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Production and Application of Keratinase Enzyme from 4 Strains of *Bacillus spp.* Isolated from Yogyakarta and Garut City

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ABSTRACT: Processing of waste chicken feathers can be used as a biological treatment with keratinase enzyme. Keratinase enzyme can be produced by microorganisms. Keratinase enzyme expected to be produced from *Bacillus spp.* which has been previously isolated from Yogyakarta and Garut City. The purpose of this research was to determine the production of keratinase enzyme produced from *Bacillus spp.* and apply the keratinase enzyme in the process of degradation of chicken feathers. Research consists to measuring the growth of *Bacillus spp.* and investigation of degradation of chicken feathers by *Bacillus spp.* were analyzed descriptively, while data of digested protein by *Bacillus spp.* analyzed using a split plot design, if there are differences followed by Duncan New Multiple Range Test (DMRT). The results obtained bacterial growth and the ability of degradation was found on *Bacillus sp*. TD5B. Increasing of growth rate was followed by faster degradation time. Concentration soluble protein by *Bacillus* meganterium capable of producing higher compared with any others strains. *Bacillus* meganterium has had highest soluble protein (2,030 mg/ml). The longer degradation time followed by highest concentration soluble protein of feathers. The best incubation time at 8 hours that containing 2,256 mg/ml of soluble protein.

Keywords: *Bacillus spp.*, Feathers, Keratinase Enzyme

INTRODUCTION

Poultry feathers contain more than 90% of crude protein in keratin form, found as wastes or by-products at poultry processing plants (Howie *et al*., 1996). Increasing quantities of feathers could effect to the environmental pollution (Rajput and Gupta, 2013). The crude protein content in feather wastes could have a great potential nutrient value and may have some advantage as a protein sources for substitute from more expensive dietary ingredients for animal feed such as poultry and ruminant animal (Xie *et al.*, 2010). Worldwide, commercial poultry processing generates 5 millions of tons of feathers per year, which are currently converted to feather meal through steam pressure and chemical treatment (Freeman *et al.*, 2009). Including in Indonesia, poultry industries are growing faster comparing to the other livestock industry due to the high demand of poultry meat as cheap and high quality protein sources for human consumption. Furthermore, making keratin waste more digestible, established chemical treatment process such as alkali hydrolysis and steam pressure cooking, is both high cost processes and destructive to certain amino acids from feathers such as methionine, lysine, and tryptophan (Tork *et al.*, 2012). The nutritional upgrading of feather as animal feed, especially amino acids content with the treatment of microbial keratinase might lead to a significant increase in the availability of certain amino acids in feather keratin (Joshi *et al.*, 2007).

MATERIALS AND METHODS

Source of Keratin and Preparation of chicken feathers as substrate

Chicken feathers (whole feathers) were collected from chicken slaughterhouse at Yogyakarta district. Feathers were then extensively washed in tap water continued by sterilization with

autoclaved and then dried in hot air oven for 48 h. They were stored at 28°C until used.

Microbial Culture

The organisms was grown in basal salt medium (g/L): meat extract, 1.0; biological peptone, 1.0; NaCl, 0.5; and feathers, 1.0. For submerged fermentation, 24 h grown seed culture was used at 5% (v/v) concentration. The cultivation was performed at 28°C at 120 rpm on a shaking incubator for 24 h. Every 6 h, sample was grown by spread plate methods. After 3 d incubation, the bacteria were able to count of colonies.

Feathers Degradation

The success rate of substrate degradation was measured by medium turbidity and amount of feathers in medium. Feathers 0.5 g/L to be completed degraded by four strain bacteria in different time. The cultivation was performed at 28°C at 120 rpm on a shaking incubator for 3 d.

Keratinase Production

The cultivation was performed at 28°C at 120 rpm on a shaking incubator for 8 h. Every 2 h, sample was centrifuged at 4°C at 3,000 rpm for 15 min. The supernatant was used as crude enzyme source. Crude enzyme protein assayed using Lowry methods.

Data Analysis

Data from protein concentration of hydrolyzed feather produced were analyzed using a split plot design. Furthermore, if there are differences between the mean, analyses will be continued with Duncan's New Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

According to the measurement of the growth in liquid medium of four-isolated strains, which confirmed to be belong to *Bacillus spp.*, it was showed different profiles from one to the others. Without the addition of feather as substrate, the growth of *Bacillus sp*. TD5B showed higher pattern in liquid medium compared to *Bacillus sp*. TD5K. Furthermore, *Bacillus sp.* LS2B showed higher growth compare with *Bacillus meganterium* (Figure. 1).

