A REVIEW ON FUNGAL ALKALINE PROTEASE

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Abstract

The use of enzymes around the world in different industries has increased tremendously. Various enzymes, specifically proteases are the most essentially used in different industries, such as textile, detergent, leather, feed, waste, dairy and others. Proteases are found ubiquitously, such as in plants, animals, and microbes. Among different producers of proteases microbes are mostly commercially exploited for proteases. Even though a good number of bacterial alkaline proteases are commercially available, fungi exhibit a wider variety of proteases than bacteria. The current review focuses on the comparison among different fungal alkaline proteases and application at the industrial level. The effect of various physiochemical parameters on alkaline protease is also discussed.

Key terms- Alkaline protease, Fungal protease.

Introduction

Fungi grow in the environment where the nutrients are macromolecular in nature. These nutrients are not utilizable by the fungi as such if they are not cleaved into smaller molecules by the action of enzymes secreted by fungi themselves. So for the cleavage of proteins or polypeptides in the environment, fungi produce extracellular proteases. The extracellular proteases produced by fungi have some advantages over other sources due to easy removal of mycelium from the growth medium and their downstream processing is very easy in comparison to bacterial proteases. Furthermore, fungi are normally GRAS (generally regarded as safe) strains and they produce extracellular enzymes, which are easier to be recovered from fermentation broth (Sandhya *et al*., 2005).

Of the different types of proteases the alkaline proteases are of considerable interest because they are most commercially important ones. Proteases account for the 60-65% of the global industrial enzyme market and out of this 25% is constituted by alkaline protease (Bhosale *et al*., 1995, Rao *et al.,* 1998).

As per my knowledge concern no such report was found for fungal alkaline protease. Therefore in present review, primary emphasis has been placed on the isolation, production and characterization of fungal alkaline protease. Additionally the applications of fungal alkaline protease have also been included in this review.

Screening and selection of extracellular alkaline protease producing fungal strain:

Alkaline protease producing fungi have been found in different in habitats such as garden soil, agricultural soil, decaying wheat straw, spoiled cheese, spoiled meat, waste water etc. Proteolytic fungi from the soil samples were isolated on Reese agar plate containing casein as the protein substrate by Choudhary and Jain (2012). A few mould cultures of *Rhizopus* sp. were isolated from soil samples of Lahore area by pour plate method by Ikram-Ul-Haq (2003). *Aspergillus flavus* strain IMI327634 was isolated from soils of Madras by Malathi and Chakraborty (1991). Nehra *et al*., (2002) used wheat straw, decomposing poultry waste and spoiled meat for isolation of proteolytic *Aspergillus* sp. In Thailand, a yellow-green *Aspergillus flavus* var *columnaris* was isolated from the soy sauce industry and used in production of protease by Impoolsup *et al.,* (1981). Mulimani *et al*., (2002) isolated a bleach stable and alkali-tolerant protease producing fungus from spoiled casein.

Several fungal strains producing extracellular proteases were screened from different biotopes by Siala *et al.,* (2009). Of these isolate 11 exhibited a large zone of hydrolysis on skim milk agar plates.

An Extracellular bleach stable proteases from the fungus *Aspergillus clavatus* ESI isolated from waste water by Hajji *et al*., (2007).

The fungal populations in the alkaline soil samples collected from Eastern Ghats, Kolli hills region was studied by using PDA medium. Among these population 23 strains of fungi belong to 5 genus such as *Trichoderma, Aspergillus sp. Penicillium sp. Rhizopus, Mucor* showed protease production on casein and skimmed milk agar (Palanivel., 2013).

An alkaline protease producing *Aspergillus* sp. was isolated from agricultural soil of Jabalpur MP India by Sharma *et al*., (2012). *Aspergillus oryzae* AWT20 was isolated from spoiled cheese showed alkaline protease production (Sharma *et al*., 2005).

Table: 1- Major fungal species employed in alkaline protease production.

