World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 5.088

# THE EXISTENCE OF INDIGENOUS MICROFLORA FOR PULP KAKAO (THEOBROMA CACAO, L) FROM THREE VARIETIES IN WEST SUMATERA

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**Article Received on 11/06/2019** **Article Revised on 01/07/2019** **Article Accepted on 21/07/2019**

## ABSTRACT

The processing of cocoa by farmers is still classified traditionally. So, the quality of Indonesian cocoa is classified as very low and this is because the product has no specifications and there are post-harvest impurities. Therefore, the study of the presence of indigenous microflora of cocoa pulp fermentation (Theobroma cacao, L) of three varieties in West Sumatra was carried out to determine the presence of indigenous microflora fermenting cocoa pulp from three varieties in West Sumatra, determining the presence of bacteria (Amilolytic, Proteolytic, and Cellulolytic) and determine the characteristics of bacterial isolates contained in Cocoa pulp. This research was carried out by using a survey method whose results were presented descriptively. The results showed that the pulp of three cocoa varieties in West Sumatra found the presence of Indigenous Bacteria and the absence of yeast, in the pulp of three West Sumatra cocoa varieties the presence of bacteria (acidification, amylolytic, Proteolytic and cellulolytic) was highest in the Scavina sample and the lowest in 85 TSH samples and 12 West Sumatra cacao varieties were isolated as many as 12 bacterial isolates with different characters consisting mostly of gram-positive bacteria.

**KEYWORDS:** Fermentation, Indigenous Microflora, *Theobroma cacao*.

## INTRODUCTION

Cocoa is one of the export products that has been determined by the Ministry of Trade of the Republic of Indonesia. Along with in 2006, Indonesia was ranked 3rd in the world as the largest cocoa production (worth 639,140 tons with an area of 1,723,804 hectares) after the beaches of Ivory and Ghana (BPS, 2017 cit Rahmi et al, 2018).

Cocoa (Theobroma cacao, L) has a role in developing regions and agro-industries in Indonesia. Carrying out the plantation revitalization program until 2010 which reached 200 thousand hectares. With hope in the future, the Indonesian cocoa production will get a good value and be equal to the yields of other plantations. But seeing the current condition of the European market assessing cocoa quality is still very low, this is because the product does not have specifications and there are still post-harvest impurities (Towaha, 2012).

Basically fermentation is the main key that determines the final quality of cocoa beans. Fermented cocoa beans are classified as spontaneous fermentation involving indigenous microflora. Indigenous microflora is a natural microflora that exists in an ecosystem, such as fruit. The microflora will be active during the fruit ripening process, so the role of the microflora is hydrolyzing organic matter. This is in accordance with the statement of Periadnadi and Nurmiati (2010), that in fruits there are indigenous microflora. Indigenous microflora which produces acid which is known to use CaCo3 in a medium where acid is a characteristic of fermentation.

## RESEARCH METHODS

This study used an experimental method on the existence of indigenous microflora of fermentation from three cocoa varieties in West Sumatra, then the data obtained were analyzed descriptively. The samples used in this study were three cocoa varieties obtained from the plantations of PT. Inang Sari Padang Mardani, Lubuk Basung District, Agam Regency, West Sumatra. Cocoa used is in the pulp or fruit flesh. Furthermore, isolation of indigenous microflora from cocoa fruit pulp through dilution carried out aseptically. Dilution results were planted into GPA media, GPA CaCo3, Ehanol CaCo3, APB, SMA, and CMC with the pour plate method. So that the halo area will form around the bacterial colonies. Then the growing colonies were observed macroscopically and microscopically.

## RESULTS AND DISCUSSION

The existence of Indigenous Microflora of Cocoa Pulp Fermentation of Three Varieties in West Sumatra.

