A review on alkaline protease producers and their biotechnological perspectives

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The primary sources for occurrence of alkaline protease producers were found alkaline habitats viz. soil and water of soda lakes and deserts beside alkaline springs. Among bacteria, many efficient alkaline protease producers belong to the genus Bacillus. Alkaline proteases are the most versatile industrial enzymes that execute a variety of functions and have tremendous important biotechnological applications. Keeping in view the industrial importance of the alkaline proteases and their producers, research is now being focusing on the discovery of the alkaline proteases with novel intrinsic properties.

[Keywords: Alkaline protease based products, Alkaline protease producers, Alkaline habitats, Biotechnological applications, Classification of alkaline proteases, Efforts in production]

Introduction

The organisms producing alkaline proteases are called as alkaline protease producers. Most of the alkaline protease producers are alkaliphiles which is a group of microorganisms that grow optimally at or above pH 9.0, but cannot grow or grow slowly at near neutral pH.

First few reports about the isolation of alkaliphiles (1889-1959)

Horikoshi and coworkers have reported the first comprehensive data about alkaliphiles in that they have included many pioneer researchers and their work.1,2. The pioneer researchers like Miquel (1889), Meek and Lipman (1922), and Downie and Cruickshank (1928) have reported for the first time about alkaliphiles.3,4 Amongst these pioneer researchers Miquel (1889) described the first alkaliphilic bacterial species Urobacillus pasteurii. Later on this species was renamed as Bacillus pasteurii and at present this species is known as Sporosarcina pasteurii. Meek and Lipman (1922) and Downie and Cruickshank (1928) have reported microorganisms living in alkaline environment viz. Nitrosomonas sp., Nitrobacter sp. and Streptococcus faecalis.5,6 In 1956, antifungal activity of alkaliphiles like Bacillus circulans was first time recorded by Horikoshi.7 Beside natural alkaline ecosystem, research has also shown the existence of alkaliphiles in clinical samples viz. fecal sample. In 1959, Chesbro and coworkers have isolated Streptococcus faecalis and Streptococcus faecium from fecal sample that showed luxurious growth in highly alkaline media.8 Kushner and Lisson (1959) have used media having gradual increment in pH for subculturing of Bacillus cereus and observed enhanced resistance towards alkaline pH.

Potential search with reference to various application oriented studies in early stages (1959-1980)

The early pioneering studies of alkaliphiles from a growing number of researchers and organizations, contributed in the new application oriented and emerging fields like biotechnology. In Japan indigo has been naturally reduced by alkaliphilic bacteria in presence of sodium carbonate from ancient times. In 1959, for the first time a bacterial protease subtilisin Carlsberg from a neutrophilic Bacillus licheniformis was added into a commercial detergent. Since the neutral proteases were very unstable enzymes in detergents, therefore only the alkaline proteases were the need for various industrial applications. Takahara and coworkers (1960, 1961, and 1962) used alkaliphilic Bacillus sp. no. S-8 and improved the indigo fermentation process.8,9,10 Therefore indigo dyeing is the first recorded industrial application of alkaliphilic bacteria in the world. Later on, Horikoshi has reported most of the alkaline enzymes between the 1960 and 1980; however, he has not reported any application before 1969.11 In 1968, Aunstrup et al.
have filed a patent for preparation of proteases having greatest activity at high alkalinity. In 1971, the first report about the use of alkaline protease from *Bacillus clausii* 221 was published by Horikoshi. Later on many new types of alkaline proteases have been isolated by many researchers. In 1972, Aunstrup and coworkers carried out a large screening program to find alkaline protease producers to be used for industrial applications. Andressen et al. (1972) filed a patent for dehairing of leather by high alkaline protease that was active and stable at pH 12.5 in saturated lime solution. In 1973, Viccaro published a patent on a low-cost method for production of alkaline protease from *B. licheniformis*, and showed the applicability of this protease in the formulation of detergent. This enzyme showed the stability and catalytic efficiency at a greater extent than that of commercial detergents available at that time. In 1974, Tsuru et al. also published a patent on production of detergent compatible alkaline protease. Later on detergents containing alkaline enzymes have been expanded worldwide and published many research papers and patents on the same. In 1975, Hidemasa et al. published a patent on a new alkaline protease bacillopeptidase C and its production by cultivating *Bacillus* sp. No. 794. This new kind of alkaline protease exhibited a strong anti-inflammatory activity. In 1977, George patented a method using alkaline proteases for clarifying aqueous solutions of xanthan gum, beer and whole fermented broth from xanthan-producing microorganism such as *Xanthomonas campestris* and other species of *Xanthomonas*. To solve the pollution problems caused by earlier detergents, phosphates like sodium tripolyphosphate were replaced by the nature intended chemical catalyst zeolites in detergents. Furthermore, special additives like proteases, amylases and lipases were incorporated into the detergents to enhance their performance. At present, among the many alkalozymes from alkaliphiles, alkaline proteases are one of the essential biobuilders required in formulation of commercial detergents.

