Summary & Conclusions
Chapter 6

SUMMARY AND CONCLUSIONS

The present investigation “Keratinases from *Pseudomonas aeruginosa* KS-1: Characterization and degradation of surrogate prion protein Sup 35NM” was undertaken realizing the immense potential of keratinases to attack the infectious prion protein. Based on the results of the experiments carried out, the work can be summarized as follows:

Fifty keratinolytic bacterial isolates procured from the laboratory culture collection were screened for their keratinolytic potential based on three criteria ie. time taken for feather degradation, ability of cell free degradation of feather and finally ability of their extracellular broth to degrade surrogate prion protein, Sup 35NM. Out of the fifty isolates, only eleven isolates were able to degrade chicken feather within 48 h. These eleven isolates were identified on the basis of partial 16S rDNA gene sequence to be belonging to various species of *Bacillus* (*B. pumilus, B. licheniformis, B. subtilis, B. megaterium*) and *Pseudomonas aeruginosa*. Out of these eleven selected isolates, extracellular broth of nine isolates could carry out cell free degradation of chicken feather within 16 h. Keratinase production was variable in all the cases and it did not correlate with feather degradation. Out of the nine selected isolates, KS-1 could maximally degrade Sup 35NM as determined by congo red binding assay and thus it was selected for further studies. The selected bacterium was identified as *Pseudomonas aeruginosa* on the basis of biochemical and complete 16S rDNA gene sequence identification. It was designated as *Pseudomonas aeruginosa* KS-1 and the 16S rDNA gene sequence has been deposited to GenBank under accession no. GQ203616. The culture has been deposited to the microbial type culture collection under the accession no. MTCC 10775.

Keratinase was produced by *Pseudomonas aeruginosa* KS-1 in feather peptone medium containing 0.5% chicken feather and it was further subjected to purification. The enzyme was concentrated by acetone precipitation and purified by Q-sepharose anion exchange chromatography. Two keratinases were purified from the concentrated extracellular broth. Major keratinase peak eluted in 0.6 M fraction was designated as keratinase KP1 and minor keratinase peak eluted in 0.1 M fraction was designated as keratinase KP2. The purity of the enzymes was confirmed by HPLC by
passing through a C18 column. SDS and Native-PAGE analysis also confirmed the purity of the enzymes and SDS PAGE indicated that keratinase KP1 and KP2 had a molecular weight of approximately 45 and 33kDa respectively. The pI of keratinase KP1 and KP2 was found to be 4.8 and 5.8 respectively based on isoelectric focusing analysis. MALDI-TOF analysis revealed keratinase KP1 to have homology with putative aminopeptidase and keratinase KP2 to have homology with pseudolysin.

Biochemical characterization of the purified keratinases was carried out with respect to reaction parameters viz. pH, temperature, inhibitors, metal ions and substrate specificities.

Keratinase KP1 and KP2 had maximum activity at pH 9.0 and pH 7.0 respectively. Keratinase KP1 was stable over a pH range of 5.0-9.0 whereas keratinase KP2 was stable over a broad pH range from 2.0-11.0. Keratinase KP1 and KP2 had optimum activity at 60°C and 50°C respectively. Both the keratinases were highly thermostable with a t_{1/2} of 3.6 h and 2.3 h at 80°C for keratinase KP1 and KP2 respectively.

Both the keratinases were completely inhibited by PMSF indicating that they are serine proteases. Keratinase KP1 was a metal activated enzyme whereas keratinase KP2 was thiol activated keratinase. Keratinase KP1 was activated in presence of Mn^{2+} and inhibited by Cu^{2+}, Cd^{2+} whereas keratinase KP2 was activated in presence of Ca^{2+}, Cu^{2+}, Mg^{2+}, Zn^{2+} and Co^{2+}.

