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Isolation and Characterization of Collagenase from *Bacillus thuringiensis* for Degrading Fish Skin Collagen of Cirata Reservoir Waste

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Abstract

The objective of this research was to isolate and characterize collagenase of *Bacillus thuringiensis* obtained from the collection of Aquatic Biotechnology Laboratory, Faculty of Fisheries and Marine Science, Padjadjaran University. The substrate of collagen was derived from skin Tilapia waste of Cirata Reservoar. This study showed the presence of clear zone which is a sign of colagenolitik activity of *B. thuringiensis*. The optimum production time of collagenase was 24 hours of incubation. Collagenase of crude extract had collagenase activity of 0.181 units/ml with the protein concentration was 0.640 mg/ml. It was also found that the optimum temperature of collagenase derived from crude extract was 50°C and the optimum pH was 7-9.

Keywords: collagenase; *Bacillus thuringiensis*; skin, waste.

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1. Introduction

Enzymatic hydrolisis are commonly used to increase nutritional and functional properties from food protein [1]. Fish protein hydrolisate has been known to have antioxidative, antihypertension, antimicrobial and immunomodulatory properties. Antioxidative properties of protein hydrolisate have been a major topic which attract attention from pharmacy, food and health field. Protein hydrolisates from fish which show antioxidant activity include Alaska Pollack skin gelatin [2], yellowfin sole [3] and Allaska Pollack [4].

Main source of collagen production until now are cow's and pork's skin and bones. However, the spread of mad cow disease becomes a concern for the consumers of cow's collagen. In addition, 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 a safer alternative.

Collagen is generally produced thermochemically with strong alkali and high temperatures. The product from this process has not fully satisfied due to varying collagen quality produced. Furthermore, thermochemical process requires energy in high amount to produce and maintain high temperature. It also produces waste and by product in form of high concentration of alkali which has a potential to be toxic to the environment. As an alternative, collagen hydrolisis can be done enzymatically using collagenase enzym from microbial source. 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 *Bacillus subtilis FS-2* [5], *Bacillus licheniformis* F11.4 from shrimp shell waste [6], and *Bacillus pumilus COI-J* [7]. The bacteria which have the potential as collagenase's source are *Bacillus thuringiensis* which isolated from Rambatan river, Indramayu This isolate have been a collection of Laboratorium of Aquatic Biotechnology, Faculty of Fisheries and Marine Science, Padjadjaran University collection.

Considering its importance described above and also as and effort to handle environmental problem, enzymatic collagen hydrolisate production from fish waste source is important to be studied. The use of enzymatic technology to manage fish waste (skin, bone and scale) are expected to increase its selling value. This research was expected to find a new collagen hydrolisate product which has antioxidant value for food and nutraceutical application.

2. Materials and Method

The study was conducted at Fisheries Product Processing Laboratory, Aquatic Biotechnology Laboratory and Biochemical Laboratory Padjadjaran University. The main materials used in this research were collagen from skin of Tilapia fish, *B. thuringiensis* isolate and various chemical reagents which were used to produce and measure collagenase activity (tryptone, *yeast extract, HCl*, BSA standard). The main equipments used in this research were spectrophotometer, cold centrifuge, incubator, homogenizer. The research stages comprise of isolating collagenase from *B. thuringiensis* isolate (Figure 1), collagenolitic test, and characterization of collagenase of *B. thuringiensis* in different environment (temperature, optimum pH).

3. Results and Discussion

3.1. Extraction of collagen to a substrate on the collagenolitik test

Extraction is the proces of separating a substance based on the difference in its solubility in solvent such as water and other organic solvents. Enzyme extraction method

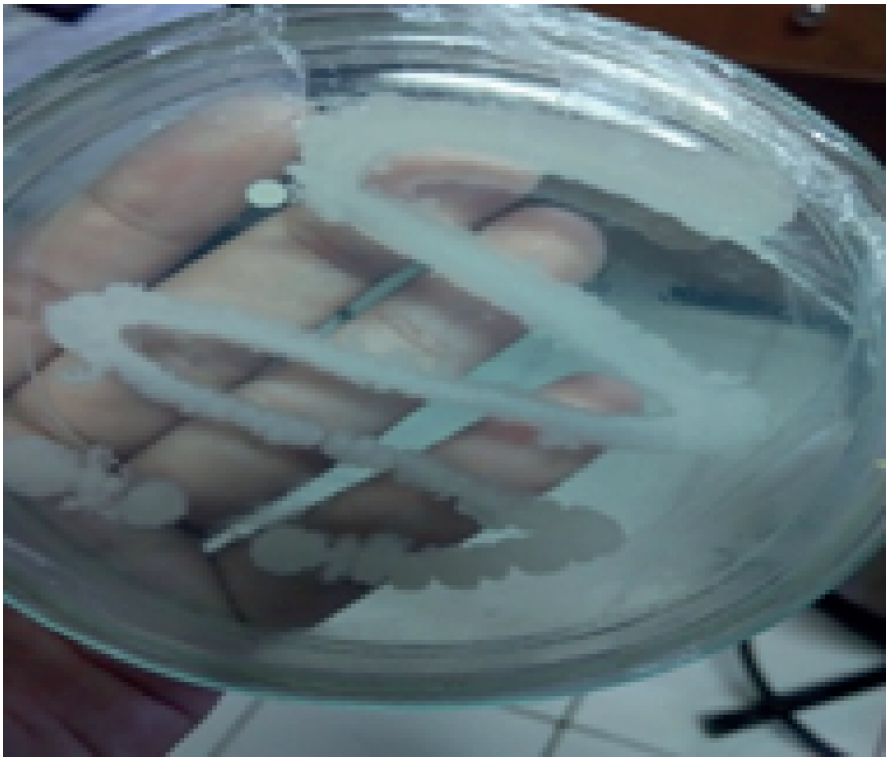

Figure 1: Clear zone of *Bacillus thuringiensis* collagenase.

