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Research Article

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Isolation and Screening Protease Acid, Neutral and Alkaline Producing Bacteria from Dadih (Traditional Indonesian Food)

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ABSTRACT

Protease is an enzyme that is economical and very widely used in various fields of industry. This study aims to isolate and screen the bacteria producing acid, neutral and alkaline protease enzymes from the dadih. Isolation of bacteria from the dadih using the method plate count while screening bacteria producing protease enzymes by using Skim Milk Agar selection medium at various levels of acidity. The results showed that 111 bacterial isolates from dadih, 27 isolates of bacteria produce protease, where 5 bacterial isolates have the potential to produce acid protease, 6 isolate of protease neutral and 16 isolates as alkaline protease producer.

Keywords: Isolation; Screening; Protease; Dadih

INTRODUCTION

Proteases are enzymes that have catalytic to peptide bonds of a polypeptide or protein molecule producing amino acids or peptides with the help of water molecules [1]. Proteases that include proteinases, peptidases or proteolytic enzymes are one of the three largest industrial enzyme groups, accounting for about 60% of enzyme sales worldwide [2]. Based on its acidity, proteases are grouped into three groups, namely acid proteases its best performed in the pH range 2.0-5.0; neutral protease that have an optimal pH in the range of 7.0 or around and alkaline proteases having optimum activity in the pH range of 8 or more [3]. Acid proteases or aspartat protease, have aspartic acid residues for their catalytic activity [4]. The active-site aspartic acid residue is situated within the motive Asp –Xaa - Gly, in which Xaa can be Ser or Thr [5]. The application of acid protease use is in the dairy industry, to coagulate casein to form curd in the preparation of cheese making by removing whey [6]. The neutral proteases include cysteine proteases, metalloproteases, and some of the serine proteases. Neutral protease applications are in the cakes, beer and food processing industries [1]. Alkali protease has serine active side and has a very important commercial value, one of them as an additive in detergent [7]. Dadih is a product of fermented buffalo milk stored for 1-2 days using a bamboo tube. The Dadih comes from West Sumatera, Indonesia made from buffalo milk and then put into bamboo tube and covered with banana leaf and then fermented at room temperature for 1-2 days to form a clot. The purpose of this study was to obtain bacterial isolates of curd which produce acidic, neutral and alkaline proteases.

EXPERIMENTAL SECTION

Preparation of Dadih

Dadih is prepared in bamboo after incubation at room temperature for a certain time. The strategy to obtain alkaline protease-producing bacteria is to use the curd that has been incubated one day, for the neutral protease producing

bacteria, curd which has been incubated 2 days while the alkaline protease-producing bacteria used curds were incubated four days.

Isolation of Bacteria from the Curds

The isolation of bacteria from the curds is done by plate count method, the curd sample dilution to 10-6. One ml samples were inoculated in a petri dish and then poured medium Nutrient Agar furthermore incubated at room temperature for 24 hours. Medium NA used with pH 4; pH 7.0 and pH 8.0.

Purification and Culture Slant

Purification of bacterial isolates was done by inoculation of bacterial colonies on NA medium with the quadrant method. Cultures were incubated at room temperature for 24 hours. A single colony of bacteria was inoculated in a test tube and incubated at room temperature for 24 hours, as a culture slant.

Screening of Proteolytic Bacteria

Screening of bacteria producing protease performed in vitro, which are used medium Skim Milk Agar (SMA) with a medium pH 4.0; 7.0 and 8.0. Bacterial isolates were inoculated on medium high school and incubated at room temperature for 48 hours. Measurement of colony diameter and the diameter of clear zone is formed.

Screening of Bacteria Producing Acid Protease, Neutral Protease and Alkaline Protease

Acid protease producing bacteria screening is done by using SMA medium with various pH: 4.0; 4.5; 5.0; 5.5 and 6.0. Bacterial cultures were inoculated on SMA medium incubated at room temperature for 24 hours. Measurement of colony diameter and the diameter of clear zone is formed. The same thing is done to screen bacteria producing neutral protease with SMA medium pH variation: 6.0; 6.5; 7.0; 7; 7 and 8.0 while screening alkaline protease producing bacteria by using SMA medium on pH: 8.0; 8.5; 9.0; 9.5 and 10.

RESULTS AND DISCUSSION

Isolation of Bacteria from Dadih

The isolation of bacteria from the curd was carried out at Medium NA, obtained by 111 bacterial isolates with details of twenty-five isolates on NA medium, pH 4.0; Forty two isolates in NA medium, pH 7.0 and forty four isolates on NA medium, pH 8.0 (Figure 1). The presence of bacteria in the curd is due to the high content of nutrients in the curd, and the bacteria contained in the curd comes from the surrounding environment at the time of milking, from bamboo used as a container or from the leaves of the bamboo tube. Based on the pH of the medium, 25 isolates (22.5%), have the ability to live and survive at acidic pH (acidophile), Forty two or 37.8% isolates are neutralphile and isolate indicated alkaliphile is forty four or 39.7%. The percentage of bacterial presence of neutral and alkaline pH is very different from bacteria living at acidic pH (Figure 2). This shows that the pH of a substrate or medium greatly affects bacterial life.