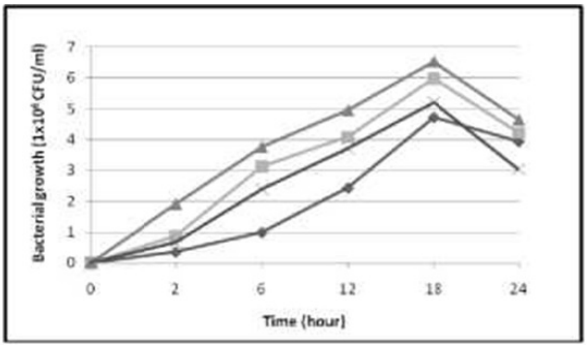

Figure1. Comparison the Growth of bacteria *Bacillus sp*. TD5K (square), *Bacillus sp*. TD5B

The growth of all strains hours, and continued by stationary phase. Based on the Figurew 2, log phase of the growth showed at 2 h until 18 h. In that moment the number of colonies that formed on the agar medium increased significantly. By the addition of poultry feathers in the liquid medium, the growth of *Bacillus spp.* confirmed faster compared without the addition of poultry feathers as a substrate (Figure. 2). There are differences in the growth profiles of the isolated strain when growing with and without poultry feathers in liquid medium. Medium with the addition of chicken feathers substrate begins with the log phase at 0 h up to 18 h and can reach about 6 x 105 CFU/ml, while the medium without the addition of feather substrate the growth just reach 2 x 105

CFU/ml (Figure 2). This was due to the addition of feather substrate that causes isolates of *Bacillus spp.* regenerate faster against the time. After 24 h incubation, the color in a liquid medium was changes from yellow into a murky brown on medium.

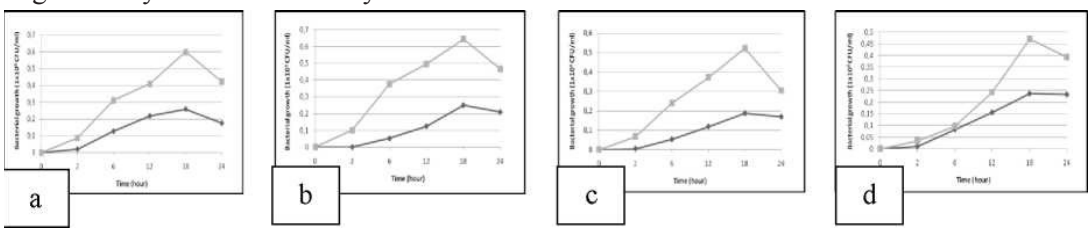

Figure 2 Comparison of bacterial growth a) *Bacillus sp.* TD5K; b) *Bacillus sp*. TD5B; c) *Bacillus sp*. LS2B; d) *Bacillus* meganterium with feathers (square) and without feathers (diamond) in the culture medium

Bacterial growth can be measured by calculation of bacteria growth in the agar medium or colony forming units (CFU). Discoloration on medium with the addition of chicken feathers a sign that chicken feathers contained in the medium hydrolyzed by isolates of *Bacillus spp*. Keratinolitik extracellular enzyme produced by each isolate *Bacillus spp.*, keratin found in chicken feathers will be hydrolyzed into peptides and amino acids that dissolve (Mazzoto *et al.*, 2011).

Feather substrate degradation by *Bacillus spp.*

Results of the feather degradation by *Bacillus spp.* showed the different in the degradation time. *Bacillus sp.* TD5B showed degraded the feathers at about 65 hours, it was more quickly in degrading from *Bacillus sp*. TD5K that completely degraded the feathers at about 68 hours. The substrate degradation by *Bacillus sp.* LS2B was performed at about 71 hours, and *Bacillus meganterium* need about 72 hours to completely degraded the feathers.

In addition of poultry feathers, which completely degraded by the keratinase enzyme, was also indicated by the changed of the color in a liquid medium. It was suggested that the murky yellow which appear in the medium as the result of hydrolysis process of proteins into peptides and amino acids. It was totally different in color medium at 0 hour which appears as clear yellow (Figure 3).

Bacillus spp. both can multiply and produce a keratinase enzyme in medium supplemented with chicken feathers, because the feathers are one of the extra nutrients for bacterial cells of as a source of carbon and nitrogen. Carbon and nitrogen are needed by cells of *Bacillus spp.* to produce more keratinase enzyme that can break down keratin contained in chicken feathers (Ali *et al.*, 2011). Keratinase will be produced in large quantities when there is a keratin substrate in the medium (Gupta and Ramnani, 2006).

