CHAPTER I

INTRODUCTION

1.1 Environmental significance of the feather (keratin) waste

Keratin is a major structural protein and is abundantly found in nature as hair, feather, nail, horn, hoof, scales and wool. The consumption of chicken and its products by people of modernized world is increasing day by day due to the technological advancement in food processing and preparation. Meanwhile, chicken feather waste, a byproduct of commercial poultry processing plants as well as chicken slaughter houses is accumulating at a higher rate (Brandelli et al, 2008). Feathers alone constitute nearly 8.5% of the total weight of chicken. The amount of solid waste generated in India from around 823.5 million chickens with an individual body weight of 2 kg would be approximately 140 million kg (Sarita and Wadhwa, 2010; Khardenavis et al, 2009). Management of this poultry solid waste is one of the major concerns for many nations (Lateef et al, 2010) and it results in environmental pollution and protein wastage (Schmidt and Barone, 2004). Hence there is a need for this feather waste management by ecofriendly methods.

This need calls for the clear understanding of the structure and composition of feather. Feathers are made up of β-keratin amd melanin pigments. It is also necessary to distinguish α- keratin from β-keratin in order to evolve the strategy for their management and degradation.

1.2 Keratin

Keratin belongs to a family of fibrous structural protein and it is the key material making up the outer layer of human skin and is also the key component of feather, scales, hair and nails. Keratin monomers assemble into bundles to form intermediate filaments, which are tough and insoluble and form strong un-mineralized tissues found in reptiles, birds, amphibians, and mammals (Gupta and Ramnani, 2006; Mc Kittrick et al, 2012).

1.2.1 Structure and unique biochemical properties of Keratin

Structurally, keratin protein has the following unique characteristics;

Keratin is a rigid and intact protein due to several hydrogen bonds and hydrophobic interactions. It has a sulfur content of 3 to 5 % due to the presence of
cysteine residues. This amino acid cross links the polypeptide chains of mature hair. Chicken feathers are composed of over 90% of keratin protein, small amounts of lipids and water, (Onifade et al, 1998; Yamamura et al, 2002; Riffel et al, 2003; Gupta and Ramnani, 2006).

Feathers keratin consists of high quantities of small and essential amino acid residues such as glycyl, alanyl and seryl as well as cysteinyl and valyl. Although they are protein in nature, they are not digested by commonly known proteolytic enzymes like trypsin, pepsin and papain due to their hard and recalcitrant nature. Based on their secondary conformation, keratins are further classified into α- keratins and β- keratins (Voet and Voet, 1995; Gupta and Ramnani, 2006; Mc Kittrick et al, 2012).

1.2.2 Structure of α- keratin

α- keratins occur in higher animals like mammals. The hair, nail, horn and wool of these animals are made up of α- keratin. α- keratins consists of a hierarchy of structures as illustrated in Fig.1a, b and c. A typical hair is ~20 µm in diameter and is constructed from dead cells, each of which contains packed macrofibrils (~2000 Å in diameter) that are oriented parallel to the hair fiber. The macrofibrils are constructed from microfibrils (80 Å wide) that are cemented together by an amorphous protein matrix of high sulphur content. α-keratin is rich in cysteine residues, which form disulfide bonds that cross link adjacent polypeptide chains. This accounts for α-keratin’s insolubility and resistance to stretching, two of its most important biological properties. The α-keratins are classified as “hard” or “soft” according to whether they have high or low sulfur content. Hard keratins, such as those of hair, horn, and nail, are less pliable which has a high concentration of mercaptans. The springiness of hair and wool fibers is a consequence of the coiled coil’s tendency to untwist when stretched and to recover its original conformation when the external force is relaxed. After some of its disulfide bonds have been cleaved, however, α-keratin fiber can be stretched to over twice its original length by the application of moist heat.
Fig. 1 (a) Structure of $\alpha$-keratin: (left to right): (i) space-filling ball model.21 (ii) Two keratin polypeptides form a dimeric coiled coil. (iii) Proto filaments form from two staggered rows of tail-to-head associated coiled coils. (iv) Proto filaments bimerize to form a protofibril, eight of which form an intermediate filament.22 (b) TEM micrograph of $\alpha$-keratin intermediate filament from a sheep horn. The strongly diffracting core of crystalline keratin is surrounded by an amorphous matrix.24 (c) $\beta$-Pleated sheet configuration. Hydrogen bonding holds the protein chains together. R groups extend to opposite sides of the sheet are in register on adjacent chains (Voet and Voet, 1995; Figure © Irving Geis).
1.2.3 Structure of \( \beta \)-keratin