Keratinases KP1 and KP2 efficiently hydrolyzed a variety of complex protein substrates. Keratinase KP1 preferentially cleaved insoluble substrates more with order of hydrolysis being meat protein > gelatin > fibrin > feather > elastin > casein whereas keratinase KP2 exhibited maximum hydrolysis of casein with the order of hydrolysis being casein > fibrin > feather > nail = meat protein > gelatin > elastin. The K:C (keratinolytic : caseinolytic) ratio of keratinase KP1 and KP2 was 2.5 and 0.5 respectively. Keratinase KP1 and KP2 were also capable of hydrolyzing synthetic para-nitroanilide substrates with best activity on N-Suc-Ala-Ala-Pro-Phe-pNA (100%) followed by D-Val-Leu-Lys-pNA (96%). In addition, keratinase KP1 efficiently cleaved N-Suc-Ala-Ala-Pro-Leu-pNA (60%) and keratinase KP2 efficiently cleaved N-Benzoyl-DL-Arg-pNA (93%).
The kinetic constants of the enzymes were determined for casein and N-Suc-Ala-Ala-Pro-Phe-pNA. Keratinase KP1 and KP2 had a \( K_m \) of 27.7 mgml\(^{-1}\) and 14.2 mgml\(^{-1}\) and \( V_{\text{max}} \) of 4.54 x 10\(^2\) \( \mu \)g ml\(^{-1}\)min\(^{-1}\) and 8.19 x 10\(^2\) \( \mu \)g ml\(^{-1}\)min\(^{-1}\) on casein respectively. The \( K_m \) and \( V_{\text{max}} \) of keratinase KP1 and KP2 on N-Suc-Ala-Ala-Pro-Phe-pNA was 1.69 mM; 3.8 mM and 5.42 x 10\(^7\); 9.76 x 10\(^2\) respectively.

Hydrolysis of insulin B chain revealed that keratinase KP1 cleaved between Val\(^{12}\)-Glu\(^{13}\), Ala\(^{14}\)-Leu\(^{15}\), Gly\(^{20}\)-Glu\(^{21}\) and Arg\(^{22}\)-Gly\(^{23}\) and keratinase KP2 cleaved between Phe\(^{1}\)-Val\(^{2}\), Cys\(^{7}\) – Gly\(^{8}\), Gly\(^{8}\) – Ser\(^{9}\), His\(^{10}\)-Leu\(^{11}\), Leu\(^{11}\)-Val\(^{12}\), Phe\(^{24}\)-Phe\(^{25}\).

The genes encoding keratinases from *Pseudomonas aeruginosa* KS-1 were cloned using PCR amplification based on primers of putative aminopeptidase and pseudolysin genes of *Pseudomonas aeruginosa* POA-1. Sequence analysis of the keratinase genes revealed keratinase KP1 to have an ORF of 1611 bp which encoded for 536 amino acid residues and keratinase KP2 to have an ORF of 1497 bp which encoded for 498 amino acid residues. The sequence of KP1 showed 99% homology with probable aminopeptidase and sequence of KP2 showed 99% homology with pseudolysin precursor of *Pseudomonas aeruginosa*. The sequences have been deposited in Genbank under the accession no. HM452164 and HM452163 respectively.

Both the keratinases were constitutively expressed as extracellular proteins (rKP1 and rKP2) in *E. coli* HB101 using pEZZ 18 vector in two forms i.e. IgG fused protein and processed protein. The tagged protein was inactive however, the processed protein was active. This functional protein was purified by Q-sepharose chromatography. Biochemical characterization revealed the pH and temperature optima for rKP1 and rKP2 to be pH 10.0/60\(^0\)C and 9.0/ 50\(^0\)C respectively. Both rKP1 and rKP2 were highly stable over a broad range of pH varying from 2.0 to 11.0. Keratinase rKP1 was comparatively more thermostable than keratinase rKP2 with a \( t_{1/2} \) of 20 min at 70\(^0\)C compared to a \( t_{1/2} \) of 12 min for rKP2.

Both rKP1 and rKP2 hydrolysed a variety of complex proteinaceous substrates with maximum hydrolysis of fibrin and haemoglobin respectively. Among synthetic substrates, both rKP1 and rKP2 had maximum activity on N-Suc-Ala-Ala-Pro-Phe-pNA which varied depending on P2 position residues. The \( K_m \) and \( V_{\text{max}} \) of keratinase
rKP1 and rKP2 on N-Suc-Ala-Ala-Pro-Phe-pNA was 9.09 mM; 7.69 mM and 21.27 x 10^2; 38.91 x 10^2 µmole ml^-1 min^-1 respectively.