Fungal species	Reference
1. *Aspergillus flavus*	Malathi and Chakraborty, 1991
2. *Aspergillus fumigatus*	Monod et al., 1991, Larcher *et al*., 1992
3. *Aspergillus melleus*	Luisetti *et al*., 1991
4. *Aspergillus niger*	Barthomeuf *et al*., 1992 Devi et al., 2008 Sharma and De, 2011
6. *Aspergillus sojae*	Hayashi *et al*., 1967
7. *Aspergilus sydowi*	Danno and Yoshimura, 1967
8. *Cephalosporium*	Tsuchiya *et al.*, 1987
9. *Aspergillus terreus*	Chellapandi, 2010 Sharma, 2013 Ali, 2008, Wu *et al*., 2006
10.*Aspergillus nidulans HA10*	Charles et al., 2008
11.*Aspergillus funiculosus*	Shumi *et al*., 2004
12.*Aspergillus tamarii*	Boer and Peralta, 2000
13.*Aspergillus clavatus*	Tremacoldi *et al*., 2007 Ogundero and Osunlaja, 1986
14.*Cephalosporium*	Tsuchiya *et al*., 1987
15.*Chrysosporium*	Dozie *et al*., 1994
16.*Conidiobolus coronatus*	Sutar *et al*., 1991 Srinivasan *et al*., 1983 Phadatare *et al*., 1993
17.*Entomophthora coronala*	Jonsson, 1986
18.*Engyodontium BTMFS10*	Sreeja and Chandrasekaran, 2005
19.*Funsarium graminearum*	McKay, 1992
20.*Fusarium sp.*	Nakao *et al*., 1973 Kitano *et al*., 1992
21.*Fusarium tumidium*	Shumi *et al*., 2003
22.*Penicillium griseofulvin*	Dixit and Verma, 1993
23.*Pencillium expansum*	Dahot, 1994
24.*Rhizopus oligosporus*	Rao *et al*., 1998
25.*Rhizopus oryzae*	Banerjee and Bhattacharya, 1992
26.*Trichoderma koningii*	Manonmani and Joseph, 1993
27.*Neurospora crassa*	Lindberg *et al*., 1981

Type of fermentation-

Microbial proteases are predominantly extracellular and can be secreted in the fermentation medium. Fungal alkaline proteases are generally produced by both submerged and solid-state fermentation (Devi *et al*., 2008, Sindhu *et al*., 2009, Impoolsup *et al*., 1981, Chutmanop *et al*.,2008, Anandan *et al*., 2007). SmF processes due to increased fermentation time with low productivity has resulted is a shift toward the SSF systems but the advantage of better monitoring and handling are still associated with the submerged cultures. Pandey *et al*., (1999) also describes the tremendous potential of SSF for the production of various enzymes of industrial importance and their direct agro-biotechnological applications. From commercial point of view SSF offers

many advantages including superior volumetric productivity, use of cheaper substrates, simple downstream processing, use of simpler machinery and lower energy requirements when compared with submerged fermentation (Paranthaman *et al*., 2009).

In the production of protease it has been shown to be inducible and was affected by the nature of the substrate used in fermentation. Therefore, the choice of an appropriate induction is of a great importance. Different carbon sources such as wheat bran, rice bran, rice straw, MOC and Coffee waste have been studied for the induction and biosynthesis of fungal alkaline protease (Sharma ; 2013, Chutmanop *et al*.,2008, Anandan *et al*., 2007,Murthy and Naidu, 2010).

Physical parameters-

Physical parameters like pH and temperature of the fermentation medium play a vital role in the growth and metabolic production of fungal population. The pH of the fermentation medium is reported to have substantial effect on the production of proteases (Yadav *et al*., 2011, Sindhu *et al*., 2009, Hajji *et al*., 2008, Hajji *et al*., 2007). It may affect growth of the fungi either indirectly by affecting the availability of nutrients or directly by action on cell surfaces.

Higher temperature may have some adverse effects on metabolic activities of microorganisms producing proteolytic enzymes (Tunga, 1995). However, some fungi produce heat stable proteases which are active at higher temperatures (Rani *et al*., 2012, Devi *et al*., 2008, Haq *et al*., 2006).

Protease production in fungal cultures is growth associated and is influenced by various factors and their interaction can affect protease productivity.

