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Keratinase a Feather Degrading Enzyme Useful for Mosquito Control

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Introduction

'Keratin' is the major structural protein present in animal feathers, hair, wool, horn and skin. Feather includes above 90% of crude protein in the form of keratin[1]. The mechanical strength of the keratin by the tight packaging of proteins in α-helix or β-sheet (α-keratin or β-keratin respectively) structures and their high level of cross-linkages by hydrogen and disulfide bonds[2]. Feather waste represents a potential protein alternative to more expensive dietary ingredients for animal feed[3]. But some keratinase producing microorganisms are converted native keratin to smaller molecular entities and consequently that can utilizes nutrient sources for the growth of bacteria[4]. In recent years, there have been several reports on the purification of Keratinase from diverse microorganisms[5]. Therefore, there is urgent requirement to find out novel enzyme which is having the ability to degrade keratin containing substrate from various sources such as poultry form and leather industries and develop enzyme purification methods. It is vital role to improve the non-polluting practice[6]. Keratinase properties based on the producers. It is generally a serine protease[7]. Occasionally, keratinase has been found to be a serine protease with a cysteine protease and a metallo protease[8]. Its optimum temperatures and pH values were reported to be 40 – 80°C and 6 – 10, respectively[7].

Bacillus thuringiensis serovar *israelensis* (*Bti*) a highly toxic bio-pesticide to dipteran larvae, opened up the probability of the use of these bio-larvicides in mosquito eradication programmes[9]. The major advantage of *Bti* over other mosquitocidal bacteria is that the *Bti* produce multiple intracellular crystal (134, 125, 67, and 27 kilo Daltons) inclusions in sporulation[10]. The various waste materials (industrial waste and agricultural derivatives) have used effectively as a substitute to commercial culture media for producing mosquitocidal bacteria (*Bs* and *Bti*) to utilize vector control practice. The present research in our laboratory is effectively used chicken feather waste from poultry forms to produce bacterial (*B. thuringiensis* subsp *israelensis*) culture for bio-pesticide[11]. The purification and characterization of the enzyme, produced by Bti, responsible for the biodegradation of feather waste is reported[12].

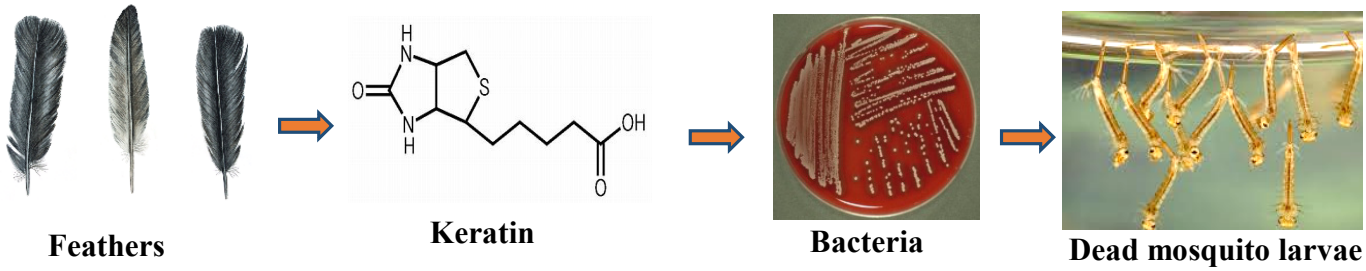
For the first time, the enzyme was purified and characterized by examined exploitation of Keratinase industrially in hydrolysis, the keratin substrate by using the *Bti* to produce the mosquitocidal toxins. Thus, the earlier report showing some *Bacillus* strains produced keratinolytic enzyme[8]. However, there is no report existing as on date on keratinolytic action of *Bti* to degrade keratin from feathers as functional unit. Most microbial keratinases are inducible enzymes[13]. Various keratinous materials such as chicken feather, feather, wool, meal and bovine hair have been employed as inducers of keratinases[14]. In the present study, we have purified the keratinase and it was similar to purification made from *Bacillus sp*[15]. The current investigation was successfully explored purification of Keratinase enzyme by the presence of distinct band on the SDS-PAGE which showed approximately 40 kD and results contemporaneous similar with previous reports were displayed by Keratinase from *B. licheniformis* FK14 (35 kDa), *Streptomyces Pactum* DSM 40530 and *Doratomyces microsporus* (30 – 33 kDa)[16]. However, keratinase with wide molecular mass range (18, 40 and 130 kDa were reported from *Streptomyces albidoflavus, Streptomyces thermoviolaceus* and *Fervidobacterium pennavorans* respectively[17] and *Chrysosporium keratinophilum* (69 kDa).

The examination of sequence for characterization of Keratinase in present study has showed that it is a surface layer protein, having advanced binding affinity towards keratin. Because, keratin proteins have preserved molecular structure (*α-helical*) having the property of multiple adherence activities with surface protein. Similar studies on the association between surface layer protein and keratin were reported previously from *Staphylococcus aureus* and *Streptococcus agalactiae*[18]. Hence, our results are concurrence with previous reports.

The appropriate pH of *Bti* Keratinase was found at neutral pH which was similar to earlier report by[19] for *Bacillus sp*. This is in difference from other alkaline keratinases. The enzyme was stable at the range of 6 - 8[8,13] *Bacillus sp*., FK 46 at 37 °C[20], *B. licheniformis* PWD-1 at 50°C[21]. But in contrast to this alkaline keratinases with optimal pH 11.0 were also observed from Bacillus halodurans[15]. They are effectively active approximately pH10 with a molecular range between 10 and 30 kDa and isoelectric point around pH 9.0. This type of protease is produced by the variety of *Bacillus* species like *B. halodurans, B. subtilis* and *B. licheniformis*[22].

The concentration PMSF and EDTA can inhibit purified Keratinase activity even at 1mM, which reveals the serine type protease inhibitor. Similar examination was accounted for proteases exhibiting keratinolytic activity from *Streptomyces* and *Bacillus* species[22]. The strong inhibitory effect by EDTA indicated the importance of metal ions for activity/stability[23]. Present results indicated the magnitude of $MgCl_2$ in feather degradation which was acknowledged by the results demonstrated that Amazonian bacterium (*Bacillus sp*. P45) enhanced the feather degradation by the presence of $MgCl_2$ in feather media[23]. Similarly, protease production by a haloalkaliphilic bacterium was shown to be slightly stimulated by $MgCl_2$, but inhibited $MgCl_2$[24]. The majority of Keratinase enzymes produced from bacteria are discharged in extracellular. Enzymatic degradation of chicken feather waste required the combined effect of reducing agents, because of single agent alone cannot be feasible to improve the enzymatic activity. The admiring feather concentration for the Keratinase was decided to be 4 mg/ml. Earlier report showed that the high concentration of feather waste was the reason for substrate inhibition or suppression of fabrication[20], on the other hand, high substrate concentration might enhance the medium viscosity, which possibly results in oxygen limitation for bacterial growth[25].

The new Keratinase enzyme was isolated, purified and characterized from *B. thuringiesis* serovar *israelensis* to degrade the keratin containing waste. The outcome recommended the prospective utilization of Keratinase in industrial to hydrolyze keratin for the fabrication of bacterial toxin (134, 125, 67 and 27 kDa). This bacterium can make hydrolytic enzyme and the most favorable conditions required by the enzyme for its proteolytic action was characterized and recommended that the mosquitocidal toxin production by using poultry processing waste in throughout the world, under the favorable condition for appropriate degradation of feather wastes. Hence, this finding is a great deal with production of bio-pesticide from mosquitocidal bacteria and other hands switch off the environmental waste.



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