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Insect gut as a bioresource for potential enzymes - an unexploited area for industrial biotechnology

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ABSTRACT

Recently, insect gut protease has gained great interest in the field of food and industrial biotechnology due to their invisible characteristics and also their ability to act as an alternative source for microbial protease. The insect gut proteases are produced either by themselves or by gut symbiotic microbes, that utilize it for their metabolism. In this review, the importance of insect gut proteases was highlighted in terms of general physio-chemical properties (pH, temperature and metal) and their compatibility with detergents and resistance to solvents with broad applications in various industries such as laundry detergents, bio-medical, food industry and bio-ethanol production. The production of insect gut protease can be increased through emerging biotechnological techniques to meet out the demand for protease in future.

1. Introduction

Most of the industries like leather, food, textile, organic synthesis, pharmaceutical, silk degumming, silver salvage and detergent industry partly depend on enzymatic process which ultimately increases the demand for protease production. Approximately, 60% of industrial enzymes were sold worldwide (Kumar and Takagi, 1999; Souza and Martins, 2001; Otten and Quax, 2005; Zambare et al., 2007; Gupta et al., 2002; Dewan, 2011). Microbes remain a major source for obtaining industrially important proteases (Maurer, 2004). However, most of the alkaline protease from microbes applied in industrial purpose have few limitations, which include low activity and stability towards anionic surfactant (SDS) and oxidizing agent (bleaching agent and hydrogen peroxidase) that are common ingredients in modern detergent formulations (Joo et al., 2003; Sanatan et al., 2013). In addition, the use of bacterial and fungal protease in the detergent additives has a main drawback in terms of the requirement of cost-intensive filtrations to obtain microbial free preparations (Phadtare et al., 1996). Unlikely, the production cost of industrially important microbial enzymes accounts for about 30–40% of the growth medium from the profit. Therefore, to meet the industrial demand of highly active preparations of proteolytic enzyme with appropriate specificity and chemo stability, researchers are searching for alternative source for industrially important protease.

Many insects are polyphagous or monophagous in nature and they are categorized as beneficial and harmful insects (Fig. 1). Till date, insect gut proteases are of main interest among the industrial biotechnologists because of their unusual alkaline micro-environment with midgut pH ranging from 10 to 12 (Christeller et al., 1992). Therefore, insect gut can be used as a promising source for isolating industrially important enzymes (Ahmad et al., 1980; Patankar et al., 2001; Anwar and Saleemuddin, 2000, 2002; Srinivasan et al., 2006; Chougule et al., 2008; Parde et al., 2012; Tabatabaei et al., 2011; Sanatan et al., 2013; Visweshwar et al., 2015). Akbar and Sharma (2017) recommended the use of insect as bio-resource for isolation of insect gut protease due to the short life cycle, voracious feeding of different host plants and production of diverse multiple enzymes (trypsin, chymotrypsin, elastase, cathepsin, aminopeptidase, carboxypeptidase and many serine proteases) from the gut with rapid catalytic and chemo-stable activity.

Some researchers have studied the biochemical characteristics and industrial application of alkaline serine protease (27 kDa) from the gut of *P. americana* (Hivrale et al., 2005, 2011; Sanatan et al., 2013).

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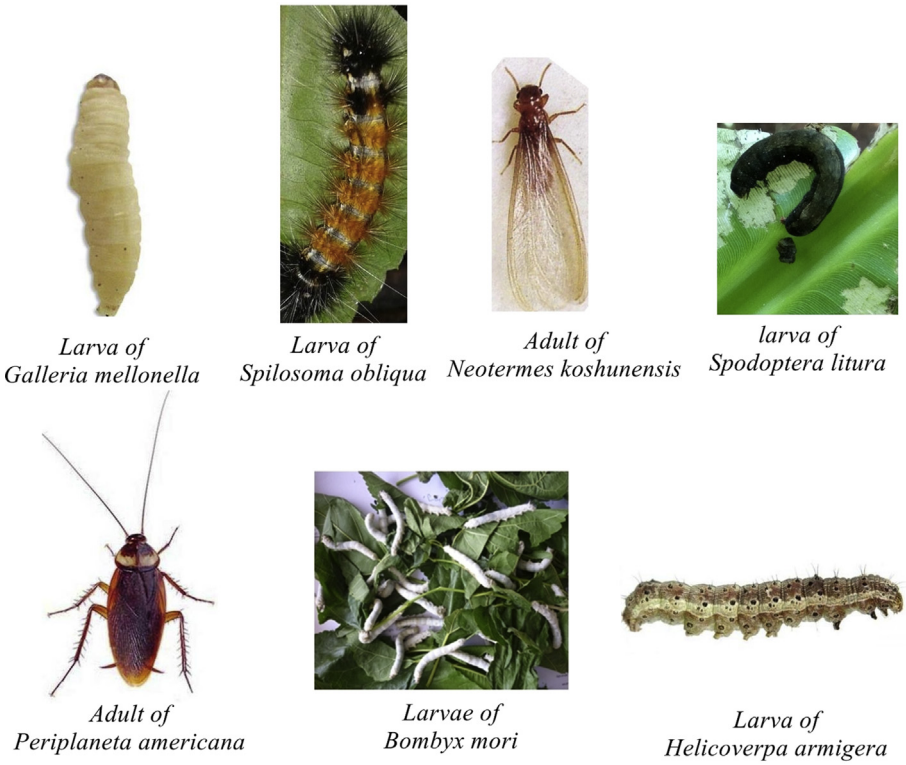

