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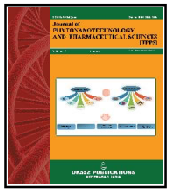

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Cost-effective production of collagenase from *Chryseobacterium contaminans* KU665299

Shikha Chauhan, Priyanka and Wamik Azmi◆

Department of Biotechnology, Himachal Pradesh University, Summer Hill, Shimla-171005 H.P., India

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Abstract

Collagenase is an endopeptidase responsible for the hydrolysis of native collagen into peptide fragments. Microbial collagenases are truly promising enzymes in relation to their extensive biological and industrial applications. Collagen being the most abundant constituent of extracellular matrix (ECM) in vertebrates opens a wide array of biotechnological and therapeutic applications for microbial collagenases. In the present study, a cost-effective and eco-friendly production of extracellular collagenase from novel, non-pathogenic, *Chryseobacterium contaminans* KU665299 was carried out utilizing slaughter house waste material as sole source. The complete digestion of waste material resulted in good yield of collagenase utilizing goat skin and auricular cartilage.

1. Introduction

Proteases render substantial variety of functions and have significant biotechnological applications. Collagenase is a commercially important proteolytic enzyme that carries out the hydrolysis of substrate collagen, most widely distributed class of proteins in vertebrates (Chauhan *et al*., 2017; Yang *et al*., 2017; Berkova *et al*., 2018; Hoppe *et al*., 2021). Collagen is an extensive fibrous constituent of extracellular connective tissue such as skin, bones, cartilage, tendons, blood vessels and teeth found in all multicellular organisms (Kate and Pethe, 2015). The enzymatic digestion of collagen results in the production of collagen peptides. Hydrolyzed collagen is produced from collagen found in the bones, skin and connective tissue of animals such as cattle, fish, horses, pigs and rabbits. Bacterial collagenases are metalloproteinases that are involved in the degeneration of extracellular matrices of animal tissues, due to their competency to digest native collagen (Duarte *et al*., 2016). There is a wide range of industrial applications of collagenase including food (Zhao *et al*., 2012; Pal and Suresh, 2016), cosmetic (Demina, 2009) tannery and meat industries (Dettmer *et al*., 2011; Duarte *et al*., 2016; Pal and Suresh, 2016). Besides industrial applications, collagenase has been indispensible for medical purposes including isolation of pancreatic islets for diabetes treatment (Berkova *et al*., 2018). Collagenases have various non-invasive therapeutic applications such as treatment of Dupuytren's (Degreef, 2016; Verstreken *et al*., 2016) and Peyronie's disease (Traore *et al*., 2016), burns (Rashaan *et al*., 2014; Sharp *et al*., 2014), wound healing (Das *et al*., 2018), intervertebral disc herniation, chronic total occlusions, glaucoma, cartilage repair, uterine fibroid, cellulite, keloid, nipple pain and degradation of human retained placenta (Kaur and Azmi, 2013a; Alipour *et al*., 2016; Chauhan *et al*., 2017) and cancer gene therapy (Cemazar *et al*., 2012; Kato *et al*., 2012).

The enduring drive to boost meat production for the protein needs of invariably increasing world population has latched onto some pollution problems. Meat industry is one of the largest producers of organic waste in the food processing sector. Slaughter house waste comprises of the inedible parts of animals elicited from production of meat and other animal by-products (Franke-Whittle and Insam, 2013). These materials have potential value as protein and natural products source but are only used minimally. Collagenases are important virulence factors which play a crucial role in the global degradation of the extracellular matrices of animals, due to their collagen degradation ability (Pal and Suresh, 2016). Several pathogenic microorganisms are reported to produce collagenase principally *Clostridium histolyticum*, which is the extensively used commercial source (Daboor *et al*., 2010; Wanderley *et al*., 2017; Berkova *et al*., 2018). Pathogenicity restricts the use of microorganism for bioprocess development (Kaur and Azmi, 2013b; Chauhan and Azmi, 2017) and also raise the cost of an enzyme production. Moreover, their toxin producing ability limits the use of their collagenases (Bhagwat *et al*., 2015). So, there is necessity of alternative non-pathogenic microbial sources for collagenase production in a cost-effective manner. Thus, the collagenase production from *C. contaminans* by the use of wastes of slaughter house as raw material was objectified in the present study.

2. Materials and Methods

2.1 Microorganism and culture conditions

Chryseobacterium contaminans KU665299 (Figure 1) used in this study, has been isolated from soil samples of local meat market

Corresponding author: Dr. Wamik Azmi

Department of Biotechnology, Himachal Pradesh University, Summer Hill, Shimla-171005, H.P., India

E-mail: wamikazmi@rediffmail.com

Tel.: +91-1772831948

(Chauhan and Azmi, 2017). The bacterial cells were grown in selective medium (pH 6.5) containing (%, w/v) peptone 1.0, Na_2HPO_4 0.2; Na_2CO_3 0.25 and gelatin 0.3 at 30°C and 150 rpm in a temperature controlled orbital shaker.

