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Impact of industrial microbial alkaline proteases from isolated *bacillus* strains

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ABSTRACT

Proteases of commercial importance are produced from microbial, animal and plant sources. They constitute a very large and complex group of enzymes with different properties of substrate specificity, active site and catalytic mechanism, pH, temperature activity and stability profiles. Industrial proteases have application in a range of process taking advantage of the unique physical and catalytic properties of individual proteolytic enzyme types. This vast diversity of proteases, in contrast to the specificity of their action has attracted worldwide attention in attempts to exploit their physiological and biotechnological applications. Proteases represent one of the three largest groups of industrial enzymes and have traditionally held the predominant share accounting for about 60% of total worldwide sale of enzymes. The overall protease enzyme market showing a compound annual growth rate of 6.3% from 2024 to 2030 to reach 11.42 billion US$ by 2030.

Key words: *Industrial protease, Substrate specificity, Catalytic mechanism, Stability.*

Introduction

Development Proteases (EC 3.4.21-24 and 99; peptidyl-peptide hydrolases) are enzymes that hydrolyse proteins via the addition of water across peptide bonds and catalyse peptide synthesis in organic solvents and in solvents with low water content. The hydrolysis of peptide bonds by proteases is termed as proteolysis; the products of proteolysis are protein and peptide fragments, and free amino acids (Sookkheo *et al.,* 2000 and Beg *et al.,* 2003).

According to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology, proteases are classified in subgroup 4 of group 3 (hydrolases). Proteases can be classified according to 3 major criteria. Such as; the reaction catalysed, the chemical nature of the catalytic site and the evolutionary relationship, as revealed by the structure.

Proteases are broadly classified as endo- or exoenzymes. On the basis of their site of action on protein substrates. They are further categorized as serine proteases, aspartic proteases, cysteine Proteases or metallo proteases depending on their catalytic mechanism. They are also classified into different families and clans depending on their amino acid sequences and evolutionary relationships. Based on the pH of their optimal activity, they are referred to as acidic, neutral, or alkaline proteases (Rao *et al.,* 1998).

Applications of Alkaline Proteases in various industries

Food Industry

Alkaline proteases can hydrolyse proteins from plants and animals to produce hydrolysates of well-defined peptide profiles. These protein hydrolysates play an important role in blood pressure regulation

and are used in infant food formulations specific therapeutic dietary products and the fortification of fruit juices and soft drinks. In recent years there has been substantial interest in developing enzymatic methods for the hydrolysis of soya protein, gelatin, casein, whey and other proteins in order to prepare protein hydrolysates of high nutritional value. In developing commercial products from these proteins, emphasis is placed on achieving a consistent product in high yields, having desirable flavour, nutritional and/or functional properties (Ward *et al.,* 1991).

Alkaline protease from *B. licheniformis* is used for the production of highly functional protein hydrolysates. This commercial alkaline protease, Alcalase, has a broad specificity with some preference for terminal hydrophobic amino acids. Using this enzyme, a less bitter hydrolysate and a debittered enzymatic whey protein hydrolysate were produced. Soluble meat hydrolysate can also be derived from lean meat wastes and from bone residues after mechanical deboning by solubilization with proteolytic enzymes. Alcalase has been found to be the most appropriate enzyme in terms of cost, solubilization, and other relevant factors. In an optimized process with Alcalase at a pH of 8.5 and temperature of 55-60ºC, a solubilization of 94 % was achieved. The resulting meat slurry was further pasteurised to inactivate the enzyme and found wide application in canned meat production, soups and seasoning (Kumar and Takagi *et al.,* 1999).

Pseudomonas aeruginosa was used to produce alkaline protease by utilizing available agricultural residues (wheat bran) via solid state fermentation (SSF). The selected orthogonal array was L-8 and optimum factors for alkaline protease production by chosen strain were found to be pH 9.0, NaCl (12.5%), temperature (45 1C), inoculum size (5 ml) and agitation speed (0.78g). Under this optimum condition, alkaline protease production by *Pseudomonas aeruginosa* was found to be 582.2579.2 U/ml. The experimental data showed 97.24% resemblance with the expected data (Meena *et al.,* 2013).

