ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2016; 4(5): 123-126

www.fisheriesjournal.com
Received: 19-07-2016
Accepted: 20-08-2016

Nadia Sekar Ayuningtyas
Faculty of Fishery and Marine Sciences Universitas Padjadjaran Jl. Raya Bandung-Sumedang km. Jatinangor West Java, Indonesia

Junianto
Faculty of Fishery and Marine Sciences Universitas Padjadjaran Jl. Raya Bandung-Sumedang km. Jatinangor West Java, Indonesia

Emma Rochima
Faculty of Fishery and Marine Sciences Universitas Padjadjaran Jl. Raya Bandung-Sumedang km. Jatinangor West Java, Indonesia

Correspondence
Nadia Sekar Ayuningtyas
Faculty of Fishery and Marine Sciences Universitas Padjadjaran Jl. Raya Bandung-Sumedang km.21 Jatinangor West Java, Indonesia

Water soluble collagen of *Oreochromis niloticus* skin as substrate for collagenase produced by *Bacillus subtilis* ATCC 6633

Nadia Sekar Ayuningtyas, Junianto and Emma Rochima

Abstract
Collagen is the major insoluble fibrous protein in the extra cellular matrix and connective tissues. It has a wide range of application in cosmetics, biomedical, pharmaceutical, leather and food industries. Collagen can be extracted from fish scales and skin by enzymatic digestion methods. The aim of this research was to study the water soluble collagen isolated from *Oreochromis niloticus* skin with collagenase produced by *Bacillus subtilis* ATCC 6633 (a collection of Microbiology Laboratory, Faculty of Pharmacy, Universitas Padjadjaran, Indonesia). *Oreochromis niloticus* was selected as collagen source due to its high content of collagen. Results showed that water soluble collagen (WSC) substrate isolated from *Oreochromis niloticus* skin contained 1.978 mg/mL of protein, whilst collagenase extracted from *Bacillus subtilis* ATCC 6633 contained 0.326 mg/mL of protein. Furthermore, the highest collagenolytic activity was obtained at 50 °C (1.298 U/ml) and pH 8 (1.696 U/ml). Water soluble collagen of *Oreochromis niloticus* skin as substrate for collagenase extracted from *Bacillus subtilis* ATCC 6633 positively exerted collagenolytic activity. It can be concluded that the optimum condition for collagenolytic activity of the enzyme is at temperature 50 ºC and pH 8.

Keywords: Collagenolytic activity, eukaryotic enzyme, freshwater fish, Nile tilapia, tilapia

1. Introduction
Collagen is the major insoluble fibrous protein in the extra cellular matrix and connective tissues. It has a wide range of application in cosmetics, biomedical, pharmaceutical, leather and food industries [1], whilst collagenases are active proteinases that degrade collagen and proteoglycan. These enzymes are necessary to initiate collagen turnover in connective tissue at both normal and abnormal conditions [2].
Collagen could be extracted from fish scales and skin by enzymatic digestion methods [3-5]. *Oreochromis niloticus* was selected as collagen source due to its high content of collagen [6]. The work of Sujithra (2013) concluded that the fish wastes contained 22% of acid solubilized collagen (ASC) based on the lyophilized dry weight and 60% of pepsin solubilized collagen (PSC) on the same basis [7], while Potaros and colleagues calculated the dry weight yields of ASC using Noitup Method and Ogawa Method, were 38.84 and 20.70%, respectively. The same two methods were used to calculate the dry weight yields of PSC, and resulted 48.21 and 38.27%, respectively [4].
Baehaki and colleagues (2012) who studied about extracellular collagenase produced by *Bacillus licheniformis* F11.4, concluded that the enzyme could be purified using ammonium sulfate and DEAE Sephadex A-50 at 50 °C pH 7. According to their work, there were metal ions that decreased collagenase activity, e.g. Fe^{2+} (1 mM), Mg^{2+} (1 mM), Mn^{2+} (1 mM), Co^{2+} (1 mM), EDTA (1 mM), and β-mercaptoethanol (1 mM), whilst Ca^{2+} (1 mM) and Cu^{2+} (1 mM) were proven could enhance the enzyme's activity [8].

2. Materials and Methods
2.1 Materials
Materials used were pure strain of *Bacillus subtilis* ATCC 6633 (a collection of Microbiology Laboratory, Faculty of Pharmacy, Universitas Padjadjaran, West Java, Indonesia), 30 g of freshly prepared *Oreochromis niloticus* skin, 1.5% acetic acid solution, 20 g of Luria Agar (contains tryptone, yeast extract, sodium chloride and agar), 20 g of Luria Broth (microbial