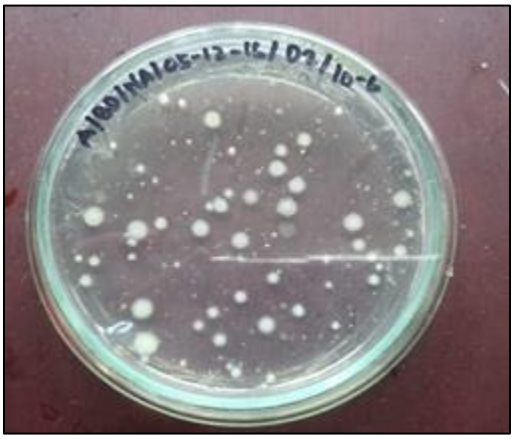

Figure 1: Bacteria colonies on NA medium pH 8.0

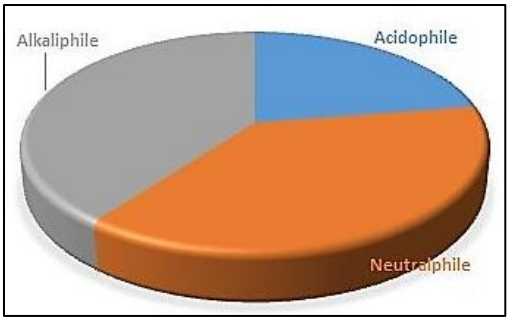

Figure 2: Percentage of acid, neutral and alkaline bacteria

Screening of Proteolytic Bacteria

One hundred eleven isolates of curds were screened using the SMA medium, earned thirty isolate bacteria producing protease enzyme indicated, where five isolates of bacteria that have a clear zone around the colony on SMA medium pH 4.0. Nine isolates on SMA medium pH 7.0 and sixteen isolates on SMA medium pH 8.0. The bacteria produce extracellular protease enzymes, which are secreted into the medium SMA bacterial cells that contain casein. Enzymes hydrolyze casein causing the medium seemed clear (Figure 3).

Figure 3: Colony bacteria with clear zone on SMA medium

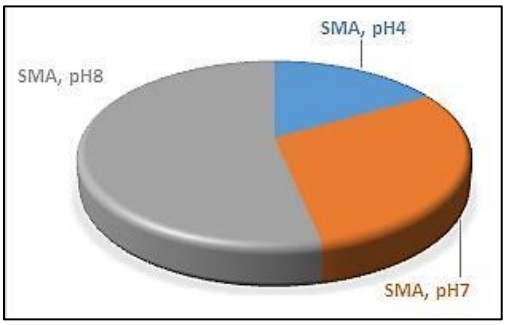

Figure 4: Percentage bacteria proteolytics

The amount of small clear zone around the colonies of bacteria indicates the size of the catalytic power of enzyme or protease enzyme activity causes the formation of clear zone around bacterial colonies. Diameter of clear zone affected by the concentration and activity of the enzyme produced by bacteria, the higher the enzyme activity, the more extensive the resulting clear zone diameter [8]. In Figure 3 is also seen that there are bacteria that grow on SMA medium but did not produce a clear zone, which means that the bacteria do not produce the protease. The growth of these bacteria is because bacteria can use the lactose contained in the medium. Bacterial isolates were able

to live and produce protease at pH 4.0 of SMA medium around 16.67%; in SMA medium at pH 7.0 about 30% and SMA medium at pH 8.0 by 53.33% (Figure 4).

Screening of Bacteria Producing Acid Protease, Neutral Protease and Alkaline Protease

Screening of five isolates was indicated of having the ability to produce protease enzyme on SMA medium pH 4.0 was then grown in medium SMA interval pH 4.0 to pH 6.0. Protease activity profile of bacterial isolates showed that the five isolates showed protease enzyme activity as acids (Figure 5).

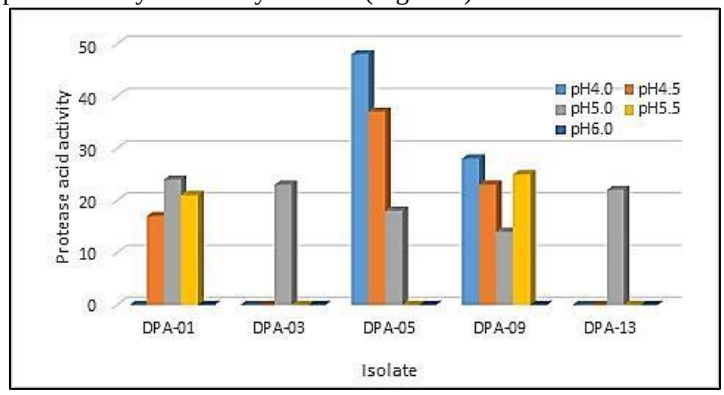

Figure 5: Histograms protease activity profile of bacterial isolates at acidic condition

Isolates DPA-05 and DPA-09 highest protease activity at pH 4.0 and While three other isolates DPA-01, DPA-03 and DPA-13 maximum enzyme activity at pH 5.0. DPA-05 isolates had the highest activity, while DPA-13 with the most low enzyme activity.