**Table 1: Average Presence of Indigenous Microflora of Cocoa Pulp Fermentation in General Medium (GPA).**

| No | Sample | Average existence | |
|---|---|---|---|
| | | Bacteria (...×$10^3$ cfu/ml) | Yeast |
| 1 | ICS 60 | 22,4 | 0 |
| 2 | Scavina | 41,2 | 0 |
| 3 | TSH 858 | 13,7 | 0 |

Indigenous bacteria that grow on the medium come from cocoa itself, so it grows naturally. The highest indigenous bacteria found in the Scavina sample was 41.2 × 103 cfu / ml, while the lowest was found in the 858 TSH sample of 13.7 × 103 cfu / ml. The cocoa pulp contains glucose, so that the glucose content contained in cocoa is used for bacterial growth.

The GPA medium is used to see the total bacteria that can grow from the sample. As shown above, it can be seen that growing colonies show the overall microorganisms originating from the cocoa sample. This is in accordance with the opinion of Periadnadi and Nurmiati (2010) stating that basically all bacteria like sugar and a little peptone for its growth, so that in the GPA medium, all types of bacteria contained in a sample are drawn, both fermenting, proteolytic and other bacteria.

**Table 2: Average Presence of Indigenous Microflora of Cocoa Pulp Fermentation in Specific Medium.**

| The existence of bacteria | Sample | | |
|---|---|---|---|
| | ICS 60 | Scavina | TSH 858 |
| lactic acid (...×$10^3$cfu/ml) | 19,4 | 29,6 | 12,1 |
| Acetic acid (...×$10^3$cfu/ml) | 12,3 | 14,9 | 10,7 |
| Amylolytic (...×$10^3$cfu/ml) | 15,5 | 43,8 | 12,9 |
| Proteolytic (...×$10^3$cfu/ml) | 44,2 | 80,1 | 27,7 |
| Cellulolytic ($10^3$cfu/ml) | 17,4 | 38,6 | 16,1 |

The natural bacteria that grow in some specific medium and form the most halo are found in the medium of SMA, this shows that in the chocolate fruit there are quite high protein-breaking bacteria. This is in accordance with the opinion of Pastor et al (2001) stating that proteolytic bacteria are simply bacteria that produce protease enzymes, namely protein-breaking enzymes. According to Karina et al. (2016), the formation of the halo zone indicates that the bacteria have the ability to hydrolyze proteins and casein present in Skim Milk Agar media to produce protease activity.

The presence of Amylolytic bacteria is the second most growing bacterium. The halo area is formed around the colony due to bacteria secreting the amylase enzyme on the APB medium and hydrolyzing the starch so that simple sugars are formed. Winarno (2002) states that microbes will secrete extracellular enzymes if they are on a hydrolyzed substrate so that a clear zone will appear around the colony. This is the activity of amylolytic bacteria. Periadnadi (2005) states that microbes can hydrolyze starch into sugar with the help of amylase enzymes derived from these microbes.

The existence of indigenous bacteria that grow on CMC medium is able to degrade cellulose into glucose and produce cellulase enzymes which are characterized by the presence of clear zones produced. This is in accordance with Hartanti's statement (2010) that bacteria will show a clear zone around the colony as a sign of cellulolytic bacterial activity. The amount of clear zone produced is the ability of bacteria to hydrolyze cellulose. The presence of cellulase enzymes is very important for the life of bacteria because it can provide energy sources.

According to Zverlova et al. (2003) that the size of the clear zone produced by cellulolytic bacteria is generally greater than the colony itself, because the cellulase enzyme is secreted to the surrounding environment by cellulose degrading bacteria. Baharuddin et al (2010) added that cellulolytic bacteria were able to degrade cellulose as a source of carbon and its energy. Cellulolytic bacteria have a faster growth rate than other microbial groups so that the time needed for enzyme production is faster.

The existence of indigenous bacteria that grows on the GPA + CaCO3 medium is used to see fermenting bacteria that have the potential as lactic acid bacteria as evidenced by the presence of clear zones. This indicates that it is highly probable that the cacao sample has bacteria that are able to break down the substrate contained in cocoa to become acidic. This is in accordance with the opinion of Periadnadi and Nurmiati (2010) as a result of hydrolysis of a microbe marked by the presence of a halo region around the colony, this is obtained in the GPA medium added with CaCO3. Calcium Carbonat functions to neutralize the lime in the

colony so that the halo area is formed. The GPA + CaCO3 medium is used to see fermenting bacteria that have potential as lactic acid bacteria.