Production protocol reference for showing efforts in production (1983-1998)
Beside natural isolates genetically engineered strains were also used by many researchers to enhance the productivity and efficiency. Many researchers have optimized alkaline protease production by adopting different techniques and designs. Phadatare et al. (1993) have observed that, proteases from fungal origin offer an advantage over the bacterial protease as the mycelia of fungi can be easily removed by filtration whereas fermented media using bacteria require cost-intensive filtration methodologies.

Characterization of alkaline proteases (1998-2001)
Various activators and inhibitors viz. phenyl methyl sulphonyl fluoride (PMSF), di-isopropyl fluorophosphates (DFP), p-chloromercuric benzoate (pCMB), disodium ethylene diamine tetra acetic acid (Na2EDTA), water soluble and water insoluble solvents and metal cations (Zn +2, Mg +2, Na +, Ca +2, Al +3, Cu +2 and Hg +2) were found to affect on the catalytic efficiencies of the alkaline proteases. Furthermore, few metal cations were found to enhance the thermal stability of alkaline proteases. Serine alkaline proteases were found to be completely inhibited by phenylmethysulfonyl fluoride (PMSF) and di-isopropyl fluorophosphate (DFP). Many researchers have recorded the molecular weights of alkaline proteases ranging from 15-30 kDa with few exceptions of 31.6, 33, 36 and 45 kDa. Anwar and Saleemuddin (1998) have given a list of some industrially important alkaline protease producers and optimum pH value and pH stability range of alkaline proteases from them. Banerjee et al. (1999) reported some characteristics of thermostable alkaline protease from *Bacillus brevis* MTCC 0016. In 1999, Samartnarn et al. have reported the pH and temperature optima of the alkaline protease from *Aspergillus oryzae* U1521 as 8-9 and 45 °C, respectively.

In 1999, Hutadilock-Towatana et al. have reported molecular mass of alkaline protease using SDS-PAGE and native-PAGE, from *Bacillus* sp. PS719, as 42 and 42.2 kDa, respectively. PS719 alkaline protease exhibited optimum activity at pH 9.0 and 75 °C temperature. Alkaline proteases may have different characteristics at free and immobilized state. Immobilization of alkaline proteases for industrial use has the potential advantages of ease in reuse and recovery.

Application of alkaline proteases (2002-2011)
Various applications of alkaline proteases related with photographic, food, feed, pharmaceutical and leather industries have been discussed below.
Pandhare (2002) has reported many applications of alkaline protease inhibitors (APIs) in basic and applied research, as well. APIs can be used in therapeutics and biocontrol agents.
In 2002, Sjodahl et al. have reported that the proteolytic enzymes help for a gentle and selective debridement, which support natural healing process in the successful local management of skin ulcerations. Alkaline proteases that have elastolytic activity were used for the treatment of burned area of body, purulent skin, carbuncled region, furuncle parts and deeply abscessed wounds. Recently a sprayable wound debrider has been developed and patented by David and Robert. Ahmed et al. (2007) have extracted collagen hydrolysate from chicken skin by using the alkaline protease from Bacillus licheniformis ATCC21415. Chunling et al. (2007) have reported that bioactive peptides were produced after digestion of shrimp (prawn) protein, proteins from spirulina, proteins from marine yeasts like Yarrowia lipolytica N3C and Hanseniaspora uvarum YA03a and casein with the purified alkaline protease. These bioactive peptides exhibited remarkable angiotensin I converting enzyme (ACE) inhibitor activity and antioxidant activity. Similar type of study was also carried out by Jing et al. (2009) using four alkaline protease producing yeasts viz. Issatchenka orientalis, Aureobasidium pullulans, Cryptococcus aureus and Yarrowia lipolytica. Protein from spirulina was hydrolyzed by the crude alkaline protease from Aureobasidium pullulans strain HN3.11 and that formed hydrolysate was found to have remarkable antioxidant and angiotensin I converting enzyme (ACE)-inhibitory activity. Alkaline protease from Fusarium sp. strain BLB showed excellent thrombolytic activity and Takumi (2009) patented a process for the preparation of the same. Parmely et al. (2009) patented a method for the treatment of inflammation. Alkaline protease formulations are used to produce tryptic and peptic casein hydrolysates which are used in cystic fibrosis treatment.

In photographic industries enzymatic hydrolysis of gelatin helped in extracting silver from used photographic films and polyester film bases. In this regard, many researchers have shown applications of alkaline proteases in photographic industries.

Gupta and Beg (2002) have published a patent for the process of production of industrially important alkaline protease that has application in detergent formulation. Alkaline protease from Bacillus PE-11 showed compatibility with selected commercial detergents in the presence of Glycine and CaCl₂. Schallmey et al. (2004) have reported some industrially important alkaline protease producers viz. Bacillus clausii, Bacillus amyloliquefaciens and Bacillus halodurans that find the application in formulation of detergents.

Kumar et al. (2008) have given the compositions of typical powder, liquid and gel form detergents. Efficacy of alkaline protease for destaining was reported by many researchers. These enzymatic properties of many alkaline proteases have suggested their suitable application as additive in detergent formulations.