Detergent Industry

Enzymes have long been of interest to the detergent industry for their ability to aid the removal of proteinaceous stains and to deliver unique benefits that cannot otherwise be obtained with conventional detergent technologies. The use of enzymes in detergent formulations is now common in developed countries, where more than half of the presently available ones contain enzymes. Detergent enzymes account for approximately 30% of total worldwide enzyme production and 89% of the total protease sales in the world; a significant share of the market is captured by subtilisins and/or alkaline proteases from many *Bacillus* species (Horikoshi *et al.,* 1996 and Gupta *et al.,* 2002b).

Ideally alkaline proteases used in detergent formulations should have high activity and stability over a broad range of pH and temperature, should be effective at low levels (0.4-0.8%) and compatible with various detergent components along with oxidizing and sequestering agents. They must also have a long shelf life (Kumar and Takagi *et al.,* 1999).

The main producers of alkaline proteases using species of *Bacillus* are the companies such as Novo Industry A.I.S. and Gist Brocades. Novo produces three proteases, Alcalase from *B. licheniformis*, Esperase from an alkalophilic strain of a *B. licheniformis* and Savinase from an alkalophilic strain of a *B. amyloliquefaciens*. Gist Brocades on the other hand produces and supplies Maxatase from *B. licheniformis*. Alcalase and Maxatase (both mainly subtilisin) are recommended to be used at 10-65ºC and pH 7-10.5. Savinase and Esperase can be used at up to pH 11 and 12 respectively (Chaplin *et al.,* 1990).

An alkaline protease produced from an alkalophilic *Bacillus* strain MK5-6. The ability of this to clean UF membranes fouled during milk processing was determined in combination with two alkaline UF membranes cleaner, Alconax and Ultrasil. The alkaline enzyme that is extremely stable in Alcanox was optimal at 5 g/l in cleaning the fouled membranes when added to the same membrane cleaner. For these reasons, they believe the alkaline protease preparation from alkalophilic *Bacillus* strain MK5-6 as an attractive candidate to be used as membrane cleaner additive (Kumar *et al.,* 1999 and Tiwari *et al.,* 1999).

Subtilisin GX from alkaliphilic *Bacillus* sp. GX6644 (ATCC 53441) produced that was found to possess properties suitable as a detergent additive (Durham *et al.,* 1987).

Leather Industry

The conventional methods in leather processing involve the use of hydrogen sulfide and other chemicals, creating environmental pollution and safety

hazards. Thus, for environmental reasons, the biotreatment of leather using enzymatic approach is preferable as it offers several advantages, e.g. easy control, speed and waste reduction. Proteases find their use in the soaking, de-hairing and bating stages of preparing skins and hides. The enzymatic treatment destroys undesirable pigments, increases the skin area and thereby clean hide is produced (Gupta *et al.,* 2002b).

Alkaline proteases with elastolytic and keratinolytic activity can be used in leather processing industries. During bating, the hide is softened by partial degradation of the interfibrillar matrix proteins (elastin & keratin). Therefore enzyme preparations with low levels of elastase and keratinase activity but no collagenase activity are particularly applicable for this process (Cowan *et al.,* 1994).

Bating is traditionally an enzymatic process involving pancreatic proteases. However, recently, the use of microbial alkaline proteases has become popular. The substitution of chemical depilatory agents in the leather industry by proteolytic enzymes produced by *Bacillus* sp. could have important economical and environmental impacts where the de-hairing process is accelerated by the use of alkaline proteases (Anwar *et al.,* 1998).

Medical usage

Alkaline proteases are also used for developing products of medical importance. It was exploited the elastolytic activity of *B. subtilis* 316M for the preparation of elastoterase, which was applied for the treatment of burns, purulent wounds, carbuncles, furuncles and deep abscesses. The use of alkaline protease from *Bacillus* sp. strain CK 11-4 as a thrombolytic agent having fibrinolytic activity. Furthermore, *Bacillus* sp. has been recognized as being safe to human (Gupta *et al.,* 2002b).

