

## PROTEOLYTIC ENZYMES FOR USE IN PROTEIN DEGRADATION, CLEANING, AND STERILIZATION PROCEDURES

### Fields of use

Sterilization of surgical equipment in hospitals, cleaning of textiles, purification in molecular biology protocols

### Current state of technology

The solution has been demonstrated and tested in the laboratory

### Type of cooperation

license agreements and/or technical cooperation agreements with industry or research partners, collaboration agreements for joint development (e.g. staff exchange), manufacturing agreements with industry partners with the capacity for manufacturing the enzyme.

### Intellectual property

Technology has been patented (EP2311323, SI24364A).

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### Summary

A technology is presented involving production and activity of a thermally stable serine protease that efficiently degrades proteins, protein aggregates, and deposits. The enzyme is produced under mild conditions, and applicable for sterilization of surgical equipment in hospitals, cleaning of textiles, and for purification in molecular biology protocols. The enzyme is efficient in a broad range of temperatures, pH values, and in presence of detergents.

### Description of the invention

**The Problem:** Hospital equipment contaminated with prion proteins represents a constant health risk. Prions are notoriously resistant to high temperature and aggressive detergent treatments, making it difficult to sterilize such equipment using standard procedures. Moreover, there is no effective way to clean protein films in internal surfaces, such as tubes and pipes. In addition, textile and surface cleaning applications make use of formulations that often include proteases; however, formulation choices are often limited by protease sensitivity to temperature and/or pH. Finally, thermally stable proteases are usually produced using extreme laboratory conditions and in low yields.

**The Solution:** The invention solves these problems by introducing a thermally stable protease pernisine. Pernisine, originating from the organism *Aeropyrum pernix*, is produced under mild conditions in a common laboratory bacterium *Escherichia coli*, and the gene sequence is specifically modified to allow for higher yields. The recombinant pernisine is robust, stable under wide temperature and pH ranges, effective against soluble proteins as well as protein deposits, and may potentially be used in combination with detergents.

### Application:

- sterilization of surgical equipment in hospitals
- cleaning of solid surfaces with protein deposits (water pipes, bioreactor walls, etc.)
- cleaning of textiles (as component of washing powders and detergents)
- replacement of proteinase K in molecular biology purification protocols

### Main Advantages

- production possible under mild conditions
- expression in *E. coli* leads to higher yields (and in shorter time) compared to *A. pernix*
- specific modifications allow for simple purification and detection

- faster action compared to other enzymes (< 10 min)
- pepsinase is efficient in a broad temperature range (50-125°C)
- pepsinase works in a wide pH range (3-10) and in presence of detergents and other denaturants
- pepsinase may be used in combination with detergents
- environmentally friendly compared to aggressive chemical cleaning agents
- high efficiency allows for degradation of higher concentrations of contaminants