The indigenous bacteria have the least amount of growth in the CaCo3 Ethanol medium. This bacterium is able to convert ethanol into acid, which is in the form of acetic acid. this is indicated by the presence of a clear zone in the CaCo3 Ethanol medium. In accordance with the opinion of Frauendorfer and Schieberle (2008) cit. Pasau (2013) added that acetic acid is formed during fermentation through degradation of pulp by enzymes and diffuses into cocoa beans. Schwan, (1998) cit. Leal et al (2008) added that traditional and spontaneous fermentation is microbial fermentation involving yeast, lactic acid bacteria (LAB), acetic acid bacteria (AAB), Bacili and filamentous mushrooms.

**Macroscopic and microscopic characters of indigenous bacterial isolates on cocoa**

**Table 3: Macroscopic and microscopic characters of indigenous bacterial isolates on cocoa.**

| sp. | Macroscopis | | | Microscopis | |
|---|---|---|---|---|---|
| | colony form | Colony Elevation | Colony color | Cell Shape | Gram Properties |
| Sp 1 | Circular | Flat | White | Basil | + |
| Sp 2 | Circular | Flat | Yellowish white | Basil | + |
| Sp 3 | Irregular | Umbonate | Yellow | Basil | + |
| Sp 4 | Irregular | Flat | white | Basil | + |
| Sp 5 | Irregular | Raised | white | Basil | + |
| Sp 6 | Circular | Flat | Transparent white | Basil | + |
| Sp 7 | Rizoid | Flat | Yellowish white | Basil | + |
| Sp 8 | Irregular | Flat | Transparent white | Basil | - |
| Sp 9 | Circular | Umbonate | Putih | Basil | + |
| Sp 10 | Circular | Umbonate | Yellow | Basil | - |
| Sp 11 | Irregular | Flat | Yellowish white | Basil | + |
| Sp 12 | Irregular | Flat | white | Basil | - |

The results of the macroscopic observation of colonies and microscopic bacterial cells against isolates of each indigenous bacteria. Each isolate has different macroscopic and microscopic morphologies. Morphological observation of bacteria was carried out using colony counter, such as counting colonies. Some macroscopic observations include colonies, elevations and colors. This is consistent with the statement of Dwijoseputro (2005) explaining that the macroscopic observation of bacterial colonies includes the form of bacterial colonies, the edge of bacterial colonies, the surface of bacterial colonies, and the color of bacterial colonies, each bacterial isolate has different characteristics.

Microscopic observations have different characteristics. In coloring gram aims to look at bacterial cell shape and the nature of gram bacteria. Gram staining that has been done is obtained by gram positive bacteria and gram negative bacteria. In addition, gram staining aims to see bacterial cell shape and gram bacterial properties. Gram positive bacteria when seen in purple and red gram negative and bacterial cell form are bacilli. This is in accordance with the opinion of Jawetz et al., (2004) stating that gram staining is one of the effective criteria for classification. The final results of gram staining will be obtained 2 groups of bacteria based on the structure of the cell wall, namely gram-positive bacteria and gram-negative bacteria. Gram positive bacteria will give a purple color because it has a thicker peptidoglycan layer while Gram negative bacteria have a pink color and a thin peptidoglycan layer.

## CONCLUSION

Based on research the existence of Indigenous Microflora Fermentation of cocoa pulp from three varieties in West Sumatra can be concluded that:

11. In the pulp of three West Sumatra cocoa varieties (ICS 60, Scavina and TSH 858), the presence of indigenous bacteria was found but no presence of yeast was found.
2. In the pulp of three West Sumatra Cocoa varieties (ICS 60, Scavina and TSH 858) the highest presence of bacteria (Acidification, Amylolytic, Cellulolytic and Proteolytic) was found in the Scavina sample, while the lowest was in the TSH 858 sample.
3. Of the three cocoa varieties 12 isolates of bacteria were isolated with different characters, most of which consisted of gram-positive bacteria.

## SUGGESTION

Further research is recommended for complete biochemical testing and bacterial identification in determining the type of bacteria that can be patented for superior isolates.

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