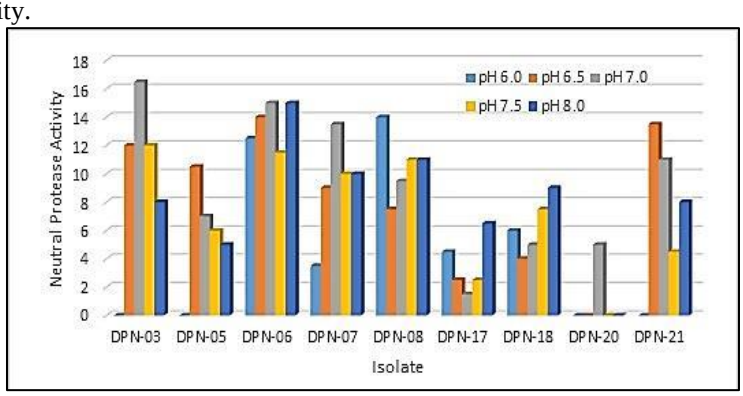

Figure 6: Histograms protease activity profile of bacterial isolates at neutral condition

Nine isolates of bacteria producing protease at pH 7.0 SMA medium grown on SMA medium pH 6.5-8.0. Six isolates showed characterized of producing neutral protease enzyme, while three isolates DPN-08, DPN-17 and DPN-18 does not characterize a producer of neutral protease (Figure 6). DPN-03 isolates had the highest enzyme activity, while the DPN-20 with the lowest enzyme activity. The neutral proteases, which are active at neutral or weakly acidic or weakly alkaline pH, include cysteine proteases, metallo proteases, and some of the serine proteases [1].

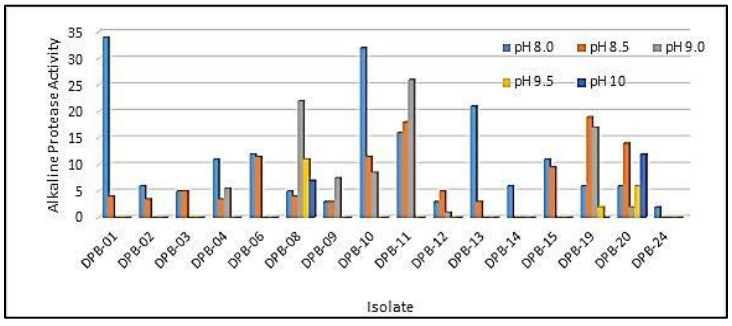

Figure 7: Histograms protease activity profile of bacterial isolates at alkaline condition

Based on the profiles of protease activity at pH 8.0 to 10.0; there are sixteen characterize bacterial isolates producing alkaline protease enzyme (Figure 7). This is due to the protease secreted by the bacteria have the ability to hydrolyze the substrate casein in alkaline conditions. Enzyme activity profile of each isolates showed different characteristics. The big difference in enzyme activity profile indicating that all sixteen isolates were also different in kind. DPB-01 isolates had protease enzyme activity and isolates the highest alkali DPB-24 with low enzyme activity. pH affects the speed of the enzyme activity in catalyzing a reaction. This is due to the concentration of hydrogen ions influence the three-dimensional structure of the enzyme and its activities. Each enzyme has a pH optimum pH at which the three-dimensional structure most conducive to bind substrate. When the hydrogen ion concentration changes of the optimal concentration, the enzyme activity is progressively lost until finally the enzyme becomes nonfunctional [9].

CONCLUSION

Isolation and screening of bacteria from the dadih, the traditional food of West Sumatra, Indonesia, obtained twenty seven isolates producing protease consisting of five bacterial isolates producing acid protease, producing neutral protease and sixteen isolates producing alkaline protease.

REFERENCES

[1] ML Rao; AM Tanksale; MS Ghatge; VV Deshpande. *Microbiol Mol Biol Rev*. **1998,** 262, 3, 597-635.
[2] A Nurullah; F Uyar. *J Biosci*. **2011**, 5, 64-72.
[3] HS Alnandi. *J Appl Pharm Sci*. **2012**, 2, 9, 71-74.
[4] AJ Barett. *Method Enzymol*. **1995**, 248, 183.
[5] DR Davies. *Rev Biophys Chem*. **1990**, 19, 189-215.
[6] S Neelakantan; AK Mohanty; JK. Kaushik. *Curr Sci*. **1999,** 77, 1, 143-148
[7] R Gupta; QK Beg; P Lorenz. *Appl Microbiol Biotechnol.* **2002**, 59, 13-32
[8] T Palmer. Understanding Enzymes, 3rd edition. Ellis Horwood Limited, Chichester, **1991**.
[9] D Nelson; M Cox. Lehninger Principles of Biochemistry, 4th edition, WH Freeman and Company, New York, **2005**.