Takahashi et al. (2004) has suggested that a soybean-milk coagulating enzyme (SMCE) from Bacillus pumilus TYO-67 can be used for the production of processed foods from soybean milk since the SMCE is a strong alkaline protease and it induces soybean-milk-coagulation by the digestion of soy-proteins. Alkaline proteases are significant for industrial perspective because of their ability to hydrolyze feather proteins and soy-proteins. Alkaline protease is used in the preparation of food additive that help to reduce blood fats. The method of preparation of the same was patented by Ethics and coworkers.

Dayanandan et al. (2003) have isolated a potent dehairing alkaline protease to be used as cleaner in leather processing from Aspergillus tamarii by solid state fermentation (SSF). This enzyme has not only reduced the pollution problem of BOD, COD and TDS but also improved the strength of the leather. Laxman et al. (2004) published a patent on the process for alkaline protease preparation. Moreover, they have used this protease preparation in the leather pretanning process. Many researchers have shown the successful application of alkaline protease in leather industries.

Rahman et al. (2005) isolated the organic solvent-stable protease producer Pseudomonas aeruginosa strain K. Kumar and Bhalla (2005) have marked the important role of microbial proteases in peptide synthesis and elucidated different types of proteases involved in synthesis of many bioactive peptides.

Alkaline protease from Bacillus licheniformis MZK-3 showed keratinolytic activity. Recently Clemmons and Holmes (2007) patented the enzyme based method for increasing the efficiency of grease traps in order to reduce the fats, oils and grease (FOG) passing through grease traps into the public wastewater collection system.

Silk degumming is a key process during which sericin is removed by thermo-chemical treatment of
the cocoon; however degumming process imposes a relatively harsh environment on the silk fibroin by changing microstructure and mechanical properties of fibroin. Keeping in view the industrial importance of alkaline proteases, researchers are more and more focusing on discovery of alkaline proteases with novel intrinsic properties and enzyme engineering to meet the industrial requirements as well as current increasing demand of global enzyme market.

Emerging potential of alkaliphiles and alkaline protease producer (2011-13)

Beside alkaline protease, alkaline protease producers have also found to produce other industrially important enzymes such as amylase, cellulase, lipase, catalase, xylanase, pectinase and chitinase. Carotenoids from alkaliphiles were also found the great importance in food industries.

Recently cells of Bacillus megaterium MTCC 2444 were immobilized by calcium alginate entrapment method and enhanced alkaline protease production was recorded.

Now a days, many diverse alkaliphilic and alkalitolerent microorganisms are used in bioremediation process such as treatment of textile effluents since these effluents have high alkaline pH. The representative example of alkaliphilic bacterial strain Bacillus sp. that showed degradation of azo dyes up to 100 mg/L in 24 h under aerobic condition. Recently, a moderate halophilic and alkalitolerent Halomonas axialensis was isolated from the salty effluent samples of textile industries from central Iran. This bacterium has shown remarkable azo dyes decolorizing ability over wide ranges of pH (7–11) and temperature (25–45°C). Similar type of aerobic decolorization of textile azo dye was reported from an alkaliphilic bacterium Bacillus cohnii MTCC 3616. Many researchers have used the consortium of different alkaliphiles for the degradation of azo dyes. Currently, immobilized bacterial cells of alkaliphiles are being exploited for degradation study of azo dyes.

Highlights of commercial products produced till the date (2013-2015)

Recently, Velloorvalappil et al. (2013) and previously, Gupta et al. (2002) have reported the names of some commercial alkaline proteases from different bacterial sources that are sold by a lot of suppliers under various trade names. Novo Nordisk, Denmark is supplier of Alcalase™, Savinase™, Esperase™, Biofeed™ pro, Durazym™, Novozyme™ 471MP, Novozyme™ 243 and Nuc™. Genencor International, USA is supplier of Purafect™ and Primatan™. Gist-Brocades, Netherlands is supplier of Subtilisin™, Maxaca™ and Maxatase™. Solvay Enzymes, Germany is supplier of Opticlean™, Optimase™, Maxapem™ and HT-proteolytic protease™. Amano Pharmaceuticals, Japan is supplier of Protease™, Collagenase™ and Amano protease S™. Enzyme Development, USA is supplier of Enzeco™ alkaline protease, Enzeco™ alkaline protease-L FG and Enzeco™ high alkaline protease. Nagase Biochemicals, Japan is supplier of Bioprase™ concentrate, Ps. Protease™, Ps. Elastase™, Cryst. Protease™, Bioprase™ and Bioprase™ SP-10. Godo Shusei, Japan is supplier of Godo-Bap™. Rohm, Germany is supplier of Corolase™ 7089. Wuxi Synder Bioproducts, China is supplier of Wuxi™. Advanced Biochemicals, India is supplier of Protosol™. Most of the above give proteases were reported from Bacillus genus. These proteases have applications in detergents, food, pharmaceutical, cosmetic and photographic industries.

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References


