PACKAGING EVALUATION RELATED TO THE SURVIVAL OF THE Acetobacter sp. RMG-2 AND BIOCELULOSE PRODUCT IN PASTE INOCULUM

Urip Perwitasari1*, Nuryati1, Ruth Melliaiwati1, and Yopi1

1Research Center for Biotechnology, Indonesian Institute of Sciences, Cibinong Science Center, Cibinong-Bogor 16911, Indonesia

*Corresponding author: uripperwitasari@gmail.com

Abstract

Packaging evaluation is essential for the survival of its microbial content. Three types of container (plastic, cup, and bottle) were evaluated in this study. The parameters analyzed were cell viability and its ability to form biocellulose of paste inoculum for 28 weeks. As a result, the cell viability of Acetobacter sp. RMG-2 packaged in three types container has opposite trends to longer storage period. Cell viability of Acetobacter sp. RMG-2 packaged in the cup reached the highest (5.8 x 10^8 cfu/mL) for 28 weeks of storage period. Thickness and dry weight of biocellulose formation inside retrieved from plastic cup were 0.1 cm and 0.4 grams respectively.

Keywords: Acetobacter sp., RMG-2, paste inoculums, 3 kinds of packaging

Introduction

Biocellulose is cellulose fiber from fermentation coconut water with Acetobacter xylinum. Biocellulose or nata de coco is indigenous food in Philippines. Technology productions of this traditional food grow rapidly in Indonesia because Indonesia has wide coconut plantations. Data from General Directorate of Plantation Ministry of Agricultural Indonesia in 2014, amount of coconut production 3.031.310 ton.

As a main content for biocellulose production, coconut water contains nutrition for support metabolism microorganism such as water, fat, and protein (Yong et al., 2009). Acetobacter sp. produce biocellulose from coconut water in addition carbon source and nitrogen (Budhiono et al., 1999). Biocellulose produced by Acetobacter after 15-20 days incubation at pH 4.0 with sucrose 10% (Jagannath et al., 2008).

The applications of biocellulose from fermentation acetic acid bacteria can be processed into high value product such as in pharmaceutical for medical implants, tissue engineering, drug delivery, wound-healing, cardiovascular applications (Jorfi and Foster, 2015), paper manufacturing, membrane filtration, acoustic transducers (Lin et al., 2013). Acetic acid bacteria such as Acetobacter sp. in Indonesia, Thailand, and Philippines mainly found in fermented foods (Lisdiyanti et al., 2003).

Preservation of starter Acetobacter becomes important it is because bacterial cellulose has a big potential value in industry application. Bacterial cellulose can production biocellulose with high purity, better crystalline property, high water absorbency, simple polymerization, stronger, and high bio-compatibility (Sukara & Melliaiwati, 2014). The immobilized cell for the production of biocellulose is one solution to the product resulted in a cell-free nata (Nugroho & Aji, 2015). Starter isolates Acetobacter sp. to producing nata de coco until today still on sale in liquid form. Liquid starter have a problem in shipment. Several carriers have been report about preservation starter bacterial such as CMC, agar, sago flour, skim milk, calcium alginate (Jagannath, 2010), biocellulose pulp (Melliaiwati, 2008). Subsequently it was report that the carrier materials (CMC and biocellulose pulp) are able to keep bacteria without loss their capability to produce cellulose (Melliaiwati, 2008). The suitable packaging for easy shipment the inoculum in matrix CMC and viability of the cell not have
been report. Therefore, the aim of this research is to evaluation the viability and activity of starter paste inoculum Acetobacter in various packaging and time incubation.

Materials and Methods

Bacterial isolate

*Acetobacter* sp. RMG-2 isolate collection of Research Center for Biotechnology, Indonesia Institute of Science was used in this research. The isolate was cultured with using media composition: HB Agar (25 g glucose, 0,15 g (NH₄)₂SO₄, 0,625 g yeast extract, 1,25 g K₂HPO₄, 0,05 g MgSO₄.7H₂O, 5 mL CH₃COOH, Bacto Agar 20 g) and adjusted pH media until 5.5. Culture was incubated for 3 days in room temperature.

Preparation of paste inoculum

*Acetobacter* sp. RMG-2 was inoculated in media contained: HB (25 g glucose, 0,15 g (NH₄)₂SO₄, 0,625 g yeast extract, 1,25 g K₂HPO₄, 0,05 g MgSO₄.7H₂O, 5 mL CH₃COOH) and adjusted pH media until 5.5. Culture was incubated for 2 days in shaker at room temperature. Culture was added to Carboxymethyl cellulose (CMC) 4% as carrier with concentration 1:1 (Melliawati, 2008).

