

Degradation of scrapie infected brain homogenate by a novel bacterial keratinase

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Abstract

Prion protein is central to Transmissible Spongiform Encephalopathy (TSE) pathogenesis. Characteristically prion is resistant to conventional methods of sterilization and the most effective means of its degradation are incineration and alkaline hydrolysis. These methods are limited by environmental acceptability, application compatibility, cost and loss of reusable materials. Enzymatic degradation provides a viable alternative for decontaminating animal carcasses, specified risk materials, as well as surgical and dentistry instruments. The objective of this research was to isolate and characterise microbial keratinases and to investigate their ability to degrade keratinaceous materials and possibly scrapie prions. Microbial isolates from farmyard waste were grown on feather meal medium and the synthesised keratinase characterised by MALDI-MS and SDS-PAGE. Keratinolytic activity was determined using keratin azure, casein and melanised feather as substrates. Degradation of scrapie prion was evaluated by western blotting analysis. One specific isolate, identified as a strain of *Bacillus licheniformis*, demonstrated considerable promise. The molecular weight of the enzyme produced by this bacteria was found to be ≈28kDa, with optimum pH and temperature at 8.0 and 50 °C respectively. This novel keratinase demonstrated significant activity on keratin azure (11 U/mL) and casein substrates, and completely degraded melanised feather within 48h. Western blotting analysis shows significant reduction in prion signal and immunoreactivity for scrapie infected mouse (ME7) brain homogenate after incubation with this keratinase. Inclusion of a biosurfactant also further enhanced degradation of scrapie prion. The ability of this novel bacterial keratinase to degrade keratin materials and scrapie prion suggests its potential use as an environmental alternative in prion decontamination and other applications.

Introduction

Enzymatic digestion of prions has been pursued as an environmentally friendly, economically sound and safe method of decontamination of prion infected materials, and was one of the science objectives and targets in UK Transmissible Spongiform Encephalopathy Directorate 2003-2006 Science Strategy [1]. A number of research on enzymatic degradation, inactivation or decontamination of prions reported different levels of success under various experimental conditions; some of these methods required augmentation by preheating of prion contaminated tissue, addition of surfactant/detergent, high alkaline pH or oxidizing agents [2-6]. A stand-alone biological alternative for prion degradation that is environmentally safe, compatible for use on sensitive materials, and commercially viable is highly desirable.

The aim of this study was to isolate keratinolytic microorganisms from farmyard wastes, and to characterise the enzyme produced; and subsequently to explore the ability of this enzyme to degrade feather keratin and scrapie prion.

Materials and Methods

Microorganism and growth condition

Isolates were obtained from farmyard waste sources and grown on a minimal medium (in g/l: NaCl 0.5; KH₂PO₄ 0.7; K₂HPO₄ 1.4; MgSO₄·7H₂O 0.1; with 1% feather meal as substrate.

Keratinase purification and characterisation

Culture supernatant was purified with HiTrap™ Blue HP affinity chromatography column (GE Healthcare Bio-sciences). The molecular weight and purity of the enzyme were determined by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) (AXIMA CFR, Shimadzu Biotech) and SDS-PAGE.

Evaluation of keratinolytic and Proteolytic activity

Proteolytic and keratinolytic activity were observed as hydrolysis ring on casein-agar plate (1% casein) and azure dye release from keratin azure incubated at 50 °C. One unit of keratinase activity (U) was defined as an increase of 0.01 in absorbance units (A₅₉₅) per hour resulting from keratinolytic action.

Evaluation of feather keratin and scrapie prion degradation

Sterile melanised wild bird feather was incubated with keratinase at 50 °C for 48h. Scrapie infected mouse (ME7) brain homogenate was treated with keratinase and incubated at 50 °C for 2h. The treated samples were separated on gel and transferred to PVDF membrane. The membrane was blocked with 5% skim milk, incubated for 1h and probed with primary antibody (SAF 83) (1:5000) and secondary antibody (anti-mouse IgG conjugated with horseradish peroxidase) (1:5000), and detected with ECL plus western blotting system.

Conclusion

A new keratinase producing bacterial strain has been isolated, identified and designated as *Bacillus licheniformis* N22. This extracellular keratinase was able to completely degrade melanised feather keratin. Preliminary results also show significant loss of PrPsc signal of scrapie prion to a level that was undetectable by western blotting analysis. Further optimisation of incubation conditions is currently ongoing, and future work will include degradation of BSE prions and validation of this method by bioassay to test for residual infectivity. This prion digestion method will provide a stand-alone biological alternative to prion decontamination that will be safe and practicable for diverse applications, environmental friendly and commercially viable.

Acknowledgement

Funding support from the Alberta Prion Research Institute Canada and Institute of Social and Health Research, Middlesex University London, UK is highly appreciated.

References

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Results

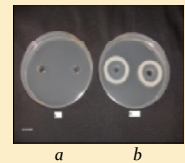
➤Thirty-two microbial strains were isolated on feather meal agar. One strain, identified and designated as *Bacillus licheniformis* N22 expressed superior proteolytic and keratinolytic activity, and showed distinguishable physiological and molecular characteristics.

➤Optimal keratinase activity (11 U/mL) was achieved at temperature (50 °C), pH (8.0) and substrate concentration (1%) over 32 h incubation.

➤Casein hydrolysis ring was indicative of proteolytic activity by keratinase (Fig.1).

Fig. 1: Casein-agar plates assay

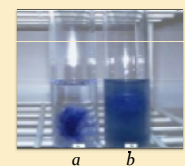
- (a) strain without proteolytic activity
(b) strain with proteolytic activity (*Bacillus licheniformis* N22).



➤Keratinolytic activity was assessed by azure dye release by keratin azure substrate (Fig. 2).

Fig. 2: Keratinolytic action of purified keratinase

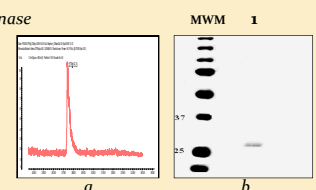
- (a) Control : without keratinase of *Bacillus licheniformis* N22
(b) with keratinase of *Bacillus licheniformis* N22



➤Molecular weight (≈28kDa) and homogeneity (single peak and band) of the purified keratinase was determined by Matrix-Assisted Laser Desorption/Ionisation Mass Spectrometry (MALDI-TOF MS) (Fig. 3a) and SDS-PAGE (Fig. 3b) respectively.

Fig. 3a: MALDI-TOF MS spectrum of purified keratinase showing a single peak

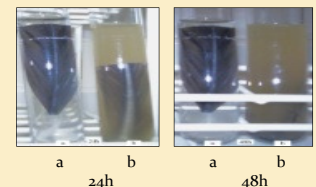
- 3b: SDS-PAGE of purified keratinase showing a single molecular weight band (lane 1).



➤Melanised feather keratin was completely degraded in 48h with both crude supernatant and purified enzyme (Fig. 4)

Fig. 4: Degradation of melanised wild bird feather

- (a) without keratinase *Bacillus licheniformis* N22
(b) with keratinase *Bacillus licheniformis* N22



➤PrPsc signal was completely lost after incubation with keratinase of *Bacillus licheniformis* N22 to a level that was undetectable within the limits of sensitivity of western blotting analysis (Fig. 5).

Fig. 5: Scrapie prion degradation analysed by Western blotting

Lanes:

- 1- Control: scrapie prion incubated without keratinase
- 2- Scrapie prion incubated with Proteinase K (100µg/ml)
- 3 & 4- Scrapie prion incubated with purified keratinase of *Bacillus licheniformis* N22

