likely determined by the density of carotene colour extracted from the cells based on spectrophotometry results.

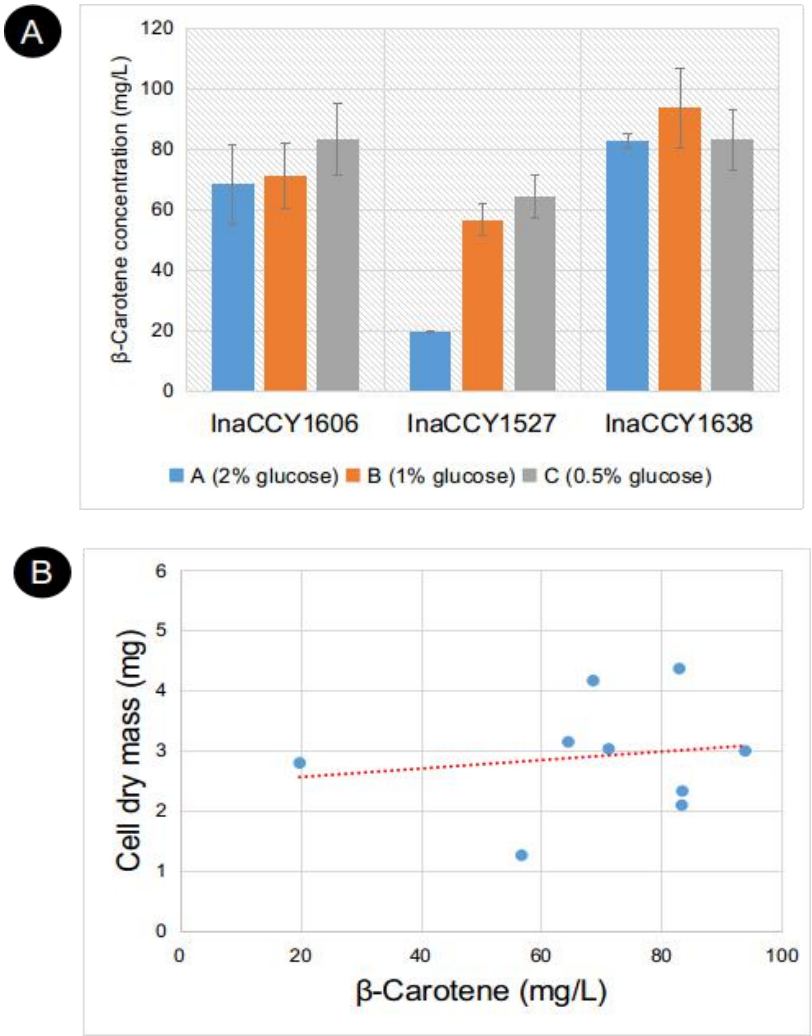

Figure 2. The *β-carotene* production by three species of red-yeasts and phylogenetic tree. A. the production of *β-carotene* in different glucose concentrations; B. The correlation pattern between dry cell mass and *β*-carotene production (R^2=0.0252).

In order to explain the relationship among red-yeasts species, the phylogenetic tree was constructed (Fig. 3). This tree has clearly differentiated the relationship among red yeasts which clustered into two clades on Sphaerocarpum and Ruineniae of Basidiomycetes yeasts group. With the 99-100% identity after the homology test of BLAStn, the relationship between InaCCY 1606 and 1638 was closer than 1527 which was still clustered in the group on a monophyletic tree. On the other hand, the InaCCY 1606 was previously originated from the sister clade, Johnsonii since the yeasts taxonomist has already moved this strain toward *Rhodosporidiobolus* genera. Its molecular profile seemed prone to Ruineniae clade that

genera name from *Sporobolomyces* changed to *Rhodosporidiobolus poonsokiae*. This tree also emphasized the strong bootstrap value in a certain node. In relation to a *β-carotene* potential producer, the result revealed that the strains from the same clade apparently had similar ability to produce this carotenoid.