Carbon sources-

Both biosynthesis and energy generation require a carbon substrate (Stanbury *et al*., 1995). Choice of carbon source depends on the product of fermentation as well as the availability, quality and cost of different sources. Carbohydrates are important carbon sources in the form of simple sugars or polymers but they also provide some oxygen and hydrogen (Zabriskie *et al*., 1980). Glucose has been preferred as a carbon source for protease production by several fungi under submerged conditions. (Srinubabu *et al*., 2007, Devi *et al*.,2008). Suitability of glucose as carbon source for the protease production has been reported by some other workers also (Andrade Barata et al., 2002, Chellpandi Dayandan; 2010). Sucrose was favored for alkaline protease production by *Conidiobolus coronatus* (Bhosale *et al*., 1995) and *Aspergillus flavus* (Oyeleke *et al*., 2010). Gradually metabolized carbon source like glycerol may be selected to slow down vegetative growth in order to increase protease production. Devi *et al*., (2008) reported mannitol as a good carbon source for alkaline protease production by *Aspergillus niger*. Other investigation has been reported glucose as representing the carbon source for protease production (Sharma and Tasin 2009). Increased yield of alkaline protease was reported by Malathi and Chakraborty (1991) who used lactose as a carbon source. Whey, a waste by product of the dairy industry containing mainly lactose and salts has been demonstrated as a potential substrate for alkaline protease production (Mckay A.M. 1992). Paraffin was also found to use by *Fusarium sp*. for the production of increased amount of alkaline protease (Nakao *et al*., 1973).

Nitrogen Source-

The type of nitrogen sources also affected the production of protease. Organic nitrogen sources have been found to be better nitrogen sources for growth and protease production in some fungi (Aleksieva et al., 1981, Rani et. al., 2012, Hajji et ai.,2007, Anandan et al., 2007) and inorganic sources (ammonium sulphate and potassium nitrate) have better enzyme yields in other organisms (Sinha et. al., 1991). Organic nitrogen may be supplied to microorganisms, in the form of amino acids, proteins or urea (Stanbury *et al*., 1995, Zabriskie *et al*., 1980).

Tryptone is a complex nitrogen source that is essentially a peptone produced by proteolytic digestion of casein by the protease trypsin, resulting in a mixture of peptides, amino acids and water soluble vitamins. Phadatare *et al*., (1993), reported tryptone followed by peptone and yeast extract to be good organic nitrogen sources for protease production by *Conidiobolus coronatus*. Casein was found to be an inducer for protein syntheis by *Aspergillus tamarii* [EF661565.1] (Sharma and De, 2011), *A. fumigatus* (Oyeleke *et al*., 2010).

Low level of alkaline protease production was reported with the use of inorganic nitrogen sources in the medium (Ire et al., 2011). However an increase in protease production by the addition of ammonium sulphate was observed by Devi *et al*., (2008). Similarly sodium nitrate was found to be stimulant for alkaline protease production (Choudhary and Jain, 2012). Ammonium nitrate was also found to increase enzyme production (Sindhu *et al*., 2009).

A comprehensive account of culture conditions for fugal alkaline protease production is listed in Table-2.