Feather of birds and scales of reptiles are made up of \( \beta \)-keratin. Feathers are the most complex integumentary appendages of all birds. They serve a variety of functions that includes flight, camouflage, courtship, thermal insulation, and water resistance. Feathers form from follicles in the epidermis that are periodically replaced by molting. Feathers are comprised of beta keratin and melanin (which provides color).

The feather has a hierarchical construction based on a primary shaft, or rachis (as shown in Fig.2) consisting of a cortex that encloses a cellular core, composed of uniformly sized cells of \(~20 \mu m\) in diameter. The rachis supports barbs, which are secondary keratinous features that form the herring bone pattern of the vane (Fig.2&3). Similarly, the barbs support tertiary features, including barbules. The bulk of the cortex (Fig.3) is constructed of fibers that measure 6 \( \mu m \) in diameter, which are aligned predominantly along the length of the shaft. These fibers are comprised of fibrils measuring 300–500 nm in diameter. The most superficial layer (cuticle) of the cortex is distinguishable from the bulk of the cortex in that it consists of circumferentially oriented fibers.

![Fig.2. Macro structure of a feather](image-url)
1.2.4 Composition of feather keratin

The average young feather consists of the following chemical substances: moisture, 6.72%; protein, 81.46%; fat, 11.36%; fiber, 0.31% (Grazziotin et al 2006; Saha, 2012a). Nitrogen is the most abundant element present in the feather. In protein, almost all different amino acids are present in feather such as taurine 0.01%, hydroxyproline 0.23%, aspartic acid 5.33%, threonine 3.70%, serine 7.88%, glutamic acid 8.13%, proline 8.14%, lanthionine 1.65%, glycine 6.25%, alanine 3.57%, cysteine 4.99%, valine 6.28%, methionine 0.57%, isoleucine 3.79%, leucine 6.59%, tyrosine 2.33%, phenyl alanine 3.97%, hydroxylysine 0.01%, histidine 0.61%, ornithine 0.30%, lysine 1.79%, arginine 5.68% and tryptophan 0.47% (Onifade et al, 1998; Grazziotin et al, 2006). Other elements found in feathers are sulphur (2.57%), chlorine (0.53%), phosphorus, in the form of pentoxide (0.34%), silicon, in the form of silicic acid (0.22%) and calcium, as oxide (0.10%). A crystalline sulphuric amino acid called cystine can be extracted from feathers. One kind of feathers may differ slightly from another in its chemical compositions. For example the fat content of the feathers of geese and ducks is greater than that of the feathers of hens and turkeys (Saha, 2012a).
1.3 Impact of current feather waste treatment methods on environment

The management of huge quantity of feather wastes generated in the poultry industries is a major concern of many nations across the globe (Schmidt and Barone, 2004 and Lateef et al, 2010). Number of methods are available for the management of this waste. In conventional methods, feathers are cooked under high temperature and pressure to produce feather meal which is used as animal dietary ingredient. Feather meal treatment methods are usually carried out by two methods like hydrothermal treatments and microbial degradation. Among them hydrothermal method is costly and also lead to denaturation of certain essential amino acids during processing, which in turn yields a product with poor digestibility and low nutritional value (Onifade et al, 1998; Gupta and Ramnani, 2006; Gurav and Jadhav, 2012; Saha, 2012a). Hydrothermal pretreatment also includes thermo-chemical treatment methods (such as acidic hydrolysis and alkali hydrolysis), and also steam pressure cooking. Typical steps involved in hydrothermal and chemical processes are depicted in Fig.4

![Typical steps involved in protein waste (feather) conversion to feedstuffs by hydrothermal and chemical treatment methods](image)

Thermal and chemical methods usually need high temperatures or high pressure with addition of diluted acids such as hydrochloric acid or alkali such as sodium hydroxide. Acidic solutions promote the loss of some amino acids such as tryptophan. Alkaline reactions are slow and degradation of some amino acids with hydroxide is less. Moreover these methods require more time, chemicals and energy with expensive industrial equipment for processing (Gupta and Ramnani, 2006; Sarita, 2012; Saha et al, 2012a). Other
techniques for feather disposing in practice include incineration, which demands more energy for processing and land filling, which can lead to environmental pollution (Khardenavis et al, 2009; Lateef et al, 2010; Brandelli, 2010).

