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Isolation and Characterization of Collagenase from *Bacillus subtilis* (Ehrenberg, 1835); ATCC 6633 for Degrading Fish Skin Collagen Waste from Cirata Reservoir, Indonesia

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Abstract

The objective of this research was to isolate and characterize collagenase from *Bacillus subtilis* ATCC 6633 collection of Microbiology Laboratory, Department Pharmacy Biology, Faculty Pharmacy Padjadjaran University. The substrate collagen derived of Tilapia fish skin waste from Cirata Reservoar which has'nt exploited fully yet. The experimental design used and the data analysed descriptively. Collagen as substrate from Tilapia skin waste had extracted by Yuniarti (2010) method in Luria Broth media. The production time of collagenase used Rahmayanti (2014) methods which incubated the isolate for 24 hours and the OD of absorbances from 0.2 to 0.8 evaluated. The effect of temperature on collagenase activity evaluated by temperature from 20 to 70^{o}C. The effect of pH collagenase activity evaluated pH from 5 to 10. The conclusion of the research that *B. subtilis* ATCC 6633 has colagenolitik activity showed by the clear zone in the Luria media. The optimum production time of collagenase was 24 h of incubation. Collagenase activity reached the optimum temperature was 50 ° C (1,298 Unit mL^{-1}), while the pH optimum collagenase obtained in the range of 7-9 (from 1.298 Unit mL^{-1} to 1,321 U mL^{-1}.

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Keywords: Bacillus subtilis (Ehrenberg, 1835); Cirata; collagenase; reservoir.

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1. Introduction

Enzymatic hydrolisis are commonly used to increase nutritional and functional properties from food protein (Zu et al., 2006). Fish protein hydrolisate has been known to have antioxidative, antihypertension, antimicrobial and immunomodulatory properties (Fujita and Yoshikawa, 1999; Shahidi et al., 1995). Protein hydrolisate antioxidative properties specifically has been a major topic which attract attention from pharmacy, food and health field (Alasalvar et al., 2002; Hagen and Sandes, 2004). Protein hydrolisate with fish as its raw material which showed antioxidant activity were Alaska Pollack skin gelatin (Buusan et al., 2008); yellowfin sole (Jun et al., 2004), Allaska Pollack, tuna backbone, yellow stripe trevally (Je et al., 2005), round scad (Thuansiakul et al., 2007), gelatin hydrolisate (Khantaphant and Benjakul, 2008).

Collagen manufactures main source up until now are derrived from cow's and pork's skin and bones. However, since the spreading of mad cow disease, the consumers of cow's collagen have grow worry, and other than that the consumption of pork's collagen have been banned in some areas due to religion reason. Therefore fish waste such as bones, scales and skin which contain many collagen are now becoming safer alternative choice.

Collagen in industry is produced thermochemically with strong alkali and high temperature. The product from this process has not fully satisfying due to varying collagen quality produced. Further than that, thermochemical process requires energy in great amount to produce and maintain high temperature and also it produces waste and by product in form of high concentration alkali which could have the potential to become toxic to environment.

As an alternative, collagen hydrolisis is able to accomplish enzymatically using collagenase enzym from microbial sources. Protein hydrolisate produced by this enzymatic process is expected to be more controlable, more efficient, specific and enviromental friendly. Several bacterial isolates which produce collagenase are *Clostridium perfringens* and *Clostridium histolica*, *Bacillus subtilis* FS-2 (Nagano, 1999), *Bacillus subtilis* CN (Tran and Nagano, 2002), *Bacillus subtilis* AS1.398, *(*Rui et al., 2009), *Bacillus pumilus* Co-J (Wu et al., 2010) , *Bacillus cereus (*Liu et al., 2010) and *Streptomyces* sp. Strain 3B (Petrova, 2006). The bacteria which have the potential as collagenase's source are *Bacillus subtilis* which isolated from Rambatan river, Indramayu, Indonesia. This isolate was the collection of Laboratorium of Aquatic Biotechnology Faculty of Fisheries and Marine Science Padjadjaran University collection.

Considering the importance of all of those things describes, collagen hydrolisate production from fish waste source enzymatically as an effort to handle enviromental problem and to increase economicalvalue is important to perform. Technological fish waste (skin, bone and scale) enzymatically from local isolates are expected to increase its selling value. Through this research, isolation and characterization of collagenase from *Bacillus subtilis* ATCC 6633 (especilly to evaluate the effect of temperature and pH on collagenase activity) has been conducted.

2. Materials and methods

The main research location for samples producing and testing were at Fishery Product Processing Laboratory, Aquatic Biotechnology Laboratorium and Biochemical Laboratory Padjadjaran University. The main materials used in this research were: collagen from Tilapia fish skin, *B. subtilis* ATCC 6633 and various chemical reagents: 100 mL acetc acid 1.5 %, 20 g Luria Agar, 20 g Luria Broth, 10 mL buffer fosfat, 10 mL leusin 5 mM, 15 mL TCA 0.5 %, 15 mL ninhidrin 0.1 %; 30 ml iso-propanol 50 %, 100 mg C*omassie Briliant Blue* (CBB) G-250, 50 mL ethanol 50 %, 100 mL asam fosfat 85 %, 100 mg BSA, aquadest, spirtus, alcohol, aluminium foil, plastic wrap, filter paper and cotton.

The main equipments used: spectrophotometer Genesis 10 UV, cold centrifuge Sigma, incubator VWR Scientific, homogenizer, vorex Digisystem. The experimental methods used and the data analysed descriptively. Collagen as substrate from Tilapia skin waste had extracted by Yuniarti (2010) method in Luria Broth media. The production time of collagenase used Rahmayanti (2014) methods which incubated the isolate for 24 h and the OD of absorbances from 0.2 to 0.8 evaluated. The effect of temperature on collagenase activity evaluated by temperature from 20 ^{o}C to 70 ^{o}C.