Isolation and Characterization of Lipid Degraded Bacteria from Galamai Leftovers

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Abstract. Galamai is one of traditional food originated from West Sumatera Indonesia. The Minangese favor galamai for its chewy texture, sweet taste and its oil content. Galamai shelf life is relatively limited; signed by odor transformation followed by the appearance of white layer on its surface. The transformation that occur in galamai possibly caused by biological degradation and, or hydrolysis and oxidation. Biological degradation as a process by which organic substances are broken down by the enzymes produced by living organism. Typical biodegradation process involves the use of enzymes produced by a microorganism on its target compound and transforming it chemically into other compounds that is less or not harmfull. The research objective is to identify the characters of lipid degraded bacteria that present on Galamai Leftovers (GL). GL samples were derived from souvenir shops in Payakumbuh City. Sample was carried under aseptic condition. 14 isolated bacteria that previously cultured on Nutrient Broth were subjected to morphology tests and then cultured on selective medium for lipolytic activity. Gram staining, characteristic tests and biochemical test were performed to pure isolates. There were 14 isolates found on GL. 3 isolates (KSKP1, KSP1, dan KB2) shown lipid degraded activity. The entire colonies had smooth and glistening surface, the color range from white to yellowish and produce clear zone on Rhodamin-B medium. The entire colonies were gram positive, catalase negative and capable of fermenting sugar. KSKP1 was aerob facultative and produced no endospore. While KSP1 and KB2 were aerobic bacteria with central type endospore and terminal type endospore, subsequently. Verification by Bergey’s Manual of Determinative Bacteriology suggested that KSKP1 was Staphylococcus, sp, while others were Bacillus, sp.

Keywords: Isolation; Characterization; Lipid Degraded Bacteria; Galamai

1. Introduction

One of traditional food from West Sumatera, Indonesia is galamai. Galamai is presented as snack with sweet and savory taste. It made by heating the mixture of rice flour, palm sugar, and coconut milk. Therefore, immediate spoilage is inevitable. The deterioration is indicated by unpleasant smell and white powdery surface [1].

The defection of galamai was showed to by the appearance of a rancid smell and white color like cotton on the surface of the galamai. Rancid odour is often used as a sign that foods with high fat content have been defect [2]. This statement that the eminent defect to galamai. Is caused by high fat content. Then microorganism that grows on the surface of galamai include groups pf gram negative bacteria and mold.

The transformation that occur in galamai possibly caused by biological degradation and, or hydrolysis and oxidation. Lipid metabolism involves several steps including emulsifying and
degradation. After degradation pathway, lipid are brothen down into glycerol and fatty acid(s). The fatty acid(s) are then converted to acetyl-CoA via the beta oxidation pathway and finally enter the TCA cycle [3]. Biological degradation is a disintegration of organic components by enzyme that provided by living organism [4]. Typical biodegradation process involves the use of enzymes produced by a microorganism on its target compound and transforming it chemically into other compounds that is less or not harmfull. Reference [5] stated that hydrolysis of short chain lipid resulted in production of free fatty acid, the cause of rancidness; which probably produced by the hydrolysis of lipid, phospholipid and its derivatives. Oil or lipid hydrolysis frequently occurred as a result of lipase activity from microorganism (bacteria or molds). Reference [6] stated that hydrolysis is accelerated by temperature, moisture content and relative humidity. Lipolytic microbes are capable to endure processing condition.

Lipid degraded bacteria are able to produce lipase, an enzyme to hydrolyze oil and lipid. Based on physiological function, lipase has a vital role to hydrolyze lipid into fatty acid and glycerol which is needed in metabolism. Lipase is able to disrupt ester bond of lipid become fatty acid and glycerol [7]. Isolation and initial characteristics are carried out to discriminate microorganism that have the ability to fat degrade at potential to be developed as biological agents producing lipase enzymes and further utilized. Reference [2] during 72 hours of incubation, bacteria was the first microbe inhibiting GL while molds emerged after 72 hours of incubation. Most of the bacteria were gram negative. Reference [8] bacterial isolation is an option to isolate or to transfer selected bacteria from its environment to gain bacterial pure culture. This research aim to identify the characteristics of lipid degraded bacteria isolated from GL.

2. Materials and Methods

2.1 Sample collection

GL samples were derived from souvenir shops in Payakumbuh City. Sample was transported under aseptic condition. The swabs containing the inoculum were streaked on sterile Nutrient Broth medium. The inoculated medium were incubated at 37°C for 24 h. Consequently, the cultivated bacteria were propagated on selective medium (Na + tween 20) to observe its lipid degraded ability.

2.2 Grams differentiation

A gram staining assay was performed according to [9] a slide of cell sample from 24 h old culture was air dried and heat fixed. It then flooded by primary stain (crystal violet) for 1 min, mordant (Lugols Iodine) for 1 min, decolourizer (70 % Ethanol) for 30 sec and counterstain (Safranin) for 1 min, respectively; each solution was rinsed off gently with water in between the application. The air dried slide was studied under the microscope with immersion oil application on objective lens.

2.3 Endospore Staining

Aseptically transfer one species of bacteria with an inoculating loop to each of the respective slides, air dry (or use a slide warmer), and heat-fix. Then place the slide to be stained on a hot plate or boiling water bath. Cover the smear with paper toweling that has been cut the same size as the microscope slide. Soak the paper with the malachite green and heat on the hot plate until the stain steams and keep it for 5 to 6 minutes. Replace the malachite green solution as it evaporates so that the paper remains saturated during heating. Cool the slide and rinse with water for 30 seconds. Counterstain with safranin for 60 to 90 seconds and rinse again. The slide was observed under the microscope using the oil immersion objective lens after previously air dried [10].