Fig. 1. Various insects have industrially important gut protease.

Recently, Uchima et al. (2011) reported heterologous expression of β-glucosidase of termite, *Neotermes koshunensis* in *Aspergillus oriyzae* and characterized their application for bio-ethanol production. Hitherto, the insect gut protease targeted for insect pest control due to their importance in food digestion, uptake of nutrients for growth and reproduction and decomposition of tissue components or tissue remodeling during metamorphosis (Terra and Ferreira, 1994; Romanelli et al., 2016; Kannan et al., 2016), now another way has opened up for exploration of insect gut protease for industrial application. Mika et al. (2013) recently reviewed that insects or insect-associated microorganisms are highly demanded by the food industry to reduce food incompatibilities such as celiac disease and to eliminate potential antinutritive factors.

2. General properties of insect gut proteases

Proteolysis is an essential part of food digestion in insects and this process is mediated by the concerted action of several digestive enzymes (Terra and Ferreira, 1994). They possess some important characteristics such as high temperature, alkaline activity etc. as depicted in Fig. 2. Therefore, the proteases with above mentioned important characteristics are promising tool for use in brewing, leather and textile, detergent, dairy and food processing industries (Anwar and Saleemuddin, 2000). Hivrale et al. (2005, 2011) reported that crude

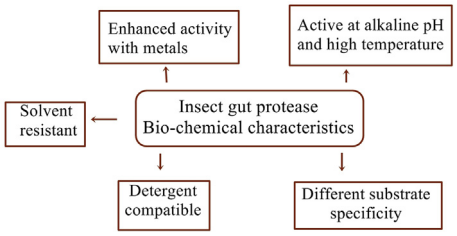

Fig. 2. Biochemical characteristics of insect gut protease.

extract from the gut of *P. americana* contained several trypsin and chymotrypsin-like proteases which are highly active at alkaline pH, therefore could be used as detergent additives. Alkaline protease finds a number of applications in various industries including waste management (Dalev, 1994), medical industry (Kudrya and Simonenko, 1994), leather processing industry (Varela et al., 1997) and food industry (Neklyudov et al., 2000). The concise information about pH and temperature of insect gut protease and its possible uses in various industries was presented in Table 1.

Two alkaline serine proteases of wax moth *G. mellonella* were purified through cation-exchange and gel filtration chromatography and the optimal activity of these proteases was found to be at pH 10.5 and 11.2 (Hamed and Attias, 1987). As above, the gut boll weevil with proteolytic activity was reported to be at pH 10.0–11.0 (Purcell et al., 1992). Likewise, Ranjbar et al. (2014) also reported the gut extract of *A. gemmatalis* showing resistance at pH 10.5.

Tsybina et al. (2005) also purified a new trypsin like protease homogeneity from the gut of *T. molitor* by ion-exchange chromatography and gel filtration chromatography. Molecular weight and isoelectric point of the purified trypsin like protease from *T. molitor* were 25.5 kDa and 7.5 respectively. The purified trypsin like protease from *T. molitor* showed its highest activity at 55 °C and the pH within the range of 5.0–9.5. Similarly, a serine protease from the gut of *H. armigera* was purified using gel filtration chromatography and its molecular weight was found to be 18.8 kDa using SDS-PAGE. The optimal temperature of enzyme was found to be 50 °C and the pH with wide range of activity between 9 and 12 indicating that midgut digestive trypsin is active at alkaline pH in *H. armigera* (Grover et al., 2018). Maase and Van Tilburg (1983) reported that the optimum temperature for Subtilisin and protease K had been determined earlier between 55 and 60 °C respectively. Anwar and Saleemuddin (2000) reported that purified alkaline protease cleaves synthetic substrates like BAEE, BAME and BApNA. *Azocasinase* like protease from the gut of Indian meal moth *P. interpunctella* showed activity at pH 9.5 and it was inhibited by serine protease inhibitors SBTI (Soybean trypsin inhibitors) and TLCK, which are specific to trypsin like

Table 1
Protease from different insect gut and its stable activity at diverse range of pH and Temperature.

