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Effect of Media Modification on Collagenase Activity from *Bacillus Licheniformis* MB-2

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ABSTRACT

The purpose of this study was to analyze media modifications to collagenase activity of Bacillus licheniformiss MB-2. The method used is assayed of the activity of collagenase in the first media (LB + collagen 5%), the second media (tryptone collagen + NaCl + 5%) and the third media (NaCl + 5% collagen). The results showed in LB media + collagen, the optimum collagenase production of B. licheniformis MB-2 was 12 h of fermentations. The results of the activity between the first media (LB + collagen 5%) with a second media (tryptone + NaCl + collagen 5%) showed a pattern similar, the optimum enzyme production was 12 h, after 12 h decreased and activity optimum occurs in exponential phases. Collagenase activity of B. licheniformis MB-2 on second and third media has lower was compared with LB + collagen media, suggesting a role as a source yeast extract. Nitrogen is essential for the growth of bacteria that affect the activity of collagenase. Collagenase activity of B. licheniformis MB-2 in the third media (NaCl + collagen) decreased over time the bacteria fermentation (Figure 3). In the third media, source of carbon and nitrogen should be provided in LB media (tryptone + NaCl + yeast extract) into reduced, carbon and nitrogen source alternatives only in collagen added, this affects the growth of bacteria B. licheniformis MB-2 and thus the activity tends to decrease during fermentation.

Keywords: *Bacillus licheniformis* MB-2, collagenase, modified media

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INTRODUCTION

Collagenase is an endopeptidase that can break down the collagen triple helix domain. Based on physiological functions, collagenase classified into two types, namely serine collagenase and metallokolagenase. Serin collagenase like all serine proteinase, has a catalytic serine residue on the side [1]. As a proteolytic enzyme, collagenase applied to many industrial fields. Collagenase is widely used in the leather tanning process [2]. Other application of collagenase is in the produces of collagen peptides with antioxidant activity [3], angiotensin I-converting enzyme (ACE) inhibitor and cancer antiproliferative activity [4].

One source of collagenase enzyme known is from bacteria. Collagenase-producing microbe research has been widely published. Some bacteria produce collagenase was *Bacillus* sp. MO-1 [5], *Bacillus subtilis* CN2 [6], *Streptomyces parvulus* [7}, *Bacillus pumilus* COI-J [8 and *Bacilus licheniformis* F11.4 [9].

MATERIALS AND METHODS

Collagenase production

The LB media contained tryptone 1 %, NaCl 1 % dan yeast extract 0.5 % (w/v). The cell growth was monitored turbidimetrically through absorbance at l = 620 nm. Incubation was conducted at 37 °C and samples were taken for analysis of enzyme activity and cell growth.

Collagenase Activity

Collagenase activity was measured according to the Bergmeyer method [10] with collagen from fish skin (5 %) as the substrate. One unit (U) of enzyme activity was defined as enzyme which produce 1 μmol of tyrosine per min.

Modification of Bacterial Growth Media

Growth media used there are first media is Lauria Bertani broth (LB) + collagen. LB medium with the composition: 1% triptone; 0.5% yeast extract and 1% NaCl. The second media is a media whose composition Triptone + NaCl + 5% collagen, and a third media is media that the composition of NaCl + 5% collagen.

RESULTS AND DISCUSSION

Collagenase production of *B. licheniformis* MB-2 in First media (LB media and addition of collagen into the media). Observations were used every 12 h for 36 h, at a temperature of 37 ^{0}C and 120 rpm. Enzymes produced by the bacteria that have been separated from bacterial cells using centrifugation. With this technique, the cells will settle by the force of gravity, while the enzyme remains present in the supernatant. Centrifugation carried out at low temperatures to prevent damage to the structure of the enzyme, further testing collagenase activity, whereas bacterial growth was observed by optical density (OD) at λ = 620 nm. Collagenase production in LB + 5% collagen media can be seen in Figure 1.

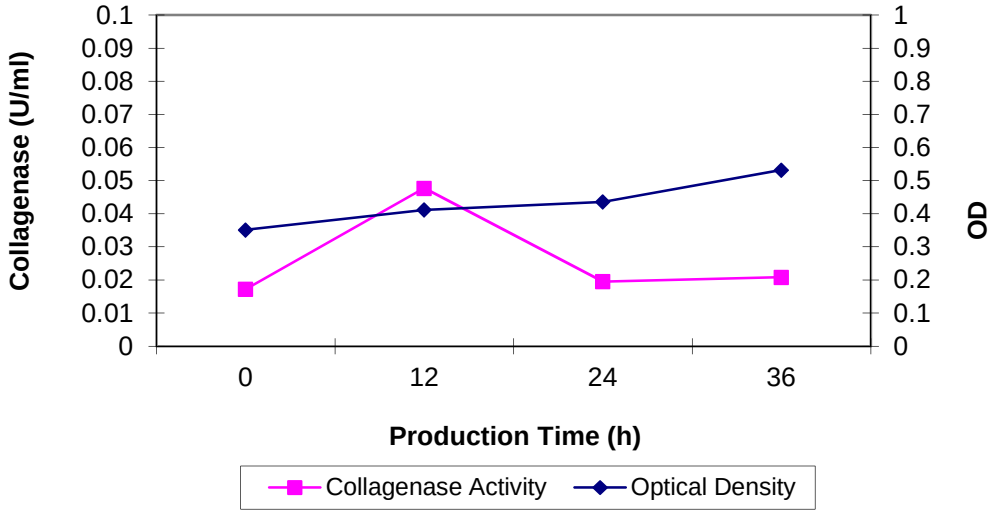

Figure 1. Collagenase activity of *B. licheniformis* MB-2 on LB + collagen media

Figure 1 showed the optimum collagenase production of *B. licheniformis* MB-2 was 12 h of fermentations with the activity of 0.048 U/ml. Collagenase activity of *B. licheniformis* MB-2 rapidly reduced following its optimum activity at 12 h of incubation. The *Bacillus licheniformis* F11.4 was reported to synthesize collagenase optimally by 20-35 h of fermentation time in LB media, addition of collagen into the media (LB + collagen), resulted in different responses with optimum production being 10 h incubation [9].