As both rKP1 and rKP2 efficiently cleaved fibrin, detailed analysis of their fibrinolytic potential was carried out and it was observed that both rKP1 and rKP2 possessed fibrinolytic, fibrinogenolytic and plasminogen-activating properties. rKP1 rapidly cleaved the α-chain of fibrin followed by slow degradation of β-chain while γ-γ chain could not be degraded. Similarly, rKP2 preferentially cleaved α-chain followed by cleavage of β-chain in 24 h. For fibrinogen, rKP1 completely cleaved Aα-chain within 10 min followed by γ-chain which was slowly cleaved with complete degradation in 3 h. In case of rKP2, Bβ-chain and γ-chain are completely cleaved within 2 h. An 1.5 fold and 2.1 fold increase in area of clear zone was observed on plasminogen-rich plate compared to plasminogen-free plate for rKP1 and rKP2 respectively thus showing their plasminogen activating activity.

Sequence analysis had revealed that keratinase rKP2 had a long pro-sequence of 174 aa. Thus, further work was undertaken to ascertain the minimum length of pro-sequence required for functional expression of keratinase rKP2 by N-terminal pro-sequence truncations. N-terminal truncation studies revealed that out of the total 520 bp long pro-sequence of rKP2, only 187 bp region is the minimum length required for the correct folding of the enzyme. The size of the pro-sequence has a direct impact on the conformation of the protein hence leading to altered thermostability of the enzyme.

Molecular characterization of keratinases was followed by their structure-function analysis using in silico approaches. Keratinase KP2 shared homology with pseudolysin which is a well worked out protein, however keratinase KP1 was a novel protein giving homology with probable aminopeptidase. In addition, its detailed characterization revealed it to possess additional protease function besides being an aminopeptidase. Thus, in the present work, attempts were made to understand the structural basis of the protease function of keratinase KP1 using in silico approaches.

First, the amino acid sequence of KP1 was blasted against the pdb database and four proteins viz. aminopeptidase from Streptomyces griseus, Aneurinibacillus sp., Vibrio proteolyticus and Peptidase precursor from Shewanella amazonensis were selected as templates. Among these, maximum sequence identity and homology of
rKP1 was found with aminopeptidase from *Streptomyces griseus* however, the length of rKP1 was much longer than that of aminopeptidase from *Streptomyces griseus*.

Next, multiple sequence alignment of KP1 with other homologs revealed that the secondary of rKP1 was more or less conserved with that of aminopeptidase of *Streptomyces griseus*. All active residues known for aminopeptidase of *Streptomyces griseus* were found to be conserved in KP1 however among other homologs from *Aneurinibacillus*, *Vibrio proteolyticus* and *Shewanella amazonensis* they were semi-conserved. The catalytic residues of aminopeptidase of *Streptomyces griseus* namely His85, Asp97, Glu131, Glu132, Asp160, Met161, Tyr172, Arg212, Ser213, Tyr246, His247 were conserved as His296, Asp308, Glu340, Glu341, Asp369, Met370, Tyr381, Arg422, Ser423, Tyr466, His467 of KP1 respectively.

Further, three dimensional model of KP1 was generated by MODELLOR tool using aminopeptidase from *Streptomyces griseus* and *Aneurinibacillus* as templates. The modeled structure of KP1 was superimposed over structure of aminopeptidase from *Streptomyces griseus* and it was observed that a particular area was highly superimposed whereas the remaining area did not superimpose due to shorter length of the template.

The pocket finder tool predicted the binding pocket of KP1 to be constituted of the following residues: His296, Asp308, Glu340, Glu341, Asp369, Met370, Tyr381, Arg422, Ser423, Tyr466, His467. This was in confirmation with the residues predicted by multiple sequence alignment. Further analysis revealed that although the protein volume of KP1 is just double than that of *Streptomyces* aminopeptidase the pocket size is 4.8 fold larger.

Superimposition of the pocket of KP1 over the pocket of aminopeptidase of *Streptomyces* revealed that the binding pocket of aminopeptidase of *Streptomyces* was partially covered by the pocket of rKP1. It had a high RMSD value as the pocket of KP1 was much larger than that of aminopeptidase of *Streptomyces*. However, the superimposition of the catalytic residues revealed perfect alignment of most of the residues with a significant RMSD value of 0.754 Å.