Packaging of paste inoculum

Subsequently, paste inoculum was packed in to three different type of packaging (plastic, cup, and bottle). The paste inoculum filled as much as 2/3 of the volume. Paste inoculum was stored in 4°C then checks the cell viability and activity to produce biocellulose.

Cell viability

One gram of pasta inoculum was added to 9 mL aquadest sterile. Cell viability was inoculated on media HB agar. The number of living cells is the number colony divided the dilution factor.

Biocellulose production

Three gram of *Acetobacter xylinum* inoculum was added in to bottle jam containing 100 mL media GAA (0.3 g of glucose, 0.5 g of (NH₄)₂SO₄, 5 mL of acetic acid was mixed in 100 mL of coconut water and adjusted to pH to 5.5). The fermentation culture was carried on bottle jam in static culture and then given a cover paper. Incubation was carried out at room temperature.

Results and Discussion

Cell Viability

*Acetobacter* are group acetic acid bacterium and includes types of aerobic obligate. It was needed oxygen for the metabolism (De Vero & Guidici, 2013; Sainz et al., 2016). Some research about this *Acetobacter* sp. RMG-2 had been done including *Acetobacter* inoculum for manufacturing of paste as biocelullose (Melliawati, 2008). This research was conducted on the packaging paste inoculum in three kinds of packaging to find out the population of *Acetobacter* sp. RMG-2 cells that can live in the packaging. Figure 1 shows the three kinds of packaging paste inoculum biocellulose. Types of packaging in this study have different materials, plastic packaging is from PE (polyethylene), cup packaging is from PP (polypropylene), and bottle packaging is from glass.
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Figure 1. Paste inoculum of *Acetobacter* sp. RMG-2 on various packaging
(A: Plastic, B: Cup, C: Bottle)

*Acetobacter* sp. RMG-2 population at the beginning of storage in three packs is $9.1 \times 10^8$ cfu/mL. *Acetobacter* sp RMG-2 cells population in paste inculum is shown in Figure 2. Population cell for 28 weeks incubation at 4°C in plastic packaging is $1.6 \times 10^7$ cfu/mL, in cup packaging is $5 \times 10^6$ cfu/mL, and in bottle packaging is $3.9 \times 10^6$ cfu/mL (*Figure 2*). Population of the cell in plastic, cup, and bottle packaging are decline after 28 weeks incubation in 4 °C. Declining population number, possibly because oxygen in media is diminishes. Then it causes the bacteria to die because *Acetobacter* needed oxygen for cell metabolism (De Vero & Guidici, 2013).

The decline of population cells from $10^9$ cfu/mL become $10^7-10^8$ cfu/mL in which 83%-90% cell of *Acetobacter* sp RMG-2 still life after 28 weeks of incubation in 4°C temperature storage. The result is similar with study that have been report, the population cell of *Acetobacter* sp. RMG-2 in CMC for 15 weeks incubation is $1.79 \times 10^8$ cfu/mL and $7.75 \times 10^7$ cfu/mL in cellulose pulp (Melliawati, 2008). The other study show viability the lactic acid bacteria in skim milk for 60 days incubation have decline population from $10^9-10^{10}$ cfu/g become $10^7$ cfu/g (Jagannath et al., 2010). It is show CMC is the suitable carrier for *Acetobacter* sp RMG-2. The low population *Acetobacter* sp. RMG-2 in glass because the density of glass more tightly than PP and PE. Glass packaging is impermeable to gases and vapors (Marsh & Bugusu, 2007).

Figure 2. Viability of paste inoculum *Acetobacter* sp. RMG-2 on various packaging and stored at 4 °C

*Cell activity*

The capability cells *Acetobacter* sp RMG-2 to produce biocellulose fibers from waste coconut water shown in *Figure 3* until *Figure 6*. Thickness and weight biocellulose from paste inoculum are not stable but pH media and water content of the biocellulose relative stable. The unstable thickness in this research can be caused by the composition of waste coconut water are different on each evaluation every month (coconut water obtained from the market). Thickness of biocellulose is affected by...
sucrose and ammonium sulphate, maximum concentration sucrose is 10% and ammonium sulphate is 0.5% (Jagganath et al., 2008). Source of sucrose for produce biocellulose in this study was derived from coconut water only. The mature coconut water is the optimum media for producing biocellulose (Mohammad et al., 2016). Young coconut (6 months) has different chemical composition with mature coconut (12 months). Sucrose content in mature coconut is 10% almost 10 folds higher than young coconut (Yong et al., 2009).