Modifications to the media in Figure 2 is only carried out the removal of elements yeats extract. The results of the activity between the first media (LB + collagen 5%) with a second media (tryptone + NaCl + collagen 5%) showed a pattern similar activity, the optimum enzyme production was 12 h, after 12 h decreased and activity optimum occurs in exponential phases. Shorter optimum fermentation time of collagenase, *i.e.* 8 h was reported when *Bacillus subtilis* CN2 [6]. *Bacillus* sp. DPUA1728 produced protease optimally at longer incubation time, that is by 24 h [11].

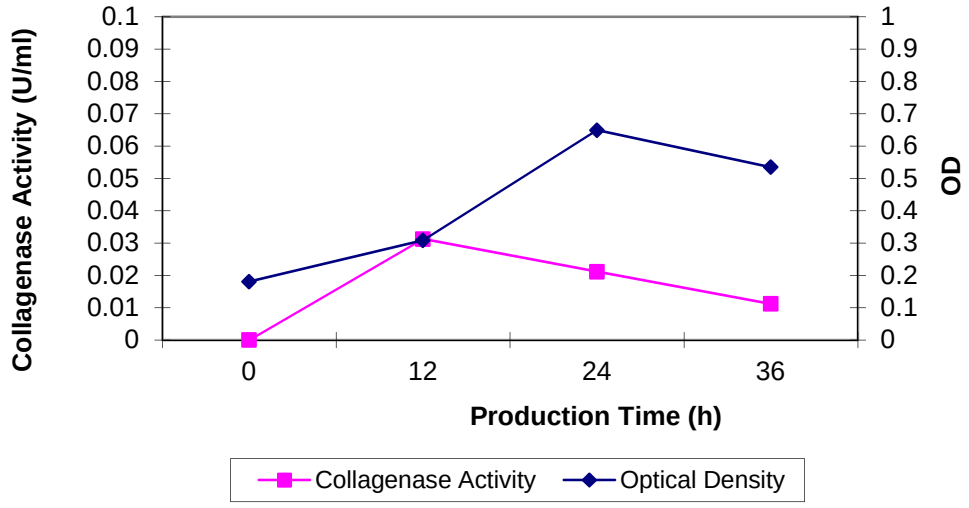

Figure 2. Collagenase activity of *B. licheniformis* MB-2 on media of tryptone + NaCl + collagen

Figure 2 showed the activity of collagenase in second media (tryptone + NaCl + collagen) was lower activity than that first media (LB + collagen), yesat extract as a source of nitrogen is very important for the growth of bacteria that affect the activity of collagenase even though alternative sources of nitrogen in the media have been available from collagen.

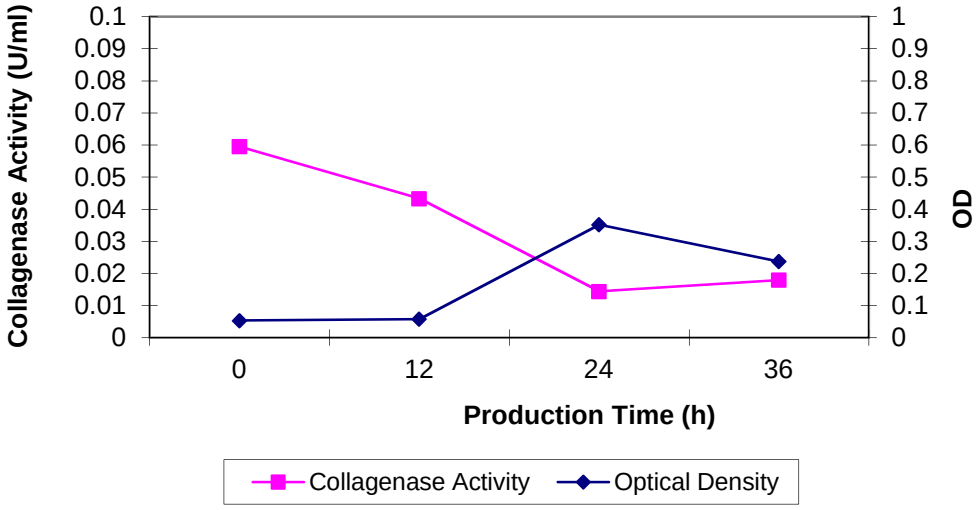

Figure 3. Collagenase activity of *B. licheniformis* MB-2 on media of NaCl + collagen

Collagenase activity of *B. licheniformis* MB-2 in the third media (NaCl + collagen) decreased over time fermentation (Figure 3). Collagenase activity of *B. licheniformis* MB-2 on second and third media has lower was compared with LB + collagen media, suggesting a role as a source yeast extract. Nitrogen is essential for the growth of bacteria that affect the activity of collagenase. In the third media, source of carbon and nitrogen should be provided in LB medium (tryptone + NaCl + yeast extract) into reduced, carbon and nitrogen source alternatives only in collagen added, this affects the growth of bacteria *B. licheniformis* MB-2 and thus the activity tends to decrease during fermentation. The decline in collagenase activity related to the availability of sources of nutrients that the collagen presence of nutrients that need to be hydrolyzed in advance so bacteria growth which eventually disrupted the synthesis of collagenase also decreased.

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