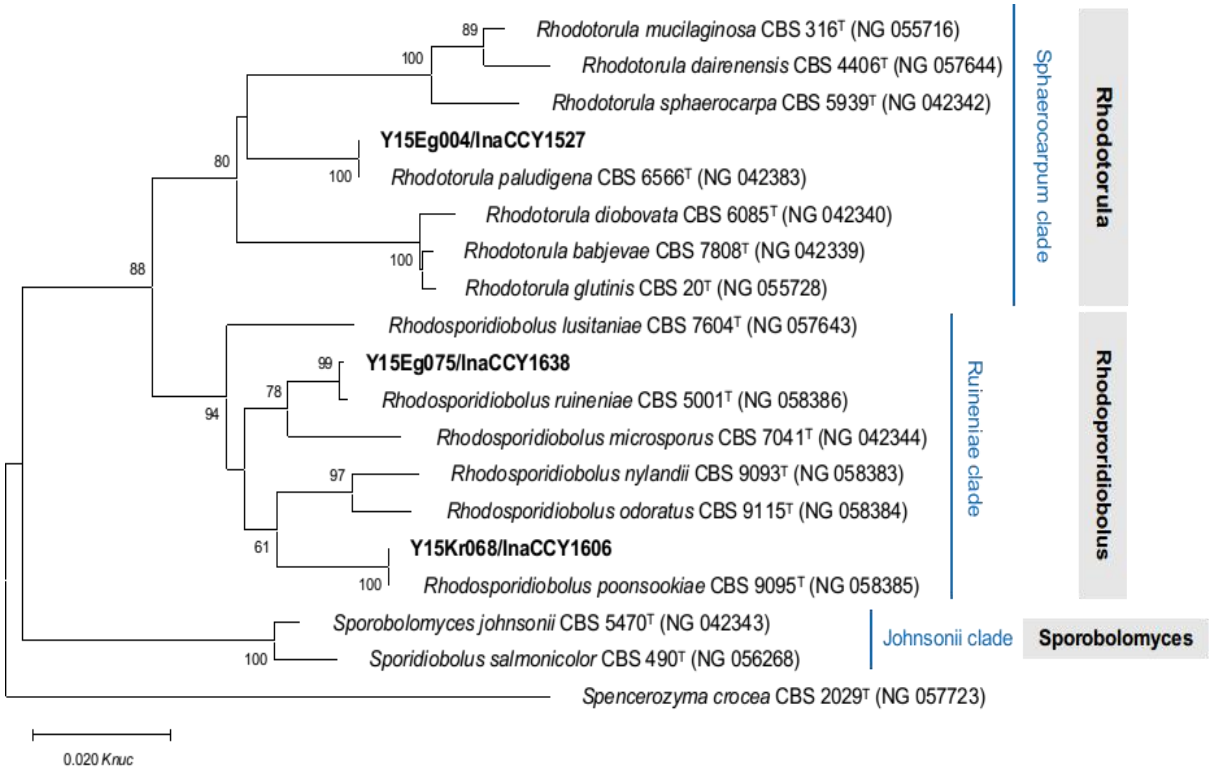

Figure 3. The relationship among red yeast was figured on the phylogenetic tree with bootstrap value.

Discussion

Looking for a new source on sustainable carotenoid production is essential for future development. Despite carotenoids are easily obtained from fruit and vegetables, another technique using bioprocess technology attracts many scientists to improve the carotenoid yield by manipulating the medium or species itself (Mussagy et al. 2019). In this study, the red-yeasts isolated from Indonesian resources which are *Rhodotorula paludigena* (InaCCY1527/Y15Eg004), *Rhodosporodiobolus poonsokiae* (InaCCY1606/Y15Kr068), *Rhodosporidiobolus ruineniae* (InaCCY1638/Y15Eg075) have a good potency to develop on a large scale production. The *β-carotene* production from *Rhdosporidiobolus* species which are from Ruineniae clade could be a good candidate for *β*-carotene production since their higher production than Sphaerocarpum clade which is *Rhodotorula* species. Therefore, by recognizing their phylogenetic tree position could be used as a tool for determining particular species producing higher carotenoid, at least from their certain group.

To achieve a better yield, the glucose content of the medium could optimize the growth and production process. It was reported that the increase in sugar content could increase the total carotenoid production and yeast growth (Aksu and Eren 2005). In fact, in terms of sugar, glucose is one of the important parts of the growth medium which also supported by the nitrogen source. Sometimes, carbon and nitrogen sources are simultaneously applied in particular concentration to obtain better growth or carotenoid production (Libkind, Brizzio, and van Broock 2004), while the glucose concentration did not exceed to 2% of the

total volume to obtain the best yield (Maldonade, Rodriguez-Amaya, and Scamparini 2012). Meanwhile, each species has a different ability to tolerate the glucose content, *Rhodosporodiobolus poonsokiae* (InaCCY1606/Y15Kr068) and *Rhodosporidiobolus ruineniae* (InaCCY1638/Y15Eg075) can tolerate better than *Rhodotorula paludigena* (InaCCY1527/Y15Eg004) which produced the lowest yield among others. In comparison, yeasts strain *Rhodotorula* sp. RY1801, was also reported that used optimum condition at 1% glucose content that *Rhodotorula paludigena* (InaCCY1527/Y15Eg004) seems similar to the previous report (Zhao et al. 2019).

In conclusion, the red-yeasts are potential for *β-carotene* producer shown by *Rhodosporodiobolus poonsokiae* (InaCCY1606/Y15Kr068) and *Rhodosporidiobolus ruineniae* (InaCCY1638/Y15Eg075) using glucose from 0.5 to 2% in the growth medium. Our finding confirms that red-yeasts are potential for *β-carotene* producer and growth medium manipulation is also crucial for *β-carotene* production.

Conflict of interest

The authors whose names are listed have no conflict of interest. Isolation and preservation were conducted by all of us, data collection and analysis are by Sumerta I N, and the manuscript was written by Sumerta I N and Kanti A.

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