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PHAs Accumulation in Bacterial Cells Isolated from Rice By-Products

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Abstract: Polyhydroxyalkanoates (PHAs) are biopolymers synthesized by microorganisms. Bacteria can accumulate PHAs in their cells and used as energy resource. In this study, PHAs-producing bacteria were isolated from rice by-products particularly Glutinous Broken Rice (GBR), non-glutinous Broken Rice (BRR), Rice Barn (RB) and Thai Rice Vermicelli (TRV). The samples were cultured on three cultural media including of Tryptic Soy Agar (TSA), Plate Count Agar (PCA) and Starch Agar (SA). The 356 of purified isolates were cultured on complex and minimal medium then screened for PHAs accumulation in their cells using Nile Red dyeing method. Detection of the intracellular granules was using florescent and transmission electron microscopy. Isolate BRR25-1 and RB36 were accumulated large areas of PHAs inclusion in their cells when cultured on SA and TSA, respectively. The isolates were sequenced based on 16S rRNA gene and submitted in GenBank with Accession No. MG745381 and MG745383. The potential isolates could be produced and accumulated PHAs in their cells when fermented in cheap carbon substrates from rice by-products.

Key words: Polyhydroxyalkanoates (PHAs), Accumulation, Rice by-products, Rice Barn (RB), Thai Rice Vermicelli (TRV), Broken Rice

INTRODUCTION

Polyhydroxyalkanoates (PHAs) are naturally occurring aliphatic polyesters. They are accumulated by a wide variety of bacteria from intracellular reserve materials and produced by different substrates (De Albuquerque *et al*., 2018). PHAs properties suitable as potential petrochemical plastic replacements but there are still bottlenecks for scaling up microbial production systems. One of the major problems is the cost of carbon substrates (Nielsen *et al*., 2017). Many research groups try to find inexpensive carbon sources for the polymer fermentation such as industrial-or agro-waste products, domestic waste water and waste food. Rice by-products are agricultural wastes from rice milling. Thailand is a major of milled rice-exporting country in Asia (Petchseechoung, 2017). Rice milling is the process that helps in removal of hulls and bran's from paddy grains to produce polished rice (Dhankhar, 2014). Rice by-products in rice milling are rice hull, rice germ and bran layers and fine broken kennels. The rice by-products are low-cost and could be used as cheap carbon sources for PHAs-producing bacteria and achieved potential bacteria from those sources as well. The objective of this study was to detected PHAs inclusion in bacterial cells that isolated from rice by-products for the further application by using rice by-products as a carbon sources.

MATERIALS AND METHODS

Sample collection: Rice by-products particularly of Glutinous Broken Rice (GBR), non-Glutinous Broken Rice (BRR), Rice Barn (RB) and Thai Rice Vermicelli (TRV) were collected from rice mills at Nong Bong Village, Suranaree Sub-district, Muaeang Nakhon Ratchasima District, Nakhon Ratchasima Province, Thailand. The samples were placed into sterile glass bottles and Thai rice vermicelli were kept in sterile plastic bags then transported to Microbiology Laboratory, Faculty of Medical Science, Nakhonratchasima College for bacterial isolation. The samples were stored at room temperature except Thai Rice Vermicelli. The TRV samples were immediately put in isolation media when arrived at the laboratory.

Bacterial isolation: The 25 g of each sample were mixed with 225 mL of phosphate buffer pH 7.2. Tenfold dilution series were prepared from 10^{-2}-10^{-7} in 9 mL of diluent buffer. Each dilution was spread on three isolation media including Tryptic Soy Agar (TSA), Plate Count Agar (PCA) and Starch Agar (SA) with duplication plates then incubated at 30°C for 24 h. Different of morphological characteristics of the isolates were purified and collected for further experiments. Purified bacterial isolates obtained from TSA, PCA and SA media were investigated for PHAs-accumulation in their cells. The isolates were

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re-cultured in Tryptic Soy Broth (TSB) and incubated at 30°C for 16-18 h. then transferred into 5% skim milk and kept in -80°C for collection of stock culture.

Bacterial cultivation: The purified isolates were streaked on their original medium agar (TSA, PCA or SA) then streaked on modified complex agar and incubated at 30°C for 24 h. Bacterial colonies from complex medium agar were streaked on modified minimal medium agar and incubated at 30°C for 24-48 h. Both complex and minimal medium agar was developed by Chansatein *et al.* (2012).

Screening of PHAs-producing Bacteria: The isolates were cultured on complex medium agar and then transferred to minimal medium agar which was supplemented with 0.5 mg/L Nile Red according to Berlanga *et al.* (2006). PHAs-producing isolates were observed under UV light with pink colony.

Detection and identification of PHAs-producing bacteria: The detection of PHAs inclusions in bacterial cells was used fluorescent and electron microscopy according to Chansatein (2018). The bacterial identification based on 16S rRNA gene was sequenced by Macrogen Inc., Seoul, Korea.