Table-2 Optimized production conditions for alkaline protease producing fungi

S.No	Fungal sp.	Type of ferment -ation	Carbon Source	Nitrogen source	Temp.	pH	Incubation period	References
1.	*Aspergillus tamarii*	SSF (wheat bran)	Glucose	Peptone	30ºC	9.0	4d	Anandan 2007
2	*Aspergillus flavus*	SmF	Fructose	Beef extract	28ºC	-	72h	Chellapandi 2009
3	*Aspergillus terreus*	SmF	Glucose	Beef extract	28ºC	-	72 h	Chellapandi 2009
4	*Aspergillus flavus AS-2 mutant*	-	Fructose	Soyabean meal + Casein	55ºC	8.5	96h	Rani *et al.*, 2012
5	*Aspergillus niger*	SmF	Glucose	Ammonium sulphate	45ºC	8.5	-	Devi *et al.*, 2008
6	*A. oryzae (Ozykat-1)*	SSF	WB:RB (0.33)	-	30ºC	7.5	60h	Chutmanop *et al.*, 2008
7	*Aspergillus flavus var columnaris*	SSF	WB Koji	Soyabean Koji	30ºC	-	32h	Impoolsup *et al.*, 1981
8	*A. clavatus*-	-	Starch	Yeast extract	30ºC	8	-	Hajji *et al.*, 2008
9	*Aspergillus flavus*	SSF	-	Cornsteep liquor	32ºC	7.5 9.5	48h	Malathi & Chakraborthy, 1991
10	*Aspergillus oryzae*	SSF (Coffee waste)	Maltose	Yeast extract	30ºC	7.0	4d	Murthy & Naidu 2010
11	*Aspergillus flavus MTCC 9952*	SSF, WB: CC 1: 1	-	-	37ºC	9.0	48h	Yadav et *al.*, 2011
12	*Aspergillus terreus*	SSF MOC	-	-	40ºC	9.5	96h	Sharma, 2012
13	*Aspergillus versicolor PF/F/107*	-	Wheat Bran	Sodium nitrate	35ºC	9.0	4d	Choudhary and Jain 2012
14	*Aspergillus flavus*	Smf	Sucrose	Casein	30ºC	8.0	144h	Oyeleke *et al.*, 2010
15	*Aspergillus sp.*	Smf SSF	Starch	Casein	35ºC	8.5	72 h Smf 96 h SSF	Nehra *et al.*, 2002
16	*Penicillium goldleuskii SBSS*	SSF	Glucose	Ammonium nitrate	35ºC	9.0	96h	Sindhu *et al.*, 2009
17	*Ophiostoma piceae*	-	Starch	Soy drink from Soyabean meal	23ºC	-	9d	Abraham and Breuial 1996
18	*Tritirachium album*	-	Glucose	Peptone. YE, $NaNO_3$	28ºC	5.9	24-120 h	Fortelius and Markkanen 2000
19	*Conidiobolus coronatus*	-	Sucrose	NH_4NO_3 tryptone, casein	28ºC	7-7.5	48 h	Bhosale *et al* 1995.
20	*Penicillum sp.*	SSF	Wheat bran	Soy protein	28ºC	-	3d	Agrawal D. *et al.*, 2004
21	*Aspergillu funiculosus G. Smith*	SmF	Lactose	yeast extract KNO_3	37ºC	7.0	-	Shumi *et al.*, 2004
22	*A. Clavatus*	-	-	-	30ºC	7.8 pH	5-7 d	Ogundero., 1986

Purification and characterization of fungal alkaline proteases

The serious purification of enzymes did not begin until after 1920. Most of the early purification was carried out by Willslatter and colleagues between 1922 and 1928.

The application of proteases in the industries require their improved properties like usage at optimum to high temperature, high alkaline pH values, stability over a range of time etc. Recently several alkaline proteases from the different fungal species were isolated in a homogenous state and characterized in detail (Table-3)

Molecular Mass-

Molecular mass range for fungal alkaline protease is very wide. The different molecular mass of the alkaline proteases were obtained from *Aspergillus* species such as 33kDA from *A. fumigatus* (Monod *et al*., 1991), 35 kDa from *A. clavatus* (Tremacoldi *et al*., 2007). The molecular weight of *Aspergillus fumigatus* TKU003 protease is approximately 124 kDa, which is apparently higher than those of other strains of *A. fumigatus* (Wang *et al*, 2005).

Optimum pH and temperature for activity-

Though the effects of pH and temperature on the activity of many alkaline proteases have been reported, a comparative study is difficult, since the conditions of reactions in which the proteases tested were different in different reports. However from an overview of literature it can be seen that the reported optimum pH for casein hydrolysis by most of the fungal alkaline protease is between 8-11pH and temperature between 40-60^{O}C. Higher pH optima 10 to 10.5 have been reported for alkaline proteases of *Aspergillus oryzae* (Babu *et al*., 2007) and *Penicillum expansum* (Dahot, 1994). *Aspergiilus clavatus* showed highest protease activity at pH 9.5 (Celia and Carmona, 2005). A maximum temperature of 55^{o}C was recorded for an alkaline protease from a new strain of *Aspergillus oryzae* AWT20 (Sharma et al., 2006). An alkaline protease purified *Aspergillus fumigatus* TKU0003 maximum activity at 50^{o}C (Wang *et al*., 2005). Damare *et al*. (2006) isolated a deep sea fungus as a source of alkaline and cold-tolerant protease.

Effect of metal ions on protease activity-

A number of peptidases contain or require metal atoms, without which they are inactive. For example carboxypeptidase specifically requires cobalt for its activity and leucine aminopeptidase requires mangneese or magnesium.

Alkaline proteases isolated from different fungal sources behaved differently in the presence of metal ions. At a concentration of 1mM Co^{+2} Fe^{+2} increased the activity of alkaline protease from *T. Koningii*, whereas Na^{+}, Ag^{+}, K^{+} and Pb^{+2} inhibited its activity (Ashour *et al*., 1996). Crude protease from *Aspergillus sp*. (AS#10) showed increased activity in presence of Mg^{+2} and Co^{+2} whereas it showed decreased activity in presence of Hg^{+2}, Ca^{+2}, Na^{+2} and Zn^{+2} (Sharma and Saretha, 2009).

