Introduction
Enzymes play an indispensible role in various biotechnological sectors by acting as efficient biocatalysts. The industrial enzyme market is predominantly occupied by hydrolytic enzymes such as proteases, amylases, lipases, esterases, amidases, cellulases, xylanases, phytases etc. Of these, proteases are at an eminent position as they occupy more than 50% of the total share of enzyme sales worldwide (Rao et al., 1998). Over the last decade, a new dimension has been added to the protease world with the advent of ‘Keratinases’ which are special proteases with the additional ability to degrade the recalcitrant, proteinase-K resistant proteins.

‘Keratinases’ are by and large serine or metallo proteases that stand apart from the other conventional proteases like trypsin, pepsin and papain etc. owing to their unique ability to attack the ‘hard-to-degrade’ keratin residues (Gupta and Ramnani, 2006). Keratin forms a major component of the epidermis and its appendages viz. hair, feather, nail, horn, hoof, scale and wool and is characterized by a high degree of cross-linked disulfide bonds along with hydrophobic interactions (Suzuki et al., 2006).

Keratinases are elaborated by compendium of micro-organisms that include bacteria, actinomycetes and fungi (Brandelli et al., 2010). The past decade has seen reports on the characterization of keratinases from several organisms including those of Bacillus sp., Streptomyces sp., Chryseobacterium sp. and Stenotrophomonas sp. (Prakash et al., 2010; Xie et al., 2010; Silveira et al., 2010; Coa et al., 2009). However, among the many keratinases reported, only keratinase from Bacillus licheniformis is produced at a commercial scale and is available under the trade name Versazyme™, Valkerase and Prionzyme.

Microbial keratinases exhibit great diversity in their biochemical properties with respect to activity and stability in various pH and temperature ranges (Brandelli et al., 2010). In addition, they also exhibit diversity in the range of recalcitrant proteins they degrade ie. feather, hair, nail, hoof etc. Owing to these diverse properties and multi-faceted biotechnological implications, keratinases find application in various industrial sectors including food, feed, leather, detergent (Onifade et al., 1998;
Brandelli et al., 2010). Feather recycling for the cleanup of the ever accumulating poultry waste in the environment has always been the major application area of keratinases. Some of the emerging pharmaceutical applications of keratinases include their use as ungual enhancers for treating nail infections, for elimination of keratin in acne, opsoradiasis, elimination of human callus and degradation of keratinized skins (Vignardet et al. 2001; Friedrich et al. 2005; Gradisar et al. 2005; Mohoricic et al. 2007; Chao et al. 2007).

In addition to these applications, the major event which brought about a paradigm shift in keratinase research was the catastrophic ‘Mad cow epidemic’ that hit the United Kingdom in 2003. Meat was banned all over world to contain the spread of prion. Ever since the mad cow epidemic hit the United Kingdom, prion diseases have become an ever- looming nightmare for people all over the world. Earlier, transmission of prion was thought to be limited by the ‘species barrier’ however in the recent years this concept has corroded with the identification of bovine spongiform encephalopathies (BSE) as the causative agent of variant Creutzfeldt-Jakob disease (vCJD) in humans (Will et al., 1996, Hill, 2004; Moore et al., 2005). The present threat for public health arises from the risks of human-to-human transmission of vCJD (Lemmer et al., 2004). This iatrogenic transmission of vCJD can occur during treatment using contaminated medical devices or through transplantation of contaminated tissue. Forensic pathologists and autopsy surgeons are also at a serious occupational risk of prion diseases as the postmortem and mortuary rooms are another potential source of infection. Controlling TSE has become a major challenge for the public health organizations. Processes are required to reduce the prion load in the environment including decontamination of carcasses of infected animals and hospital instruments. However, the major bottleneck in prion decontamination is their high level of resistance to the conventional chemical and physical procedures (Taylor, 2000). The existing decontamination methods involve the use of hazardous chemicals viz. hydrogen peroxide, phenolics, guanidine thiocyanate and peracetic acid (Rutala and Weber, 2010). Their use has numerous drawbacks including concerns about worker safety, environmental impact and risk of damaging medical instruments. Thus, there is an urgent need for alternate methods for decontamination. In this respect, enzyme based methods, mainly involving keratinases are emerging as potential green solution to curb this serious problem of prion
decontamination. The structure of prion is highly homologous to the β-pleated structure of keratin (Langeveld et al., 2003). Thus, it was hypothesized that keratinases which degrade the β-keratin can accomplish degradation of these structural proteins. Several keratinases esp. from Bacillus sp. and Streptomyces sp. have been already documented to degrade prion (Langeveld et al., 2003; Tsiroulnikov et al., 2004). However, the major bottleneck lies in the fact that most of the available keratinases require the treatment of the prion infected tissue with alkali or high temperature treatments only. Such harsh treatments cannot be used for disinfection of surgical instruments and are neither environment friendly. Thus, the current focus of keratinase research is shifting towards the search for efficient keratinases which can degrade prion under ambient conditions.

As prion are highly infectious and experimentation with prion requires BSL3 containment facilities, thus an alternate surrogate prion protein, Sup 35NM is generally used for preliminary studies. Sup 35NM is a translation termination factor of Saccharomyces cerevisae. Its property of aggregation and characteristics are highly homologous to actual infective prion and has thus been documented to be used as a model protein for prion studies (Chen et al., 2005).

Overall, looking into the urgent need for finding a safe, eco-friendly alternative for reducing the spread of prion, the present study was undertaken with the aim of looking for potential keratinase with the ability to degrade prion-like protein, Sup 35NM. The detailed objectives of the work are as follows:

**Section 1: Screening, selection and identification of a potential keratinase producer for degradation of Sup 35NM**

A. Screening and selection of potential feather degrading strains based on:
   - Time taken to degrade feather
   - Keratinolytic potential of cell free, extracellular broth

B. Evaluation of selected strains for degradation of yeast surrogate prion protein, Sup 35NM
   - Expression and purification of Sup 35NM
   - Degradation of Sup 35NM by extracellular broth of selected strains using congo red assay
Introduction

C. Identification of the selected bacterium
   ➢ Biochemical identification
   ➢ Identification by sequencing of complete 16S rDNA

Section II: Purification and biochemical characterization of keratinases
   ➢ Concentration of enzyme
   ➢ Purification by column chromatography
   ➢ Biochemical characterization with respect to pH and temperature kinetics, stability in presence of inhibitors, metal ions and substrate specificity
   ➢ MS/MS analysis and N-terminal determination

Section III: Molecular characterization of keratinases
   ➢ Cloning and sequence analysis of keratinase gene
   ➢ Expression of the cloned gene in *E. coli*
   ➢ Biochemical characterization of recombinant keratinases
   ➢ Characterization of fibrinolytic activity if any
   ➢ Identification of pro-sequence region

Section IV: Structural analysis of keratinases using *in silico* approaches
   ➢ Sequence comparison
   ➢ Multiple sequence alignment
   ➢ Homology modelling
   ➢ Binding pocket analysis and ligand binding
   ➢ Functional validation by site directed mutagenesis

Section V: Feather degradation by recombinant *E. coli*: Insight into mechanism of sulfitolysis and proteolysis
   ➢ Structural and biochemical basis

Section VI: Degradation of Sup 35NM by keratinases under ambient conditions
   ➢ Standardization of various parameters *viz.* enzyme concentration and additives