Further, as KP1 had both the characteristics of aminopeptidase and protease, the binding mode analysis was performed with substrates/inhibitors of both the functions. Amino acid substrates *viz.* leucine and methionine were taken as ligands for
checking the aminopeptidase function of KP1 and inhibitors viz. rivastigmine, N-Benzylloxycarbonyl-L-serine-Betalactone and kazal were taken as ligands for checking the protease function of KP1. Docking analysis revealed that leucine and methionone revealed them to completely fits in the binding pocket of KP1 forming four hydrogen bonds each thus validating the predicted pocket for aminopeptidase function. Next, the docking analysis with protease inhibitors revealed that all both the inhibitors docked in the same pocket identified for aminopeptidase function. These inhibitors when docked with aminopeptidase from *Streptomyces* do not even partially fit in or around its binding pocket. Thus it can be inferred that KP1 has a larger binding pocket compared to other aminopeptidases thus it can take larger proteins explaining its protease function.

Finally, the catalytic residues predicted by the above study were functionally validated using site directed mutagenesis. First four major residues namely Glu340, Glu341, Arg422 and Tyr466 were mutated individually to alanine. Out of the four mutants, complete loss of both amidolytic and proteolytic activity was observed in case of one mutant namely Glu341 (E341A) thus establishing its role in catalysis.

Kinetic comparison of the rest of the mutant proteins revealed varying effects both on amidolytic and proteolytic activity of rKP1. All residues drastically decreased the amidolytic activity whereas proteolytic/keratinolytic activity was majorly affected by Glu340 only (E340A). For amidolytic activity, the lowest $K_{cat}$ on AAPF and AAPL was observed for R422A whereas for protease activity, lowest $K_{cat}$ was observed for E341A. All residues were found important for amidolytic activity however only one residue was found to affect proteolytic activity.

To further search for residues involved in the protease function of rKP1, three more mutations were carried out mutating Ser423, His296 and His467 individually to alanine. There was complete loss of activity in case of S423A and H296A thus proving the importance of these residues in catalysis. Kinetics ($K_m$ and $V_{max}$) for the rest of the active mutants was compared with that of native rKP1 on N-Suc-Ala-Ala-Pro-Phe-$p$NA (AAPF) and N-Suc-Ala-Ala-Pro-Leu-$p$NA (AAPL) and a lowering in $K_m$ and $V_{max}$ was observed for all mutants compared to native rKP1 with $V_{max}$ in the order of H467A<E340A0<R422A=Y466A for AAPF. For AAPL, lowering in $K_m$ and $V_{max}$ was observed for E340A compared to native protein whereas an increase in $K_m$ was observed for the rest of mutant proteins.
Thus, overall form the above study, Glu340, Ser423 and His467 can be proposed as the catalytic triad involved in the protease function of keratinase KP1.

Besides biochemical, molecular and structural characterization of keratinases, an attempt was made to understand the mechanism of feather degradation using recombinant *E. coli* strains harboring rKP1 and rKP2. These recombinant strains were able to degrade feather while control *E. coli* strain harboring vector pEZZ 18 only was unable to degrade feather. Structural changes during feather degradation were studied by scanning electron microscopy. It was observed that there was scanty colonization of feather by both the recombinant as well as control strains in 6 h followed by significant colonization in 9 h. After 18 h, complete disintegration of feather was observed with degraded feather giving putty appearance in case of recombinant strains however intact feather was observed in case of control *E. coli* strain.

Role of colonization in feather degradation was studied by subjecting both un-colonized and pre-colonized feather to action by keratinases rKP1 and rKP2. It was observed that only, pre-colonized feather could be degraded by the keratinases while un-colonized feather remained intact establishing the importance of colonization for feather degradation. Further, it was observed that there was an eight fold increase in free cysteinyl group in case of pre-colonized feather as against un-colonized feather. This clearly showed that colonization led to sulfitolysis which in turn lead to subsequent degradation of feather by keratinases.

Pre-colonized feather could be degraded by keratinases only, non-keratinolytic protease such as trypsin could not degrade even pre-colonized feather proving the role of keratinases for degradation.

Established through a set of experiments, it was hypothesized that GGT-GSH mediated redox might be one of the way of sulfitolysis for feather degradation and cysteinyl glycine might be the active redox moiety. This was proved by quenching of free cysteinyl groups by DTNB which lead to reduction in feather degradation by recombinant *E. coli*. It was further substantiated by checking for feather degradation using recombinant *E. coli* GGT knockout strains. It was observed that GGT knockout strains failed to degrade feather, inspite of colonization and keratinase production thus clearly proving the role of GGT in feather degradation. Thus, GGT is involved in
sulfitolysis by its action on glutathione to release free cysteiny1 groups which are known to be much stronger reductant than glutathione alone.