Salts of heavy metals such as silver, copper, mercury and lead inactivate most enzymes in high concentration. Tsuchiya *et al*., (1987) reported protease isolated from *Cephalosporium sp*. KM 338 inhibited by Hg^{+2} Mn^{+2}, Cu^{+2} and Ca^{+2}. Nehra *et al*., (2004) reported that Mg^{+2} were found to be an activator of the alkaline protease enzyme produced by *Aspergillus sp.*

Fungal alkaline proteases are completely inhibited by phenylmethylsulfonyl fluoride (PMSF). Protease activity completely lost in presence of PMSF because it sulfonate the essential serine residue in the active site (Gold and Fahrney, 1964) .This inhibition pattern of these proteases classify them as serine protease (Morihara K 1974).

Industrial Application-

Fungal alkaline proteases have a large variety of applications, mainly in the detergent and food industries. In view of the recent trend of developing eco-friendly technologies, fungal alkaline proteases are envisaged to have extensive applications in leather treatment and in several bioremediation processes. These enzymes are also used to develop high value added products (Kumar and Takagi, 1999).

Detergent industry-

Proteases are one of the standard ingredients of all kinds of detergents ranging from those used for household laundering (Nehra *et al*., 2002) to reagents used for cleaning contact lenses (Anwar and Saleemuddin 2000) or dentures. The ideal detergent protease should possess substrate specificity to facilitate the removal of a large variety of stains due to food, blood and other body secretions. Activity and stablility at high pH and temperature and compatibility with other chelating and oxidizing agents added to the detergents are among the major prerequisites for the use of proteases in detergents.

A number of published reports are available on the compatibility of the fungal alkaline proteases with detergents (Phadatare *et al*., 1993. Hajji *et al*, 2007, 2008, Usama F. Ali 2008, Savitha *et al*., 2011 ,Devi et al., 2008, Niyonzima and More, 2015, Pundir *et al*, 2012) .

The fungal alkaline protease *Conidiobolus coronatus* was consistent with commercial detergents used in India while maintaining 43% of its activity in the presence of calcium (25mM) and glycine (M). These data imply that protease obtained from *C. coronatus* has potential for use in laundry detergents (Lourdes *et al*., 2014).

2. **Leather Industry**- Alkaline proteases with elastolytic and keratinolytic activity have been used in leather processing, especially for the dehairing and debating of skins and hides. The enzymatic process is easy to control, less time consuming and also helps in waste management and is therefore eco-friendly. In addition enzymatic treatment destroys undesirable pigments and increases the skin area, thereby producing clear hide (Dilip K. Arora, 2003).

Few fungal alkaline proteases have been reported to be suitable and find application in leather industry (Malathi & Chakraborty 1991, Pal *et al*., 1996). A protease obtained from *Conidiobolus coronatus* has potential in soaking, dehairing and bating of animal skin/ hide as reported by Laxman *et al*. (US Patent No. 6, 777, 219, 2007) .

3. **Dairy Industry**- The major application of proteases in the dairy industry, is in the manufacture of cheese, proteolysis is responsible for characteristic of most varieties and is indispensable for good flavour and textural development (Fox, 1982). Parera *et al.*, (1993) have used alkaline protease for the production of whey hydrolysate from the cheese whey although rennet is generally the enzyme of choice for cheese making.

In the dairy industry, bovine rennet is still the most widely used in making cheese fungi such as *Rhizomucor miechie, R. pusillus, A. oryzae* are extensively used for the production of proteases for use as milk coagulants (Neelakantan *et al.*, 1999). Another extract with the powder of coagulating milk, already produced industrially, is derived from the fugus *A. niger var. awamori* (Neves Souza and Silva 2005).

4. **Soy Product-** Proteases have been used from ancient times to prepare soy sauce and other soy products. The alkaline protease of fungal origin plays an important role in the hydrolysis of soy protein (Agrawal *et al.*, 2005).