1.4 Limitations of hydrothermal and chemical treatment methods

The poultry feathers are either dumped, which pollute the soil or which again pollutes the air while burning. Mammoth size of discarded feather, apart from polluting the soil or air, also causes various human ailments including chlorosis, mycoplasmosis and fowl cholera (Saha, 2012a). Currently applied feather incineration process also increases emission of certain gases like CO, SO$_2$, Volatile Organic Compounds (VOCs) and Nitrogen Oxides into environment. Consequently these pollutants have been known to cause respiratory diseases, cardiovascular diseases and cancer, among other illnesses (Food & Water watch Fact sheet, 2012). Huge feather waste generated worldwide, currently a minor quantity of waste feathers is used in other industrial applications such as clothing, insulation and bedding, producing biodegradable polymers (Schmidt and Barone, 2004), enzymes and also as a medium for culturing microbes (Saha, 2012a). Therefore, the alternative use of feather waste is very much necessary to prevent the feather waste related pollution. Hence, there is a demand for developing biotechnological and eco friendly alternatives for recycling of keratin wastes.

1.5 Alternate eco friendly technology-Microbial degradation

Use of microbes capable of producing extracellular keratinases is a possible alternative and an eco-friendly method to convert this abundant waste into low cost, value added nutrient rich animal feed (Onifade et al, 1998; Riffel et al, 2003; Refai et al, 2005; Grazziotin et al, 2006; Anbu et al, 2008; Rani Gupta et al, 2012). These alternate methods include enzymatic and/or fermentation methods. These methods produce feather meal rich in rare amino acids like cysteine, serine and proline, which can find application as feed substrate. The amino acids segment has the largest share of the market at $5.4 billion in 2008, and an expected to increase to $7.8 billion in 2013. The market for industrial enzymes was the second largest segment at $3.8 billion in 2008, with an expected rise to $4.9 billion in 2013, for a Compounded Annual Growth Rate (CAGR) of 8.9% (Sarita, 2012). A higher quantity of pretreated feather is utilized to produce a digestible dietary protein feedstuff for poultry and livestock (Papadopoulos et al, 1985; Onifade et al, 1998; Brandelli et al, 2008; Brandelli, 2010). Understandably, poultry feather locks up a great deal of potentially useful protein and amino acids that could be
beneficially harnessed as animal feedstuff. This makes recycling of feather a subject of interest among animal nutritionists, because of its potential as a cheap and alternative protein feedstuff. However, limitations to feather utilization arise from its poor digestibility and low biological value and the deficiencies of nutritionally essential amino acids such as methionine, lysine, histidine and tryptophan (Papadopoulos et al, 1985; Onifade et al, 1998; Grazziotin et al, 2006; Gradisar, 2005; Saha, 2012a). Keratinolytic enzymes offer opportunities for a low energy consuming technology for bioconversion of poultry feathers from a potent pollutant to a nutritionally upgraded protein rich feedstuff for livestock. Biological degradation of feather waste is more efficient than physical and chemical degradation, resulting in more useful and toxic free byproduct. In this scenario biological feather degradation has received the consideration from scientific research community in recent days (Refai et al, 2005; Gupta and Ramnani, 2006; Brandelli, 2010; Sarita, 2012; Saha, 2012a; Fang, 2013).

Hence it is proposed to review literature on microbial feather degradation in order to understand the microbial process, available novel strains and commercial formulations. The present study is aimed at the isolation and characterization of novel keratinolytic bacteria and optimization of the physico chemical conditions for keratin degradation. It also aims at the characterization of keratinolytic enzyme and its application.