Figure 1: Culture of *Chryseobacterium contaminans* KU665299.

2.2 Collagenase assay

Enzyme activity was measured by determining the extent of collagen/ gelatin (substrate) breakdown using Rosen's modified colorimetric ninhydrin method (Rosen, 1957). Amino acids liberated were expressed as micromoles of L-leucine released in one minute under standard assay conditions. Tris-HCl buffer 250 µl (0.1M, pH 7.5), crude enzyme 50 µl and 300 µl of 0.3% (w/v) substrate (gelatin prepared in 0.1M Tris-HCl buffer, pH 7.5) were incubated at 37°C for 30 min. The reaction was stopped by addition of 600 µl chilled TCA (50%, w/v). A set of control was also run. Then, 200 µl of the reaction mixture was withdrawn and amount of L-leucine released was measured by ninhydrin method. One unit (U) of enzyme activity has been defined as the amount of enzyme required for the release of 1µmole of L-leucine per minute as per assay conditions.

2.3 Pre-treatment of waste

The waste skin and cartilage tissue (mainly auricular cartilage) were collected from slaughter house. They were washed and surface sterilized using 70% (v/v) ethanol. The skin and cartilage tissues were dehaired and chopped into small pieces (1.0 g each). Chopped skin and cartilage tissue (3.0 g) were put in 250 ml Erlenmeyer flasks individually containing 50 ml distilled water and were autoclaved.

2.4 Cultivation of *C. contaminans* for production of collagenase utilizing waste material

The preculture of *C. contaminans* (5%, v/v) of 24 h age grown in medium (pH 6.5) containing (%, w/v) peptone 0.5, gelatin 0.3, Na_2HPO_4 0.2 and Na_2CO_3 0.25 at 30°C was used to inoculate the flasks containing the waste material. The cell growth and collagenase production by *C. contaminans* with the hydrolysis of these waste products were monitored for 30 h under the optimized conditions. A set of control was also kept to check the autolysis of the waste material under the same conditions.

3. Results and Discussion

Collagenases are truly vital enzymes in relation to their considerable therapeutic and industrial applications in pharmaceutics (Alipour *et al*., 2016; Chauhan *et al*., 2017), food and meat industries (Pal and Suresh, 2016; Duarte *et al*., 2016), leather industries (Dettmer *et al*., 2011) and bioremediation processes (Najafi *et al*., 2005). Therefore, it is requisite to produce these proteases in adequate amounts. This leads to the development of optimization approach for better production of enzymes.

3.1 Production of collagenase utilizing waste goat skin

Collagen (Type I) is the major constituent of skin, tendon, vascular ligature, organs and bone. The culture of *C. contaminans* grew well while utilizing waste goat skin and maximum cell mass 1.80 ± 0.09 mg/ml with 467 x 10^9 CFU was achieved at 27 h of incubation (Fig. 2). Although, the entire skin was utilized within 21 h but the maximum collagenase production (3.32 ± 0.04 U/ml) was exhibited at 24 h of incubation. The digestion of waste goat skin by *C. contaminans* has been shown in Figure 2 and 3. Likewise, Kaur and Azmi (2013) utilized the waste animal skin for cost effective production of collagenase from *B. tequilensis* and waste management.

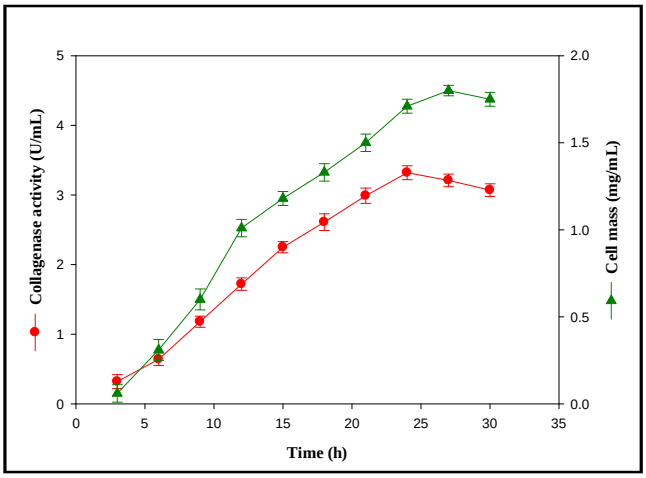

Figure 2: Growth profile and collagenase activity of *C. contaminans* by utilizing waste goat skin.

