1. INTRODUCTION

Feathers are produced in large amounts as a waste by product of poultry processing plant (Sangali and Brandelli, 2000). Worldwide, around 18,500 lakh tons of poultry feathers are generated annually, of which India's contribution alone is 3500 tons. Every year, more than 20,000 tons of feathers are produced as waste by poultry farming yet, which are hydrolyzed by mechanical or chemical treatments, can be converted into feedstuffs, fertilizers, glues and foils. These are used for the production of amino acids and peptides.

Feathers are generally land filled or burnt. They reach millions of tons per year worldwide (Williams et al., 1991). Increasing quantities of feathers can lead to environmental pollution (Gradies et al., 2000). The incremental increase in poultry industry all over the world resulted in the generation of millions of tonnes of chicken feathers waste (Vasileva-Tonkova et al., 2007).

Disposal and recycling of feather waste

Disposal of feather waste is quite challenging. A current value-added use for feathers is the conversion to feather meal, a digestible dietary protein for animal feed, using physical and chemical treatments (Papadopoulos et al., 1986). These methods can destroy certain amino acids and decrease protein quality and digestibility (Moritz and Latshaw, 2001; Anbu et al., 2005). Formerly, the production of commercial feather meals demands the use of these physicochemical methods. High cost and intensive energy are two prerequisites for the completion of this process. The traditional methods for disposal of feather wastes include incineration and land filling. Nevertheless, these methods have extensive operating costs, consume energy, result in loss of natural resources and have extreme environmental implications (Onifade et al., 1998).

Recycling of feathers is a subject of interest among animal nutritionists, because of its potential as a cheap and alternative protein feedstuff. At present, the waste feathers are either disposed by incineration, which can lead to cause environmental pollutions (Deydier et al., 2005) or transformed into animal feed by harsh treatments like high temperature and pressure that results in the loss of nutritionally important amino acids like methionine, lysine, and tryptophan, and in the
formation of non-nutritive amino acids, such as lysinoalanine and lanthionine (Dalev et al., 1997). However, a more serious problem lies in the amino acid imbalance besides the formation of non-nutritive amino acids like lysinoalanine, lanthionine, etc (Wang and Parsons, 1997). This problem can be partly circumvented by steam-treating feathers to denature the keratins of feathers. After milling, a friable meal is obtained that is 70-80% digestible (Homer and Shaible, 1980).

All together, the financial bottleneck created by thermo-energetic cost of this process has necessitated the development of cost-effective biotechnological alternatives for recycling of keratin-wastes into useful products. Therefore, this exigency for developing biotechnological alternatives for recycling of keratin-wastes creates a prodigious amplitude for microbial keratinases in present day enzyme market (Gupta and Ramnani, 2006; Brandelli et al., 2010). Additionally in the role of environmental biotechnology, keratinase-mediated degradation of feathers provides a viable alternative to alkali hydrolysis and steam pressure-cooking of feather resulting in production of better animal feed additives from feather hydrolyzates (Gupta and Ramnani, 2006; Rai et al., 2009). Therefore, the bioconversion of keratinous residues is attracted increasing biotechnological interest since it might represent an alternative way of waste management that could be coupled with the production of valuable products (Brandelli, 2008).

**Keratin contents in feathers**

Feathers are having almost 90% of pure keratin and more than 90%, which found as wastes or by products at poultry processing plants. Keratins are insoluble fibrous proteins largely found in nature constituting hair, wool, feather, nail, horns, hoofs, and other epithelial covering of vertebrates (Bradbury, 1973). Keratins are valuable but unavailable fibrous animal proteins. They are components of a range of by-products occurring especially abundantly in slaughter houses meat and poultry plants: skin remains, bristle, animal hair, horns and hooves, feathers, etc. Keratins are stable and insoluble structural proteins found in the epidermis of vertebrates and its appendages like feathers and hairs. The α-helix (α-keratin) or β-sheet (β-keratin) keratin chains are tightly packed into a supercoiled polypeptide chain extensively cross-linked with disulfide bridges, hydrogen bonds and hydrophobic interactions,
resulting in the mechanical stability of keratin and its recalcitrance to common proteolytic enzymes such as pepsin, trypsin and papain (Onifade et al., 1998).

Keratin rich wastes, mainly in the form of feathers and hair, are generated in high amounts as by-products of agroindustrial processing (Gupta and Ramnani, 2006). Keratinous wastes could have a great potential as a source of protein and amino acids for animal feed and for many other applications (Bertsch and Coello, 2005).