5. **Pharmaceutical Industry**- The wide diversity and specificity of fungal protease are used to great advantage in developing effective therapeutic agents. Oral administration of protease from *Aspergillus Oryzae* (Rani *et al.*, 2012 has been used as a digestive aid to correct certain lytic enzyme deficiency syndromes. Alkaline proteases have also been used for developing production of medical use, such as for the treatment of burns and purulent wound. *A. niger* LCF 9 alkaline protease has a high collagenolytic activity and is being used for therapeutic application (Kumar and Takagi, 1999). Proteases from the tested *Aspergillus* strains exhibited promising hydrolytic activities towards fibrinogen, fibrin and blood clot (El, Shora and Metwally, 2008). *Aspergillus* protease can be used as digestive aids in gastrointestinal disorders such as dyspepsia.

6. **Brewing Industry**- In brewing, proteases have two major applications. They can be used during the cereal mashing process to increase the yield of extract. Though papain, bromelain, and papsin are the traditionally used proteases in chill proofing process, microbial proteases also have been reported to be useful. The rennet produced by *Mucor pusillus* has been reported to be effective for beer clarification (Nelson and Witt, 1973).

7. **Silver Recovery**- Silver is one of the precious and noble metals used in photographic industry. The waste X-ray photographic films containing 1.5%-2% black metallic silver spread in gelatin is good source for silver recovery. Since silver is bound to gelatin it is possible release the found silver by hydrolyzing the gelatine by fungal alkaline protease (Shankar *et al.*, 2010, Choudhary, 2013).

8. **Waste management**- Waste from poultry and leather industry are keratin rich whose polypeptide is densely packed and stabilized by several weak interactions in addition to disulfide bounds.

Fungal keratinases from *Aspergillus oryzae, Chrysoporium inducum, Trichophyton sp, A. terreus, Microsporum gypseum, fusarium oxysporum* have also been studied towards the degradation of keratin (Ali *et al.*, 2011, Sharma *et al.*, 2011, Kim, 2003).

9. **Silk degumming**- Degumming is the process of removing the sericin, or silk gum, from silk. Removing the gum improves the sheen, color and texture of the silk. Use of fungal alkaline protease in degumming of silk is reported in the literature (Gulrajani *et al.*, 2000, More *et al*, 2013).

10. **Other uses**- Alkaline protease from *Condiobolus coronatus* was found to be able to replace trypsin in animal cell cultures (Chiplonkar *et al.*, 1985). Protease has also been used in the reduction of tissue inflammation (Bailey *et al.*, 1977, Nout *et al.*, 1990.

Table-3: Properties of some fungal alkaline proteases

Source	Mol.Wt	Opt. pH	Opt. Temp.	Effect of metal ions		Inhibtory	References
				Stimulatory	Inhibitory		
A. Oryzae	35 kDa	10.0	60ºC	Mg^{+2}, Ca^{+2}	Mn^{+2}, Co^{+2}	PMSF	Murthy & Naidu 2010
A. Clavatus	35 kDa	9.5	40ºC	-	-	PMSF & Chymostatin	Tremacoldi et.al, 2007
A. nidulans HA10	42 kDa	9.0	35ºC	-	-	PMSF	Charles *et al.*, 2008
A. fumigatus TKU003	124 kDa	8.0	40ºC	-	-	PMSF	Wang *et al*, 2004
A. tamarii	48 kDa	-	-	-	-	-	Boer & Peralta 1999
A.flavus var columnaris	-	8.5-9.0	50-55ºC	-	Na^{+2}	PMSF	Impoolsup 1981
A. fumigatus	33 kDA	9.0	-	-	$Mn^{+2,}$ $Zn^{+2,}$ $Co^{+2,}$ $Pb^{+2,}$ Hg++	PMSF antipain Chymostatin	Monod *et al.*, 1991
A. terreus	37 kDa	8.5	37ºC	-	-	-	Chakrabarty *et al*, 2000
A. flavus	46kDa	8.0	45ºC	-	-	PMSF	Hossain *et al.*, 2006
A. funiculosus G. Smith	51kDa	7.5	35ºC	-	-	-	Shumi *et al.*, 2004
A. terreus gr	16+1 kDa	11.0	-	$Ca^{+2,}$ Mg^{+2}	Hg^{+2}	PMSF	Niyonzima and More 2014
A. nigerZl	68 kDa	9.0	40ºC	-	-	PMSF	Coral *et al.*, 2003
A. niger C-15	34 kDa	8.0	60ºC	-	EDTA, HgCl3, $FeCl_3$	-	Kim 2004
A. tamarii EF 661565.1	63 kDa	8-9	60ºC	$Zn^{+2,}$ Mg^{+2}	$Hg^{+2,}$ Co^{+2} $Ca^{+2,}$	PMSF	Sharma & De 2011