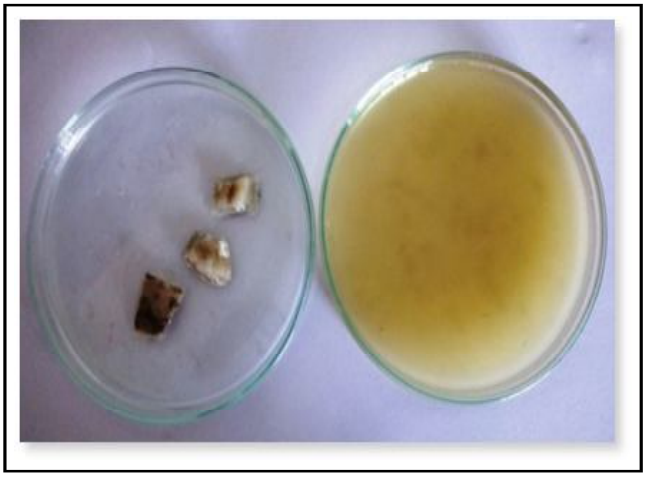

Figure 3: Digestion of waste goat skin by collagenase of *C. contaminans*.

3.2 Production of collagenase utilizing waste auricular cartilage

Cartilage is a flexible connective tissue found in many parts of the bodies of humans and other animals, including the joints between bones, rib cage, ears, nose, elbow, knee, ankle, bronchial tubes and the intervertebral discs. The complete digestion of the cartilage was observed within 18 h of cultivation. It resulted in a good yield of collagenase production (3.83 ± 0.04 U/ml) within 24 h of incubation with *C. contaminans* and maximum cell mass (1.84 ± 0.10 mg/ml) with 501 x 10^9 CFU were also attained at 24 h (Figure 4). The digestion of auricular cartilage tissue by collagenase of *C. contaminans* has been shown in Figure 5.

The culture of *C. contaminans* grew well utilizing waste goat skin and auricular cartilage. The complete digestion of waste material occurred and resulted in good yield of collagenase utilizing goat skin and auricular cartilage. Hence, cost effective production of collagenase by *C. contaminans* was achieved by utilizing wastes of slaughter house as the sole carbon and nitrogen source.

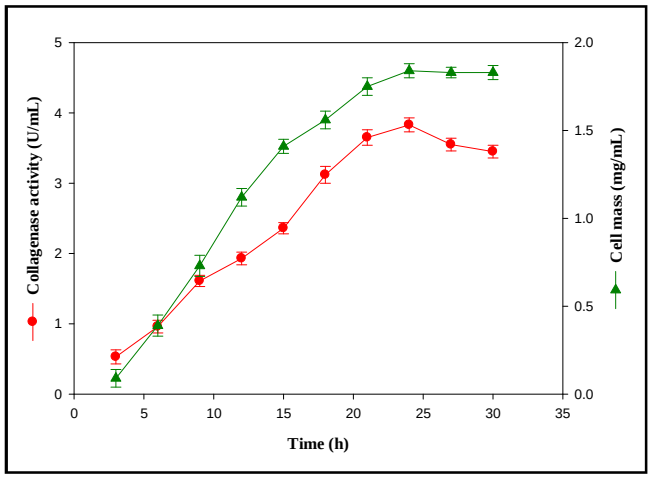

Figure 4: Growth profile and collagenase activity of *C. contaminans* by utilizing waste auricular cartilage tissue of goat.

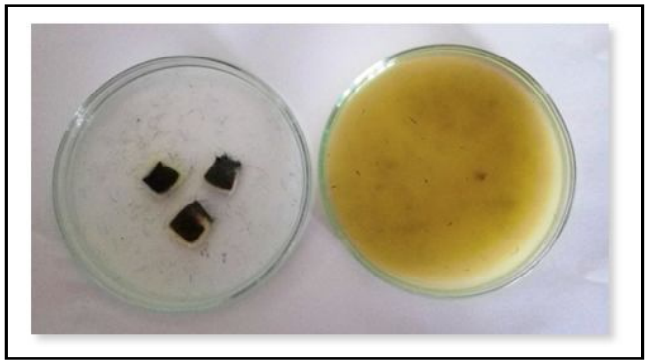

Figure 5: Digestion of auricular cartilage tissue by collagenase of *C. contaminans*.

4. Conclusion

Collagenase has potential for use as promising biotechnological product in pharmaceutical, cosmetics, food and detergent industry. The utilization of slaughter house wastes as sole carbon and nitrogen source for collagenase production by *C. contaminans* solved two purposes; cost-effective production of an important enzyme and management of waste. Thus, a novel technology harnessing collagenases could be developed for the bioremediation of slaughter house or related industrial wastes.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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