2.4 Characteristics test

The isolates were subjected to the morphological (shape, elevation, colour dan margin) and biochemical test (sugar fermentation, starch, gelatine, casein, lipid, motility, catalase test dan oxidase test) [9], [11] and [12].
3. Result and Discussion
Isolation and Purification of Lipid Degraded Bacteria: The procedure used for isolation of microorganism from a food sample will depend upon a number of factors. Isolation media and procedures are often a personal choice, but due regard should be given to their suitability for recovery of stressed organisms, which are easily inhibited by many selective agents and also by elevated incubation temperatures [13]. Morphology variations of 14 obtained isolates can be seen on Table 1.

Consequently, selective medium (NA + tween 20) was used to study isolate lipid degraded capability. The addition of oil to medium is commonly used for this purpose. Olive oil, tween 20 and tween 80 are the usual source of the oil for the goal [14].

Table 1. Morphological Characteristics of Bacterial colonies derived from GL

| Isolate code | Form  | Margin       | Approx size | Color    |
|--------------|-------|--------------|-------------|----------|
| KSKK 1       | Circular | Entire   | Small      | Yellowish|
| KSKK 2       | Irregular | Undulate | Small      | Yellowish|
| KSKP 1       | Circular | Entire   | Pin point  | White    |
| KSKP 2       | Irregular | Undulate | Pin point  | White    |
| KSKP 3       | Irregular | Undulate | Small      | White    |
| KSKP 4       | Circular | Entire   | Moderate   | White    |
| KSB 1        | Circular | Entire   | Large      | White    |
| KSB 2        | Circular | Entire   | Large      | White    |
| KSK 1        | Irregular | Undulate | Small      | White    |
| KSP 1        | Circular | Entire   | Moderate   | White    |
| KSP 2        | Circular | Entire   | Moderate   | White    |
| KB 1         | Irregular | Undulate | Moderate   | White    |
| KB 2         | Irregular | Undulate | Moderate   | White    |
| KB 3         | Circular | Entire   | Moderate   | White    |

Bacterial Lipid Degraded Ability: The presence of pin point size colonies on selective medium after 24 hours of incubation in 37°C is a sign of lipid degraded bacteria growth [12]. Most of lipid degraded bacteria produce no pigment. Thus, white and yellowish are the common color [15]. Furthermore, lipolytic bacteria with a wide clear zone has a potency as a lipase producer [16]. The occurrence of clear zone is caused by microbial activity in degrading substrate [17]. The wider the zone, the larger amount of lipase produced [18]. Fig. 1 shown clear zone around colonies. The comparison of morphological and biochemical test of lipid degraded bacteria derived from GL with Bergey’s of Manual Determinative Bacteriology can be seen on table 2.

Figure 1. Clear zone produced by lipid degraded bacteria on selective medium
Table 2. Morphological and Biochemical Test Result of Lipid Degraded Bacteria

| Characteristics     | KSKP 1 | Staphylococcus sp, based on Bargey’s of Manual determinative bacteriology | KSP 1 | KB 2 | Bacillus sp, based on Bargey’s of Manual determinative bacteriology |
|---------------------|--------|--------------------------------------------------------------------------|-------|------|---------------------------------------------------------------------|
| Colony surface      | Smooth and glistening | Smooth and glistening | Smooth and glistening | Smooth and glistening | Smooth and glistening |
| Cell shape          | Cocci  | Cocci | Bacilli | Bacilli | Bacilli |
| Gram Character      | +      | +    | Bacilli | Bacilli | Bacilli |
| Spore               | No     | No   | Central | Terminal | Central/Terminal/Sub Terminal |
| Glucose             | A      | A    | A       | AG      | + |
| Fructose            | AG     | +    | AG      | AG      | + |
| Lactose             | A      | A    | A       | A       | + |
| Dextrose            | A      | A    | A       | A       | + |
| Sucrose             | AG     | A    | A       | A       | + |
| Arabinose           | A      | A    | A       | A       | + |
| Catalase            | +      | +    | -       | +       | +/- |
| Metabolism          | Facultative anaerobe | Aerobic/Facultative anaerobe | Microaerophylic | Aerobic | Aerobic/Microaerophylic/Anaerob |

Note: A = Produce Acid and AG = Produce Acid and Gas

Based on the table, there was a similarity between KSKP1 with *Staphylococcus*, sp.; KSP1 and KB2 with *Bacillus*, sp. based on a comparison to Bargey’s Manual of Determinative Bacteriology. Family of *Micrococcaceae* is aerobic and catalase positive. While *Staphylococcus*, sp. is cocci in single, in pair, in tetra or grape-like cluster. Some species produce pigment range from yellow to orange. Those bacteria need organic nitrogen (amino acid) for its growth and are anaerobic facultative [19].

*Bacillus* consist of 22 species, mostly found in food. It is generally aerobic to anaerobic facultative; catalase positive but few are catalase negative; and produce endospore (central, terminal dan sub-terminal) depends on the species. Some *Bacillus* are lipolytic [19]. Lipid degraded bacteria that commonly found are *Bacillus*, sp., *Klebsiella*, sp. and *Staphylococcus*, sp.. Some microorganisms produce hydrolytic lipase, such as *Staphylococcus*, *Pseudomonas*, *Micrococcus*, *Aspergillus*, *Alcaligenes* [20]. *Bacillus*, sp. is capable of degrading organic compounds [21].

4. Conclusion

3 isolates of lipid degraded bacteria were obtained by the research. Isolate 1 was indicated genus of *Staphylococcus*, sp and the other two were *Bacillus*, sp. it is recommended to continue to the next step that is to isolate and characterize the enzyme on laboratory scale.

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