A. parasiticus	23 kDa	8.0	40ºC	-	-	PMSF	Rashbehari *et al.*, 2003
A. clavatus ES1	32 kDa	8.5	50ºC	$Ca^{+2,}$ Mg^{+2}	-	PMSF	Hajji *et al.*, 2007
A. fumigatus	33 kDa	9.0	37ºC	-	-	PMSF	Larcher *et al.*, 1992
A. terreus (IJIRA 6.2)	37 kDa	8.5	37ºC	-	-	-	Chakrabarti *et al.*, 2000
A. flavus	33 kDa	-	-	-	-	PMSF	Chen *et al.*, 2009
A. fumigatus	32 kDa	-	-	-	-	PMSF	Reichard *et al.*, 1990
A nidulans PW1	32 kDa	-	-	$Fe^{+2,}$ $Cu^{+2,}$ $Fe^{+3,}$ Hg^{+2}	-	-	Montes *et al*, 2008
A. niger JBPSI-1	-	10.0	55ºC	-	-	PMSF	Sharma &De 2012
Beauveria bassiana	31.5kDa	7.5-9.5	25ºC	-	-	-	Urtz & Rice 2000
F. oxysporum	41kDa	8.0	45ºC	$Ca^{+2,}$ Mg^{+2}	-	Iodoacetamide pepstatin	Barata *et al*, 2002
F.Sp. BLB	27kDa	9.5	50ºC	-	-	-	Veda *et al* 2007
Graphium putredinis	31kDa	7.8	50ºC	-	$Hg^{+2,}$ EDTA	PMSF	Savitha *et al.* 2011
Myceliophthora Sp.	28.2 kDa	9.0	40-45ºC	-	-	PMSF	Zamphorlin *et al.* 2011
Penicillium sp.	-	9.0	45ºC	$Ea^{+2,}$ $Mg^{+2,}$ Mn^{+2}	$Fe^{+3,}$ $Hg^{+2,}$ $Ca^{+2,}$	-	Agrawal *et al*, 2004
P. expansum	20.5 kDa	10.5	35ºC	Co^{+2}	$Hg^{+2,}$ Ag^{+2}	-	Dahot 1994
Trichoderma harzianum	20 kDa	7-8	60ºC	-	EDTA, Hg^{+2}	PMSF	Savitha *et al*, 2011
Trichodeema Koningii	-	10.5	50ºC	Fe^{+2} , $Co^{+2,}$ Ca^{+2}	$K^{+,}$ $Pb^{+2,}$ $Ag^{+,}$ Na^{+}	-	Manonmani & Joseph, 1993
Scopulariopsis Sp.	15+1 kDa	9.0	50ºC	$Co^{+2,}$ Mn^{+2}	$Zn^{+2,}$ Hg^{+2}	PMSF TLCK	Niyonzima & More 2013
A. nidulaus	37kDa	8.5	40ºC	Fe^{+}	$Cu^{2+,}$ $Fe^{3+,}$ Hg^{2+}	-	Pena Montes *et al.*, 2008
F. culmorum	28.7kDa	8.3-9.6	50ºC	-	-	PMSF Chymostatin	Pekkarinen *et al.*, 2002
Tritirachium album	18.5 kDa	7.5-12.0	-	-	-	DIP, PMSF	Ebeling *et al.*, 1974 (117)
Chrysosporium keratinophilum	69kDa	9.0	90ºC	Fe^{+2}	-	1,10 Phenanthroline	Dozie *et al.*, 1994
Rhizopus oligosporus	39 kDa	8.0	50ºC	-	Mn^{++2}	-	Rama Devi *et al.*, 2011

Summary

Commercial exploitation of any enzyme relies on efficient source of their production. Although proteases are ubiquitous in occurrence, fungi are the preferred source in fermentation process because they produce extracellular enzymes, which are easier to be recovered from fermentation broth. Alkaline proteases have various applications in major areas of food processing, beverage production, leather, paper and pulp, textiles, detergents, waste management etc. This review would contribute to the production, isolation and characterization of alkaline proteases from various fungal sources and for commercial application of protease.

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