RESULTS AND DISCUSSION

The 356 of purified isolates from GBR, BRR, RB and TRV were screened for PHAs accumulation in their cells. The 66 isolates gave a pink color under UV light when cultured on minimal medium supplemented with Nile Red dye. Their cell shapes were cocci and rods. All isolates was Gram-positive bacteria. Isolate BRR25-1 and RB36 were accumulated PHAs in large areas when cultured on SA and TSA, respectively. PHAs accumulations in their cells were between 1 and 11 inclusions in difference sizes. The isolates were sequenced based on 16S rRNA gene and submitted in GenBank with Accession No. MG745381 and MG745383. Both of them could be used for DNA sequence references of PHAs-producing bacteria. PHAs inclusions in bacterial cells of isolate RB 36, BRR25-1, GBR12 and TRV28 were shown as Fig. 1a-d, respectively. The production of biodegradable polyesters on a large scale is limited because of the relative expensive substrate required (Khandpur *et al.*, 2012). Researchers had been used inexpensive carbon sources for saving cost in polymer fermentation by bacteria such as rice bran and starch (Huang *et al.*, 2006; Oh *et al.*, 2015; Nagamani *et al.*, 2015; Chen *et al.*, 2015; Takahashi *et al.*, 2017) whey (Koller *et al.*, 2007) waste vegetable oil

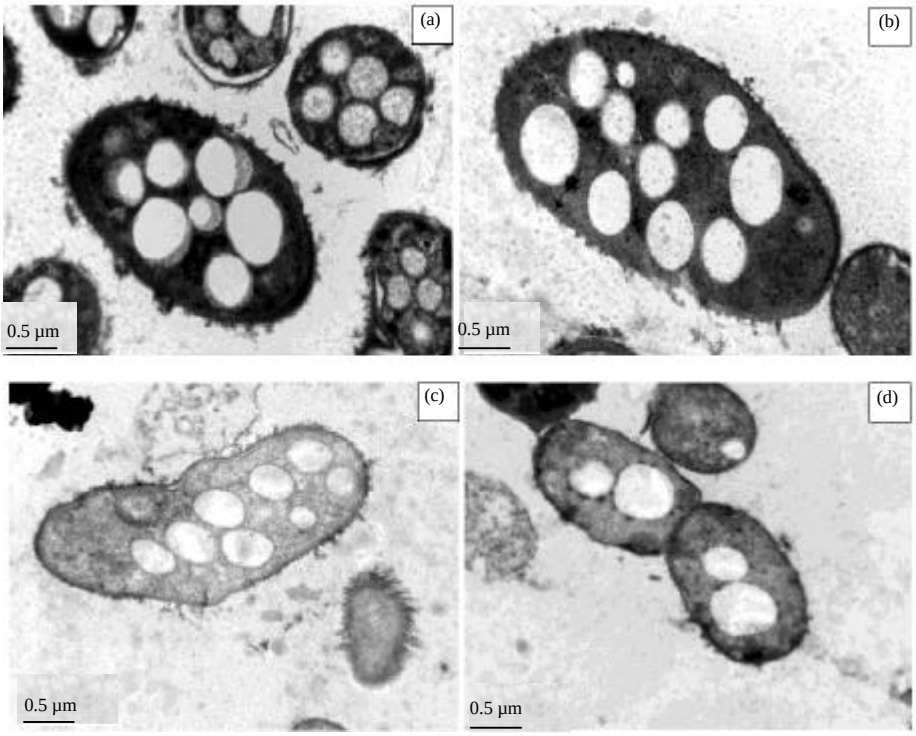

Fig. 1: TEM micrographs of granules in cells of bacterial isolate RB 36: a) BRR25-1; b) GBR12; c) TRV28 and d) when detected by TEM

(Song *et al*., 2008) dairy industrial waste water (Ahn *et al*., 2009) olive mill wastewater (Cerrone *et al*., 2010; Alsafadi and Al-Mashaqbeh, 2017) domestic wastewater (Huang *et al*., 2012) vinasse (Bhattacharyya *et al*., 2012) paper mill wastewater (Jiang *et al*., 2012) rice-based ethanol stillage (Bhattacharyya *et al*., 2004) oil extracted from spent coffee grounds (Obruca *et al*., 2014); food waste (Nielsen *et al*., 2017) and sugarcane bagasse (Salgaonkar and Braganca, 2017). Khandpur *et al*. (2012) studied of PHAs production is done in combination of the best screened substrate particularly rice bran, paddy husk, pigeon pea waste, sugarcane bagasse and waste frying oil. In Thailand, annual paddy rice production is around 30-32 or 20 mln. tons of milled rice (Petchseechoung, 2017). So, rice by-products are between 10 and 12 mln. tons a year. Rice by-products are cheap, environmental friendly and could be good sources for PHAs-producing bacteria, especially, the potential strains which were isolated from those habitats.

CONCLUSION

The 356 purified isolates were isolated from rice by-products. The 66 isolates accumulates PHAs in their cells. All isolates were Gram-positive cocci and rods. Isolate BRR25-1 and RB 36 accumulated PHAs inclusion in their cells with large areas when detected by TEM. The isolates were sequenced based on 16S rRNA gene and submitted in GenBank with Accession No. MG745381 and MG745383. The strains will be used for PHAs fermentation and using rice by-products for their substrates.

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