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Bali Nusa Dua Convention Center, 18-19 October 2018

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Temperature range and degree of acidity growth of isolate of indigenous bacteria on fermented feed “fermege”

Temperature range and degree of acidity growth of isolate of indigenous bacteria on fermented feed "fermege"

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Abstract. Fermege is a fermented feed of ruminants, especially goats made from water hyacinth (*Eichhornia crassipes*). Temperature range and pH need to know in making starter formula for acceleration of fermentation process at making ruminant feed made from this materials. The starter formula expired period can be extended by adjusting starter storage temperature and pH of the starter. This research was aimed to find the temperature and pH range for the growth of isolate of indigenous bacteria "fermege." This research is an explorative research conducted by growing bacteria isolate indigenous fermege in liquid medium with various pH and incubation in various temperature. Bacterial population was calculated based on turbidity of bacterial suspension with turbidometer. The stages of this research were to isolate the bacteria present in the fermege, purify the isolates found, and then grow the isolates in a liquid medium with various pH values. The isolated bacterials were incubated at different temperature variations. The cell population density of the isolates was calculated after incubation for 24 hours. The results showed there were eight indigenous bacterial isolates. All isolates can grow in the pH range 6 and 7. Two isolates (*Bacillus subtilis* and *B. pumilus*) can grow at 4°C. All isolates obtained can grow at a temperature of 30°C. Isolates *Bacillus badius, B. subtilis*, *B. cereus, , Pseudomonas stutzeri* and *P. diminuta* can grow at 50°C. Based on research indicates that indigenous fermege bacterial isolates have the ability to grow in the neutral pH range and temperature range between 4°C and 50°C**.**

1. Introduction

The factors that influence microbes growth included physical and chemical requirements [1]. The physical requirements for growth fall into three groups namely temperature, pH and osmotic pressure. Bacteria are found in all ranges of temperatures. Bacteria can be separated according to temperature ranges in which they grow best.There are three kinds of groups of bacteria according to the temperature ranges, psychrophiles microbes that grow at cold temperatures, mesophiles microbes that grow at moderate temperatures and the thermophiles that grow at high temperatures. Related to the temperature there are three kinds of term like these: first, the minimum growth temperature is the lowest temperature at which an organism grows. Second, the maximum growth temperature is the highest temperature at which an organism grows. Thirt, the optimum growth temperature is the temperature at which the highest rate of growth occurs.The optimum growth temperature varies between bacterial types [2]. Influences the temperature to the cell included some kinds of physiology process. Increased temperature breaks chemical bonds.This causes changes in the three dimensional structure.These changes can inhibit or destroy the ability for the molecules to function properly.

Another important factor that influence microbes growth is pH [3]. Bacteria grow in a wide range of pH values [4]. Most bacteria prefer the neutral pH of 7.0. Some bacteria are acidophiles that grow at extremely low pH values.The other bacteria grow at high pH value. The bacteria are alkaliphiles. Influences pH on the microbes growth included some cell process. An excess of hydrogen ions causes bonds to break.This changes three dimensional structure of cells component. Changes in three dimensional structure destroy protein function. Destruction of protein function can be a lethal event.

In previous research isolated indigenous micobes from feed fermentation "fermege". There are eight kinds of bacterials. The bacterials isolated from fermege included were *Bacillus badius, B. subtilis, B. brevis, B. pumilus, B. cereus, Xenorhapdus luminescens, Pseudomonas stutzeri* and *P. diminuta* (Isnawati and Trimulyono, 2016). Based on the cellulolitic activity test, all of the bacterials shown have cellulolytic activity [5].

In subsequent research future, these bacterials will be used to produce starter that can accelerate the fermentation process in the goat feed fermented making, based on water hyacinth and corncob as raw materials. There are some problems in making and storaging of starter. Varga and Papai found that compatibility between strains is important both for the production and storage of the product [6]. Because of the fact, it is very important to make the bacterials consortium that contain bacterials that are mutualy synergiatic. Also in the processing steps like pure culture making, storaging the pure culture and the thawing process influence the quality and viability of the bacterials in the starter.

One kinds of the way to maintain viability and quality bacterials in the starter is by the subculture technique. This technique is not good for several reasons, one is, with certain bacteria that make organic acids or other potentially self-toxic metabolites, they may be tolerant to some degree (aciduric) but they do not survive long in their own toxic metabolites. two reason is appearences of 'infidelity of DNA replication' phenomenon that can alter the bacterials genetics [7].

The other ways to maintain quality and viability bacterials starter is stote the culture in the specific media. Young stored the lactic acid bacterials in MRS supplemented with 40% glycerol at -70^{o}C when the starter in the rest condition [8].*Pectinatus frisingensis* is one the type of bacteria used in beer fermentation can be inhibited it's growth by regulating pH medium [9]. The phenomenon suggests that the pH can be used to maintain viability and bacterial quality in starter.

Two types of lactic acid bacteria can be maintained it's viability for 12 months by regulating storage temperature up to 4^{o}C, with a supply of lactose and glucose [10].The present study aimed finding the pH and temperature range to maintain the bacterials viability and quality in the starter. Temperature and pH and other physical and chemical included the environment of microbes [11].

2. Experiment

There are some steps in this study. First, to make fermege. Fermege making is done based on Fitrihidajati *et al*. procedure [12]. Second, to isolate the indigenous bacterials from fermege that has cellulolitic activity. In this step is used the specific media that contain some substance namely $NaNO_3$ 0,5gL^{-1} ; K_2HPO_4 1gL^{-1} ;$MgSO_4.7H_2O$ 0,5gL^{-1}; $FeSO_4.7H_2O$ 0,02gL^{-1} ;KCl 0,2gL^{-1} and agar 20gL^{-1} ajust pH 7,5, with enrichment 0,05% (w/v) AZCL HE-Celllose (Megazyme, Bray, Ireland). Thirth, to observe the pH and temperature range that can maintain the viability of the indigenous bacterials. Observation of pH and temperature range for indigenous bacterials was done by growing bacteria indigenus in liquid medium with various pH. Then, then incubated at various temperatures for 24 hours. After incubation periode was measure the turbidity of the bacterials suspension with the turbidimeter. The turbid suspension indicated the bacterials growth.