Poultry feather constitutes the most abundant keratinous material in nature. Thus this waste product carries potent ecological implications, especially with burgeoning global poultry production. However, limitations to feather utilization arise from its poor digestibility and minimal biological value due to the deficiencies of nutritionally essential amino acids, such as methionine, lysine, histidine and tryptophan (Moran et al., 1966). Nevertheless, a more serious problem lies in the amino acid imbalance besides the formation of non-nutritive amino acids, such as lysinoalanine, lanthionine, etc (Wang and Parsons, 1997).

Proteases make up 60% of the total worldwide sale of industrial enzymes, finding applications in food, detergent and leather processing industries and development of such enzyme-based processes is needed for partial or total replacement of toxic chemicals currently used in industries (Rao et al., 1998). Among these different proteases, keratinases (E.C.3.4.99.11) constitute a group of enzymes capable of disrupting the highly stable keratin structure consisting of disulphide, hydrogen, and hydrophobic bonds in the form of α-helices and β-sheets (Parry and North, 1998).

**Keratinolytic microorganisms and their enzymes on Keratin degradation**

Keratinous materials which are rich in disulfide bonds are degraded very slowly in nature (Bálint et al., 2005) because they are water insoluble and resist to the degradation by classic proteolytic enzymes (Gradisar et al., 2005). In addition, it’s a highly recalcitrant polypeptide and strongly stabilized by several hydrogen bonds, hydrophobic interactions, and several disulfide bonds, which renders the protein not easily degradable by common proteolytic enzymes such as trypsin, pepsin, and papain (Brandelli, 2008). Despite their elevated resistance to degradation, keratins do not accumulate in nature, due to the existence of natural decomposers. Huge amount of
keratin solid wastes accumulate in nature and they are dispersed off by land filling and by incineration. But, these methods have several ecological disadvantages (Deydier et al., 2005; Paisley and Hostrup-Pedersen, 2005). However, despite its recalcitrance, keratin can be efficiently hydrolyzed by keratinolytic enzymes produced by a multitude of bacteria and fungi (Friedrich et al., 1999; Lucas et al., 2003).

Keratinases are serine proteases which are capable of degrading hard and insoluble keratin proteins. A large array of microbes such as various bacteria, actinomycetes and fungi are known to secrete keratinases that degrade keratin (Gupta and Ramnani, 2006; Brandelli et al., 2010). There is growing interest in microbial proteases of commercial importance in many areas as environmental sciences, biomedicine and biotechnology. The microbial biodegradation of insoluble macromolecules like keratin, cellulose, collagen, lignin and chitin depends on the secretion of extracellular enzymes with the ability to act on compact substrates (Madavasamy and Panneerselvam, 2012). The structural protein keratin is resistant to the activity of a broad range of proteases. However, it can be degraded by some species of fungi and to a less extend in bacteria (Bernal et al., 2006), who secrete keratinolytic enzymes (keratinases), that have the ability to degrade native keratin into smaller molecular entities which can subsequently be absorbed by cells. Degradation of keratinous material is also important in keratin waste valorization. Worldwide poultry processing plants produce millions of tons of feathers as a waste product annually (Sanaa Torka et al., 2013), keratinolytic microorganism accede to the bioconversion of these keratinous wastes into recoverable products such as enzymes and feather meal.

The use of microorganisms capable of producing extracellular keratinases is a possible alternative and an eco-friendly method to convert this abundant waste into low-cost, nutrient-rich animal feed (Grazziotin et al., 2006). In addition, keratinases have applications in other industrial processes such as detergent formulation (Hoshino et al., 1995) and leather processing (Laxman et al., 2004).

Keratinolytic microorganisms and their enzymes may be used to enhance the digestibility of feather keratin. They may have important applications in processing keratin-containing wastes from poultry and leather industries through the development
of non-polluting methods (Onifade et al., 1998). Keratinous wastes represent a source of valuable proteins and amino acids and could find application as a fodder additive for animals or source of nitrogen for plants. Biodegradation by microorganisms possessing keratinolytic activity represents an alternative attractive method for improving the nutritional value of keratin wastes, as it offers cheap and mild recreation conditions for the production of valuable products (Kim et al., 2001).