![Figure 3. Thickness of biocellulose](image)

The result of average thickness of biocellulose from paste inoculum in plastic packaging is 0.41 cm, cup packaging 0.68 cm, bottle packaging 0.38 cm for 9 days. Biocellulose produced by paste inoculum Acetobacter sp. RMG-2 in cup packaging is the higher than plastic and bottle packaging. It is appropriate with the number of population cell Acetobacter sp. RMG-2 (Figure 2). The numbers of living cell increase the thickness of biocellulose production. The layers of crystalline biopaste inoculumcellulose starts to thicken after 5 days of incubation, it is because the sheets biocellulose formed subfibril then biocellulose crystallized as microfibril (Mohammad et al., 2014).

Dry weight of biocellulose of each month does not appropriate to the thickness of biocellulose produced in every month. At the 20 weeks incubation of paste inoculum Acetobacter the thickness of biocellulose is high but the dry weight of biocellulose is low. This is due to the biocellulose produced by Acetobacter sp RMG-2 has not crystallized. A layer of cellulose fibers which have not crystallized has high water content so it looks thick. Crystallization index and crystallization size will decrease with increasing growth time of the cell (Sheykhnazari et al., 2011).

![Figure 4. Dry weight of biocellulose](image)
pH culture media for producing biocellulose in present study are 5.5, it was preferred optimum pH for *Acetobacter* cell growth (Hwang et al., 1999). After production biocellulose (9 days incubation) range pH medium at the end of fermentation coconut water between 4.9-6.8 (Figure 5). The change of pH media because production gluconic acid from metabolism of Acetobacter (Masaoka et al., 1993). During conversion glucose into gluconic acid the pH medium has drop significantly from 5.0 to 4.3. Optimum production gluconic acid by *Acetobacter* is pH 4 (Hwang et al., 1999) and *Acetobacter* growth between 3.5-7.5 (Mohammad et al., 2014).

Hwang et al. (1999) explain during gluconic acid production the cell growth or cellulose concentration increased slowly. At the cellulose production phase the pH of the culture medium increased from 4.3 to 6.5, the cell grew and cellulose production increase rapidly. The accumulated of gluconic acid consume by the cell. In stationary phase the biocellulose not produced but the cell still consume gluconic acid and pH of the culture increase from 6.5 to 7.0. This explains the increase in the pH of the medium which occurred at week 20, and production of biocellulose began declining, but the number of living cells *Acetobacter* sp. RMG-2 still high.

In this study a decrease in production of biocellulose each packaging has a difference. *Acetobacter* sp. RMG-2 from paste inoculum in bottle packaging at 20 weeks incubation and plastic packaging at 24 weeks incubation could not produce biocellulose even though pH media at range for *Acetobacter* growth. It is probably due to the activity of cell *Acetobacter* sp. RMG-2 to produce biocellulose decrease, because basically *Acetobacter* is aerobic bacteria that require oxygen for metabolism and glass packaging denser than the other packaging.

![Figure 5. pH of residual medium in the end fermentation](image)

Water content biocellulose production from paste inoculum *Acetobacter* sp. RMG-2 in all type packaging relative stable (Figure 6). Biocellulose production from paste inoculum after 20 weeks incubation in cup packaging has higher water content in the amount of 94%. Study from Sheykhnazari et al. (2011) show hydrogen and C–H bonds increased with increasing growth time in bacterial cellulose. Biocellulose water content average from paste inoculum in every packaging was 91%. It shown the packaging does not significantly affecting the water content of biocellulose produced.

Total counts of cells *Acetobacter* sp. RMG-2 paste inoculum in all packaging decreased. The viability cell is relatively the same in each packaging. *Acetobacter* sp. RMG-2 in bottles and plastic packaging loses activity in producing biocellulose. *Acetobacter* sp. RMG-2 in paste inoculum in bottle packaging loses the activity to produce biocellulose after 20 weeks incubation (Figure 3 and Figure 4), while *Acetobacter* sp. RMG-2 in paste inoculum in plastic packaging loses the activity to produce biocellulose after 28 weeks incubation (Figure 3 and Figure 4).

Overall the results shows suitable packaging for paste inoculum biocellulose is plastic cup. In addition to the good viability, ease of delivery of paste inoculum plastic cup also allows for lighter
than glass packaging and production costs using a plastic cup is also more benefit (Humbert et al., 2009).

Conclusion

The packaging types influence the cell viability of *Acetobacter sp*. RMG-2 because the natures of the *Acetobacter* sp. as obligate aerobic bacteria. It was need oxygen in their life cycle. Plastic packaging and packaging cup still allow aeration of oxygen into the package even if only a little. Cell viability on a cup packaging gives better results than other packaging on both. Cell viability *Acetobacter* sp. RMG-2 on a plastic cup packaging $5.9 \times 10^8$ cfu/mL during the storage of 28 weeks. Need more research, using the coconut water with a carbon source is measured as a medium for producing biocellulose.

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References


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