Short term experiments of protein release from feather keratin by recombinant keratinases in presence of GGT-GSH redox showed an enhancement in protein release in case of simultaneous as well as pre-treatment of feather by GGT-GSH. This indicated that GGT-GSH system modifies the feather substrate by reducing di-sulfide bonds making it more vulnerable to attack by keratinase thus resulting in enhancement in protein release. Thus, overall it can be put forth that GGT-GSH system has been identified as a novel redox principle involved in feather keratin degradation.

After gathering information on the mechanism of keratinolysis, attempt was made to exploit the recombinant keratinases for degradation of prion protein under ambient conditions. As discussed earlier, due to the infectious nature of prion and lack of biosafety facilities available, a surrogate prion protein, Sup35NM was used for the present study.

The degradability of Sup35NM aggregates by rKP1 and rKP2 were compared at different enzyme concentrations (2-10 µg) at 37°C, pH 7.0 after 18h. Western blot analyses against infectivity domain i.e. anti-N clearly showed that no degradation was seen even after increasing the dosage of keratinase rKP1 and rKP2 to 10µg. Next, the effect of various additives viz. triton X-100, Tween 80, SDS, sodium carbonate and β-mercaptoethanol on degradation by rKP1 and rKP2 was studied and the results showed that the infective domain of Sup35NM was completely degraded by rKP2 in presence of 0.5% SDS. In case of rKP1, none of the additives lead to degradation of Sup35NM.

rKP2 was thus further taken up for standardization of concentration of additive and time of degradation and it was observed that complete degradation of Sup35NM was achieved by rKP2 in presence of 0.1% SDS in 15 min under ambient conditions. When compared with other proteases, it was observed that Sup35NM was only partially degraded by proteinase K (PK) and keratinase from Bacillus licheniformis (KerA) even in presence of SDS in 15 min establishing the potential of keratinase rKP2.
Besides chemical additive, attempt was also made to make the process of prion degradation totally enzymatic. In this respect, the combined action of keratinase and GGT-GSH couple was studied on degradation of Sup 35NM. Proteinase K (PK) was taken as a positive control and non-keratinolytic enzyme, trypsin was taken as a negative control. Western blot analysis revealed that rKP2 efficiently degraded Sup 35NM in 15 min at 37°C, pH 7 in presence of GGT-GSH system thus establishing the role of GGT-GSH redox for prion degradation too. PK could partially degrade and trypsin could not at all degrade Sup 35NM even in presence of GGT-GSH redox.

Thus, overall it can be stated that keratinase rKP2 can effectively degrade Sup 35NM in 15 min under ambient conditions in presence of either SDS or GGT-GSH couple.

Based on the results of the experiments carried out on keratinases from *Pseudomonas aeruginosa* KS-1, their biochemical and molecular characterization, structure and function analysis, mechanism of feather degradation and application for degradation of surrogate prion protein, Sup 35NM the following conclusions can be drawn:

- The bacterium *Pseudomonas aeruginosa* KS-1 (Accession no. MTCC 10775) is a potential keratinolytic strain with an ability to completely degrade chicken feather within 24 h.
- Keratinases from *Pseudomonas aeruginosa* KS-1 have the potential to degrade surrogate prion protein, Sup 35NM.
- Two keratinases *viz.* KP1 and KP2 can be purified from 0.6M and 0.1M fractions respectively of Q-sepharose anion exchange column. Keratinase KP1 and KP2 have a molecular weight of 45 kDa and 33 kDa respectively. The sequence of keratinase KP1 and KP2 is homologous to that of probable aminopeptidase and pseudolysin precursor of *Pseudomonas aeruginosa* respectively.
- Keratinase KP1 and KP2 are serine proteases with keratinase KP1 and KP2 being a metalloprotease and thiol activated protease respectively. Keratinase KP1 had optimal activity at pH 9.0 and 60°C whereas keratinase KP2 had optimal activity at pH 7.0 and 50°C. Both the keratinases were stable over a wide range of pH and temperature.
Both the keratinases cleaved a variety of insoluble and soluble complex protein substrates with better activity of keratinase KP1 towards insoluble proteins whereas keratinase KP2 preferred soluble proteins. K:C (keratinolytic: caseinolytic) of KP1 and KP2 was 2.5 and 0.5 respectively. They efficiently hydrolyzed hydrophobic, synthetic substrate viz. N-Suc-Ala-Ala-Pro-Phe-pNA. Keratinase KP1 and KP2 had 4 and 6 cleavage sites respectively on insulin B chain.