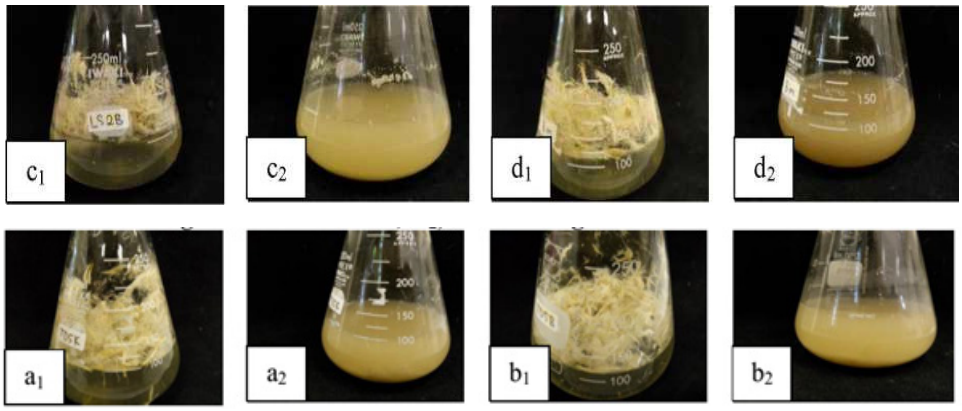

Figure 3. Degradation of chicken feathers by 4 *Bacillus strain.* a_1) *Bacillus sp*. TD5K at 0 hours; a_2) *Bacillus sp.* TD5K at 68 hours; b_1) *Bacillus sp.* TD5B at 0 hours; b_2) *Bacillus sp.* TD5B at 65 hours; c_1) *Bacillus sp.* LS2B at 0 hours; c_2) *Bacillus sp.* LS2B at 71 hours; d_1) *Bacillus* meganterium at 0 hours; d_2) *Bacillus* meganterium at 72 hours

Concentration of soluble protein by *Bacillus spp.* keratinase

Investigation of feather digested protein by keratinase from all strains was performed in submerge fermentation in liquid meat extract medium containing poultry feathers. The digested protein in the medium from feathers suggested as the action of keratinase activity against feathers keratin. The result was shown in Figure 4.

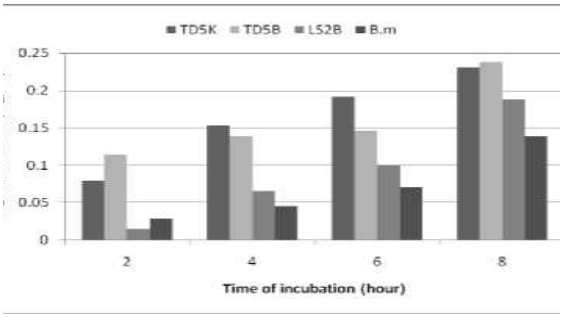

Figure 4. Graph of enzyme keratinase production by *Bacillus sp*. TD5K, *Bacillus sp*. TD5B, *Bacillus sp.* LS2B, and *Bacillus meganterium*

The data were then analyzed using split plot design. Based on the results, the different types of *Bacillus* strain effect on concentration of soluble protein (mg/ml) degraded from feather by keratinase enzyme. Furthermore, it has showed significant interaction between the substrate and the addition of different types of *Bacillus* strain. It is stated that the addition of the substrate treatment factors significantly influence the concentration of soluble protein by the strains. *Bacillus* meganterium was significantly different ($P>0.05$) with *Bacillus sp*. TD5K, *Bacillus sp*. TD5B, and *Bacillus sp*. LS2B. The difference in incubation time effect on concentration of soluble protein (mg/ml), and showed significant interaction between the addition of the substrate and the difference in incubation time. It is stated that the addition of the substrate treatment factors significantly influence the concentration of enzyme keratinase produced by the strains. The incubation time of 2 hours, incubation time of 4 hours, 6 hours of incubation time and incubation time of 8 hours was significantly different ($P<0.05$). The enzymes can be produced by making more cultures of bacterial isolates. The feather substrate suggested to be a carbon and nitrogen sources for the living of the cells. This indicates that the isolates of *Bacillus spp.* affect the concentration of enzyme produced associated with log phase in the growth phase of each strain. The increasing of incubation times, resulted in the acceleration of microbial activity and the number of microbes (Ali et al., 2011).

CONCLUSIONS

In conclusion, *Bacillus spp.* can be produced keratinase enzyme. Bacterial growth and the ability of degradation was found on *Bacillus sp.* TD5B. Isolates and incubation time work on concentration soluble protein of feathers.

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