considers several aspects including the source of enzyme, type, nature and form of extracts or preparations desired. Animal enzymes are generally located in specific organs such as the digestive organs and tissues. Enzyme extraction of organs was done by separating the crude enzymes of fat and controlling the temperature so that there was no protein denaturation of enzymes [8].

3.2. Optimum Temperature

The enzyme had maximum activity at certain temperatures. The activity increases with increasing temperature until it reaches the optimum temperature. Once the temperature rises further, it will lead to decreased activity. The optimum temperature to obtain collagenase from the crude extract and the results of precipitation was 50 °C. Collagenase of crude extracts had an activity of 0.151 units / ml at a temperature of 20 °C. Its activity increased to a temperature of 50 °C. The maximum reaction speed requires an optimum temperature. Above the optimum temperature, the reaction rate decreased sharply, mainly due to denaturation by heat.

Table 1: Catalytic properties of collagenase from various sources.

Sources	pH optimum	Temperature optimum °C	Inhibitor	Metal ion		Substrate spesificity	Molecul Weight	Type collagenase
				Inhibitor	Activator		kDa	
Invicera (*Scomber japanicus*) [10]	7.5	55	PMSF, TLCK, Soybean trypsin inhibitor	Hg^{2+}, Zn^{2+}		collagen type I	14.8	serine
Invicera filefish (Novoden modestrus) [11]	7.0-8.0	55	TLCK	Zn^{2+}, Cd^{2+}, Cu^{2+} Ni^{2+}	K^{+}, Li^{+}, Ba^{2+}, Ca^{2+}, Mg^{2+}	-	27.0	serin
Shrimp hepatopancreas (*Pandalus eous*) [12]	7.5-8.5	40-45	PMSF, antipain	-	-	collagen type I	22.0-23.0	serin
Tuna *(Thunnus thynnus)* pyloric caeca [13]	7.5	55	PMSF, TLCK, Soybean trypsin inhibitor	Hg^{2+}, Zn^{2+}	-	collagen type I	15.0	serin
Rainbow trout Oncorhynchus-mykiss tail (RTT) [14]		20	1,10-phenanthroline, cysteine ± zinc			collagen type I	29, 27, 26	metallo
Streptomyces strain 3B [15]	7.5	37	1,10-phenanthroline, EDTA	Cu^{2+}, $Zn^{2+}Hg^{2+}$, Fe^{2+}	Mg^{2+}, Ca^{2+}, Ba^{2+}	gelatine and collagen type I	116, 97	metallo
Bacillus subtillus FS-2 [5]	9	50	EDTA, Soybean tripsin inhibitor, iodoecetamida, iodoacetic acid		Ca^{2+}, Mg^{2+}, dan Zn^{2+}	gelatine	125	metallo
Clostridium perfringens [16]	7.2	42	1,10-phenanthroline		Ca^{2+}, Mg^{2+} dan Zn^{2+}		120	metallo
Photorhabdus luminescens [17]	7.0		1,10-phenanthroline and EDTA	Mn^{2+} dan Ca^{2+}	Zn^{2+}, Co^{2+}		74	metallo
Daging ikan *Pasific rockfish (Sebastes sp)* [18]	7.5-8.5	60-70	1,10-phenanthroline and EDTA		Ca^{2+}	Lingcod skin type I collagen, Gelatine	47, 95	metallo

3.3. Characterization of Collagenase

The main factors that affect the activity of the enzyme is the enzyme concentration, substrate, product, activator inhibitor compounds, pH and type of solvent contained in the environment, ionic strength and temperature [8]. Characterization of collagenase was conducted to determine the catalytic properties of the enzyme so that it can be an optimum condition for enzyme activity. Collagenase from various sources has differemt catalytic properties. The difference is caused by factors such as species, age, type of food, water quality, and ambient temperature. Differences in the catalytic properties of enzymes are also found in the same species caused by interspecies factors. These factors include age, size sex, spawning phase, spawning history, the composition of the food, a history of stress and others [9]. Differences in the catalytic properties of collagenase from various sources, including microorganisms are presented in Table 1.

4. Conclusion

This research showed a clear zone in the test which indicates the presence of colagenolitik activity of *B. thuringiensis*. The optimum production time of collagenase was 24 hours of incubation. Collagenase of crude extract had collagenase activity of 0.181 units/ml with the protein concentration was 0.640 mg/ml. It was also found that the optimum temperature of collagenase derived from crude extract was 50°C and the optimum pH was 7-9.

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