3. Result and Discussion

The results of this research were growth pH range for eight indigenous bacterials isolated from fermege and growth temperature range for these bacterials. The growth pH range displayed in Table 1 below.
Based on the data displayed in the Table 1 dan be state that the indigenus bacterials from fermege can be live in the pH range from 6 to 7. There are the effect of extreme pH to the cells. The extreme pH influence the structure of all macromolecules like carbohidrate, protein, lipids and nucleid acid. The hydrogen bonds holding together strands of DNA break up at high pH. Lipids are hydrolyzed by an extremely basic pH. The proton motive force responsible for production of ATP in cellular respiration

depends on the concentration gradient of H^+ across the plasma membrane. If H^+ ions are neutralized by hydroxide ions, the concentration gradient collapses and impairs energy production. But the component most sensitive to pH in the cell is its workhorse, the protein. Moderate changes in pH modify the ionization of amino-acid functional groups and disrupt hydrogen bonding, which, in turn, promotes changes in the folding of the molecule, promoting denaturation and destroying activity.

Table 1. The turbid score of indigenous bacterials that was grown in the various pH

No.	Species of indigenous bacterials	pH value	Turbid score
1.	*Bacillus badius*	7	0,170
2.	*B. subtilis*	6	0,343
3.	*B. brevis*	6	0,973
4.	*B. pumilus*	6	0,629
5.	*B. cereus*	6	0,288
6.	*Xenorhapdus luminescens*	6	0,756
7.	*Pseudomonas stutzeri*	6	0,531
8.	*P. diminuta*	7	0,290

There are three groups bacterials based on the growth capacity in pH value. Most bacteria are **neutrophiles**, meaning they grow optimally at a pH within one or two pH units of the neutral pH of 7. Indigenous bacterials isolated from fermege included in this group. Microorganisms that grow optimally at pH less than 5.55 are called **acidophiles**. At the other end of the spectrum are **alkaliphiles**, microorganisms that grow best at pH between 8.0 and 10.5.

Based on the data can be stated that the starter from indigenous bacterials must be maintain in the 5-8. In the 5 and 8 pH value, the indigenous bacterials will grow slow. because of that the life of the starter can be maintain for long time [13]. Following, Table 2 is the data related to growth temperature of indigenous bacterials.

Microbes, such as bacteria are more tolerant of environmental conditions than other organisms. However, each species has its own characteristic and particular range of values in which it grows and reproduces best. Some species of microorganism can grow at temperatures as low as -10^o C, and others at temperatures as high as 100^o C - or higher. These upper and lower values are a function of cell metabolism. At lower temperatures molecules move slower, enzymes cannot mediate in chemical reactions, and eventually the viscosity of the cell interior brings all activity to a halt.

As the temperature increases, molecules move faster, enzymes speed up metabolism and cells rapidly increase in size. But, above a certain value all of these activities are proceeding at such high rates, enzymes start to denature, and the total effect is detrimental. Cellular growth ceases. These boundary values define the maximum and minimum temperature at which life can exist (and grow). Each species of microbe has its own, unique upper and lower limit, which is a defining characteristic for that species.

Table 2. The turbid score of indigenous bacterials that was grown in the various temperature

No.	Species of indigenous bacterials	Temperature (°C)		
		4	30	50
1	*Bacillus badius*	None	Grow	Grow
2	*B. subtilis*	Grow	Grow	Grow
3	*B. brevis*	None	Grow	None
4	*B. pumilus*	Grow	Grow	None
5	*B. cereus*	None	Grow	Grow
6	*Xenorhapdus luminescens*	None	Grow	None
7	*Pseudomonas stutzeri*	None	Grow	Grow
8	*P. diminuta*	None	Grow	Grow

Based on the temperature growth, there are three kinds of microbe. First, the bacteria that grow at temperatures in the range of -5°C to 30°C, with optimum temperatures between 10°C and 20°C, are called **psychrophiles**. These microbes have enzymes that catalyze best when the conditions are cold, and have cell membranes that remain fluid at these lower temperatures. Second, microbes that grow at optimal temperatures in the range 20°C to 40°C, are called **mesophilic**. Important members of this group are those that live in and on warm blooded creatures, such as humans. Thirth, certain bacteria can live and grow at temperatures that exceed 50°C. These are thermophilic microbes that can tolerate the very harsh conditions decomposing organic material, the hot springs at Yellowstone National Park (where temperatures are at least 80°C to 85°C), or deep in the oceans by thermal vents bubbling up from the hot rocks just below the earth's crust

Based on the data displayed in the Table 2 can be stated that in the fermege contain three groups of microbes related to growth temperature *Bacillus subtilis* and *B. pumilus* can grow in the lower temperature. All of bacterials grow in the 30°C. Some kinds of bacterials can grow in the high temperature namely *Bacillus badius, B. subtilis, B. cereus B. cereus, Pseudomonas stutzeri,* and *P. diminuta*.

4. Summary

Based on research indicates that indigenous fermege bacterial isolates have the ability to grow in the neutral pH range and temperature range between 4°C and 50°C. These pH and temperature range suggest to use in bacterials stored condition.

5. Acknowledgement

The author would like to thank to Elfa and Kholida who had helped collecting the data.

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