Management of Industrial and Household Waste

Alkaline proteases provide potential application for the management of wastes from various food processing industries and household activities. Feather is composed of over 90% protein, the main component being keratin, a fibrous and insoluble protein. Worldwide several million tons of feathers are generated annually as waste by poultry-processing industries. Feathers constitute approximately 5% of the body weight of poultry and can be considered a high protein source for food and feed, provided their rigid keratin structure is completely destroyed. Pre-treatment with NaOH, mechanical disintegration, and enzymatic hydrolysis results in total solubilization of the feathers. The end product is a heavy, greyish powder with a very high protein content, which could be used as a feed additive (Kumar and Takagi *et al.,* 1999).

An organism isolated from an alkaline soda lake in the Ethiopian Rift Valley Area and identified as *Bacillus pseudofirmus*. *B. pseudofirmus* AL-89, and the protease it produces offers an interesting potential for the enzymatic and/or microbiological hydrolysis of feather to be used as animal feed supplement. An enzymatic process using a *B. subtilis* alkaline protease in the processing of waste feathers from poultry slaughterhouses. A formulation containing proteolytic enzymes from *B. substilis*, *B. amyloliquefaciens* and *Streptomyces* sp. and a disulfide reducing agent (thioglycolate) that enhances hair degradation, helps in clearing pipes clogged with hair-containing deposits and is currently available in the market. It was prepared and patented by Genex (Dalev *et al.,* 1994 and Gessesse *et al.,* 2003).

Photographic Industry

Alkaline proteases play a crucial role in the bioprocessing of used X-ray or photographic films for silver recovery. These waste films contain 1.5-2.0% silver by weight in their gelatin layer. Conventionally, this silver is recovered by burning the films, which causes undesirable environmental pollution. Enzymatic hydrolysis of gelatin not only helps in extracting silver, but also in obtaining polyester film base that can be recycled. Decomposition of the gelatinous coating of X-ray films, from which silver was recovered. Protease B18′ had a higher optimum pH and temperature, around 13.0 and 85ºC. The enzyme was most active toward gelatin on film at pH 10 (Gupta *et al.,* 2002b, Horikoshi *et al.,* 1999 and Fujiwara *et al.,* 1991).

An obligate alkaliphilic *Bacillus sphaericus* strain isolated from alkaline soils in the Himalayas, which produced an extracellular alkaline protease. The alkaline protease of this strain efficiently hydrolysed the gelatin layer of used X-ray films within 12 min at 50ºC and at pH 11.0 (Singh *et al.,* 1999).

Peptide Synthesis

Amino acids are of increasing importance as dietary supplements for both humans and domestic animals. Only the L-amino acids can be assimilated by living organisms, since the chemical synthesis of

amino acids produces a racemic mixture, it is necessary to separate the isomers before commercial use. Alcalase is a proteolytic enzyme isolated from a selected strain of *B. licheniformis*, its major component being subtilisin Carslberg. It was determined that Alcalase was stable in organic solvents and could be of use as a catalyst in the resolution of *N*-protected amino acids having unusual side chains (Anwar *et al.,* 1998).

Silk Degumming

One of the least explored areas for the use of proteases is in the silk industry where only a few patents have been filed describing the use of proteases for the degumming process of silk. The conventional silk degumming process is generally expensive and therefore an alternative method suggested, is the use of protease preparations for degumming the silk prior to dyeing. In a recent study, the silk degumming efficiency of an alkaline protease from *Bacillus* sp. RGR-14 was studied. After 5h of incubation of silk fiber with protease from *Bacillus* sp., the weight loss of silk fiber was 7.5%. Scanning electron microscopy of the fibers revealed that clusters of silk fibers had fallen apart as compared with the smooth and compacted structure of untreated fiber (Gupta *et al.,* 2002b).

Conclusion

Isolate *bacillus spp.* shows higher alkaline protease production at pH 9. The alkaline protease produced by this isolate was active and stable at high alkaline and salt concentration. The protease stability and high enzyme activity at moderate (25 °C-45 °C) and at room temperatures, respectively, was an attractive feature to develop enzyme based industrial processes at room temperatures. In addition production of the enzyme under SSF offer an advantage in minimizing production cost. Future research recommended as testing the alkaline protease for other suggested potential industrial applications and scale up studies.

Conflict of Interests

The authors declare that they have no conflict of interests.

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