PROTEASE

Introduction

Proteases (also called **peptidases or proteinases**) are enzymes that catalyze the **hydrolysis of peptide bonds** in proteins and polypeptides, breaking them down into smaller peptides or amino acids.

General reaction:

Protein+H2O→ProteasePeptides and/or AminoacidsProtein + H_2O \xrightarrow{\text{Protease}} Peptides \; \text{and/or} \; Amino acidsProtein+H2OProtease Peptidesand/orAminoacids

Proteases are among the **most important industrial enzymes**, accounting for **over 60% of total global enzyme sales**. They are widely used in the **detergent industry, food processing, pharmaceuticals, leather industry, and biotechnology**.

Microorganisms (bacteria and fungi) are the main source for commercial protease production due to their **low-cost cultivation, high yield, and scalability**.

Classification of Proteases

Proteases are classified based on **site of action** and **mechanism**:

1. Based on Site of Action

- **Endopeptidases:** Break internal peptide bonds (e.g., trypsin, pepsin).
- **Exopeptidases:** Act on terminal bonds to release single amino acids (e.g., carboxypeptidase).

2. Based on Catalytic Mechanism

- **Serine proteases** – Active site contains serine (e.g., subtilisin).
- **Cysteine proteases** – Use cysteine residue (e.g., papain).
- **Aspartic proteases** – Use aspartic acid residues (e.g., pepsin).
- **Metalloproteases** – Require metal ions, usually Zn^{2+} (e.g., thermolysin).

Sources of Protease

1. Animal Sources

- Pepsin (stomach of animals).
- Trypsin and chymotrypsin (pancreas).
- Rennin (used in cheese making).

2. Plant Sources

- Papain (*Carica papaya*).
- Bromelain (*Ananas comosus*, pineapple).
- Ficin (from fig tree).

3. Microbial Sources (Most Important Industrially)

- **Bacteria:** *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus amyloliquefaciens*.
- **Fungi:** *Aspergillus oryzae*, *Aspergillus niger*.
- Microbial proteases are preferred due to:
    - High productivity.
    - Genetic manipulability.
    - Easy large-scale fermentation.
    - Wide range of stability (pH, temperature).

Production of Protease

1. Selection of Microorganism

- Alkaline protease producers: *Bacillus licheniformis*, *Bacillus subtilis*.
- Acid protease producers: *Aspergillus niger*, *Mucor* spp.

2. Fermentation Process

- **Submerged Fermentation (SmF):**
    - Most common.
    - Uses liquid media containing carbon (glucose, starch) and nitrogen (soy flour, peptone).
    - Controlled aeration and agitation optimize enzyme yield.
- **Solid State Fermentation (SSF):**
    - Uses agro-wastes like wheat bran, rice husk, soybean meal.
    - Cost-effective, especially for fungal proteases.

3. Induction and Regulation

- Protease production is influenced by:
    - **Inducers:** proteins (casein, gelatin) in medium.
    - **Catabolite repression:** high glucose inhibits protease production.

4. Downstream Processing

- Biomass separated by filtration/centrifugation.
- Crude enzyme concentrated by precipitation (ammonium sulfate) or ultrafiltration.
- Purification by ion-exchange and gel-filtration chromatography.
- Enzyme formulated as liquid or powder for commercial use.

Mechanism of Action

The catalytic mechanism depends on protease type. Example: **Serine proteases** (like subtilisin).

1. **Substrate Binding** – Protein binds to enzyme active site.
2. **Nucleophilic Attack** – Active site serine attacks peptide bond.
3. **Acyl-Enzyme Intermediate** – Peptide bond cleaved, part of substrate attaches to enzyme.
4. **Hydrolysis** – Water molecule breaks the intermediate.
5. **Product Release** – Smaller peptides or amino acids released.

This allows continuous breakdown of proteins into usable products.

Applications of Protease

1. Detergent Industry (Major Use)

- **Alkaline proteases** (from *Bacillus licheniformis*) added to laundry detergents.
- Remove **proteinaceous stains** (blood, food, sweat).
- Remain active in high pH and high temperature.

2. Food Industry

- **Cheese making:** Rennin and fungal proteases for milk coagulation.
- **Meat tenderization:** Papain and bromelain used.
- **Baking:** Improves dough texture and gluten modification.
- **Beer brewing:** Prevents haze formation.

3. Pharmaceutical Industry

- Proteases used in **digestive aids** (pepsin, trypsin supplements).
- Used in **wound debridement** to remove necrotic tissue.
- Papain-based protease preparations for anti-inflammatory therapy.

4. Leather Industry

- Used in **dehairing and bating** of hides.
- Eco-friendly alternative to harsh chemicals (lime, sulfides).

5. Textile Industry

- Proteases used in **wool and silk finishing**.
- Removes impurities without damaging fabric.

6. Waste Management

- Proteases degrade protein-rich wastes (e.g., meat industry effluents).
- Used in **bioremediation** of keratin-rich wastes (feathers, hair).

Microbial Protease Production

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Microbial Strain (Bacillus / Aspergillus)
                ↓
       Fermentation (SmF / SSF)
                ↓
  Enzyme Secretion into Medium
                ↓
Filtration / Centrifugation of Broth
                ↓
   Enzyme Concentration & Purification
                ↓
    Industrial / Commercial Protease
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Future Prospects

- **Genetic engineering and protein engineering** to create thermostable and pH-stable proteases.
- Application in **biopharmaceuticals** for targeted peptide processing.
- Use in **eco-friendly industries** to replace harmful chemicals in leather and textile processing.
- **Immobilized proteases** for reusable biocatalysts in continuous industrial processes.

Conclusion

Proteases are the **largest and most commercially important group of industrial enzymes**, vital in detergents, food processing, pharmaceuticals, and leather industries. Microbial proteases, especially from *Bacillus* and *Aspergillus*, dominate global production due to high yield, stability, and scalability.

With advances in **biotechnology and protein engineering**, proteases will continue to play an increasingly significant role in **green industrial applications, medicine, and environmental management**.