The sequence analysis of the keratinases indicated that keratinase KP1 showed 99% homology with probable aminopeptidase and keratinase KP2 showed 99% homology with pseudolysin precursor of *Pseudomonas aeruginosa*. The sequences have been deposited in Genbank under the accession no. HM452164 and HM452163 respectively. The enzymes were expressed as extracellular proteins in *E.coli* HB101-pEZZ host-vector combination as rKP1 and rKP2.

Biochemical characterization revealed rKP1 and rKP2 to have a pH and temperature optima of pH 10.0/60°C and 9.0/50°C respectively. Keratinase rKP1 was comparatively more thermostable than keratinase rKP2.

rKP1 and rKP2 both possessed potential fibrinolytic, fibrinogenolytic as well as plasminogen-activating properties.

N-terminal truncation studies of rKP2 revealed that out of the total 520 bp long pro-sequence, only 187 bp region is the minimum length required for the correct folding of the enzyme and the size of the pro-sequence has a direct impact on the conformation of the protein hence leading to altered kinetics and thermostability of the enzyme.

Structural analysis of keratinase KP1 revealed that it shared maximum homology with aminopeptidase from *Streptomyces griseus*. The binding pocket of KP1 as deciphered by MSA and pocket finder constitutes the following residues: His296, Asp308, Glu340, Glu341, Asp369, Met370, Tyr381, Arg422, Ser423, Tyr466, His467. Leucine and methioine, substrates for aminopeptidases completely fits in the predicted binding pocket of KP1.

Protease character of keratinase KP1 was validated as the pocket size of KP1 is 4.8 fold larger than that of *Streptomyces* aminopeptidase indicating it can
bind larger substrates and also because protease inhibitors namely rivastigmine, N-Benzylloxy carbonyl-L-serine-Betalactone and kazal perfectly docked in the binding pocket of KP1.

- Glu341, Ser423 and His296 were functionally validated to constitute the catalytic triad of keratinase KP1.

- Feather degradation studies by recombinant *E. coli* strains indicated that keratinolysis is a co-operative action of sulfitolysis and proteolysis. GGT-GSH has been established as a novel redox assisting feather degradation. GGT-GSH couple generates free cysteiny l group which is a strong reductant that accelerates sulfitolysis of recalcitrant proteins making them more vulnerable to attack by keratinases. Subsequent attack of these tough proteins is possible only by keratinases and not by non-keratinolytic proteases.

- Application of keratinases rKP1 and rKP2 for degradation of surrogate prion protein, Sup 35NM revealed that rKP1 and rKP2 alone cannot degrade the infectivity domain of Sup 35NM at 37°C, pH 7 after 18h however rKP2 was able to completely degrade Sup 35NM in presence of 0.5% SDS. Complete degradation was achieved at a concentration of 0.1% SDS and in 15 min. Proteinase K partially degraded Sup 35NM even in presence of SDS.

- rKP2-GGT-GSH combination was also demonstrated to completely degrade Sup 35NM in 15 min under ambient conditions.

- An enzymatic formulation of rKP2 alongwith SDS or GGT-GSH can completely degrade Sup 35NM under ambient conditions in 15 min.

Thus in a nutshell, it can be put forth that the present strain of *Pseudomonas aeruginosa* KS1, is highly keratinolytic, capable of completely degrading chicken feather within 24h. It elaborates two potential keratinases with broad pH-temperature stability and substrate specificity. Both the keratinases are efficient fibrin(ogen)olytic enzymes possessing plasminogen activating activities. Protease function of keratinase KP1 annotated as putative aminopeptidase has been established and its catalytic triad has been identified as Glu340, Ser423 and His467. Coupled action of GGT-GSH and keratinase during degradation of feather keratin and prion protein has been demonstrated in detail and these findings can be taken as a lead to develop prion degrading enzymatic formulation.
MAJOR CONTRIBUTIONS OF THE PRESENT STUDY

- A novel protein of *Pseudomonas aeruginosa* annotated as putative aminopeptidase has been characterized to be keratinase KPI possessing amidolytic as well as protease function. Results have been supported by *in silico* and wet studies.

- Coupled action of γ-glutamyl transpeptidase-glutathione and keratinase has been demonstrated to effectively degrade feather keratin and surrogate prion protein, Sup 35NM