The physicochemical diversity of habitats has challenged nature to develop equally numerous molecular adaptations in the microbial world; thus, microbial diversity is a major resource for biotechnological products and processes (Gupta et al., 2002a). Although microorganisms capable to degrade keratinous substrates are generally isolated from soil and poultry wastes (Riffel and Brandelli, 2002, Brandelli, 2008), these microorganisms are almost ubiquitous in nature, thriving under diverse ecological and environmental conditions (Onifade et al., 1998).

Keratinases have many industrial and biotechnological applications including detergent industry, medicine, cosmetics, prion degradation in mad cow disease and leather industries (Langeveld et al., 2003; Yoshioka, et al., 2007). In addition, keratinases can also be considered as desirable detergent additives, which could replace the traditional proteases (Gupta and Ramnani, 2006; Brandelli et al., 2010). Keratinases with strong characteristics like oxidation and alkaline stability, detergent compatibility, and temperature tolerance are considered good market among detergent proteases.

Although, researchers have paid a great attention to this keratinous waste based on its high protein content, the high recalcitrant nature of this waste greatly hinders its utilization in the native state as an animal feedstuff unless it had been undergone physicochemical treatments. Currently, the production of commercial feather meals demands the use of these physicochemical methods. High cost and intensive energy are two prerequisites for the completion of this process. However, the resulting product has a low nutritional value and is poor in some essential amino acids such as methionine and histidine (Papadopoulos et al., 1986).

Research on keratinolytic microorganisms has been focused mostly on biotechnological applications involving the hydrolysis of keratinous by-products,
although promising new applications related to drug delivery and hydrolysis of prion proteins have been described (Brandelli, 2008; Brandelli et al., 2010). However, the ecological relevance of these organisms in natural systems and how widely the feather-degrading ability is distributed through the bacteria are questions that have been poorly investigated (Lucas et al., 2003).

Keratinase research has gained momentum because of its additional industrial and biotechnological applications other than those in the conventional sectors of proteases (Gupta and Ramnani, 2006). Keratinases find application in feed industry, fertilizers, pharmaceutical sector and as de-hairing enzyme in leather industry (Allpress et al., 2004; Anbu et al., 2005; Dastager et al., 2009). Keratinous poultry waste, especially recycled feathers, can be used in feed and fertilizer industry (Brandelli, 2008).

Studies on microbial keratinases have been focused mainly on their production from fungi due to the ease of downstream processing and the ability of fungi to grow on low-cost substrates (Anbu et al., 2005; Friedrich et al., 2005). In addition to fungal sources, keratinases have also been produced from diverse groups of bacteria such as Bacillus, Kocuria, Clostridium, Vibrio and Chryseobacterium (Bernal et al., 2006; Grazziotin et al., 2006).

Biodegradation of feathers by microorganisms is method for increasing their used as fertilizer. As they are environmentally friendly, keratinolytic enzymes are used more and more often in the production of fertilizers for agro industrial applications. The manure is stockpiled to be solid in either untreated or treated forms to consumers as fertilizer or disposed as landfill. Disposal pits and trench burial incineration are also common methods used for disposal of disease mortality (Environmental protection agency, 1999). In each of these processes, however, the outcome has limitations with respect to quality, cost efficiency as well as environmental management (Kim and Patterson, 2000).

By keeping the above facts in mind the present research has understandably designed with the following objectives.

✓ The chicken feather contaminated soil and chicken feathers collected from local slaughter house at Orathanadu (TK), Thanjavur District.
✓ To study the Physico-chemical parameters of collected soil.
✓ To isolate the feather degrading bacteria and fungi from feather contaminated poultry soil.
✓ To identify the bacteria by using cultural characterization and biochemical method.
✓ To identify the fungi by using lactophenol cotton blue staining method.
✓ To screen the keratinolytic activity by using isolated bacterial and fungal strains.
✓ To validate the keratinase enzyme action from selective strains.
✓ To optimize the production, conditions of keratinase enzyme by submerged fermentation method.
   i. pH
   ii. Temperature
   iii. Carbon sources
   Iv. Nitrogen sources
✓ To perform the flask experiment method for feather degradation by using bacterial and fungal strains.
✓ To study the roll towel method for plant growth.
✓ To study the microbial consortia method.
✓ To study the pot culture technique for plant growth.
✓ To study the effect of plant growth including seed germination, root, shoot and vigour index.
✓ To study the effect of feather waste compost on growth performance of plant.