Name of the gut protease	Source of protease	pH	Temperature (°C)	Application	Reference
Alkaline protease	*Spilosoma obliqua*	9–11	30–60	Addictives in detergent	Anwar and Saleemuddin (2000)
Trypsin like serine peptidase	*Cuelx quinquefasciatus*	7.5–10	25–60	Protease inhibitor based pest control	Borges-Veloso et al., 2012
Trypsin like serine peptidase	*Aedes albopictus*	7.5–10	37–50		Saboia-Vahia et al., 2014
Trypsin like protease	*Helicoverpa armigera*	9–12	37–50		Grover et al. (2018)
Gut protease	*Choreutis nemorana*	11	45		Chitgar et al., 2013
Serine alkaline protease	*Periplaneta americana*	8	60	Addictives in detergent/medical/chemical synthesis	Sanatan et al. (2013)
Alkaline serine protease	*Helicoverpa armigera*	10	50	Addictives in detergent	Akbar and Sharma, 2017
Pupal gut serine protease	*Bombyx mori*	9–11	45–60	Addictives in detergent/medical/chemical synthesis	Kannan et al. (2017)
B-glucosidase	*Neoterms koshunensis*	5–9	50	Bio-ethanol production	Uchima et al. (2011)
Laccase		5–6	30–50	Production of middle density fiber board/decolorization of dyes	Dittmer et al. (2009)
Prolyl-specific peptidase	*Rhizopertha dominica*	4.00	30–40	Food/medical industry	Mika et al. (2015)

enzyme at 96 and 89% respectively (Mahdavi et al., 2013).

Metal ions play an important role in enhancing the enzyme activity and stability. Joshi and Satyanarayana (2013) reported that Co^{2+} and Ca^{2+} excreted stimulatory effect on bacterial alkaline serine protease, while Hg^{2+} completely inhibited the activity at 5 mM. Similarly, the pupal gut serine protease of *B. mori* was also inhibited by Hg^{2+}, suggesting that carboxyl groups and aromatic rings of protease are perturbed through interaction by Hg^{2+} metal ions (Kannan et al., 2017). Mahdavi et al. (2013) reported that NaCl, $CaCl_2$ (5 and 10 mM) and $MnCl_2$ (5 mM) reduced the protease activity of lesser mulberry pyralid *Glyphodes pyloalis*. Therefore, the metal ions such as NaCl, $CaCl_2$ and $MnCl_2$ are act as an important co-factor to maintain and protect the proteolytic activity of the enzyme against thermal denaturation by maintaining the active conformation at higher temperature (Kumar and Takagi, 1999; Gupta et al., 2002). The overall general characteristic of insect gut protease revealed that it has essential properties required for crucial application in various industries.

3. Industrial characteristic of insect gut protease

Several proteases have been identified and characterized from bacteria, fungi, plant and other higher animal tissues towards industrial application. This review presents the importance of insect gut protease for various industrial applications (Fig. 3).

3.1. Detergent industry

Alkaline proteases are used in the detergent industry, because the pH of cleaning producers is usually in the range from 9.0 to 12.0. The use of alkaline protease along with cleaning solutions allows the use of fewer toxic chemicals like solvents and corrosive substance, decreasing their environmental impact (Castro et al., 2004). The enzyme for detergent industry should have stability and activity in typical detergent ingredients, such as surfactants, builders, bleaching agents, bleach activators, fillers, fabric softener and other formulations aids. Detergents available in the international markets, such as Dynamo, Era plus and Tide contain bacterial proteolytic enzymes (Anstrup and Anderson, 1974).

Anwar and Saleemuddin (2000) reported that alkaline protease showed activity towards commercial detergents like Surf, Nirma and Ariel. Gut protease from insect *Spilosoma obliqua* could be used as detergent addictive (biological detergent), enzymatic debriders and contact lens cleaning agent (Anwar and Saleemuddin, 2000). Jain et al. (2012) reported a serine protease from *Bacillus* sp. showing stable activity against various organic solvents up to 50% concentration. Sakiyama et al. (1998) and Showell (2016) reported that the protease can be used in detergents and cleaning agent for a long time. Kannan et al. (2017) demonstrated de-staining activity of blood soiled cloth using silkworm pupal gut serine protease along with Rin detergent

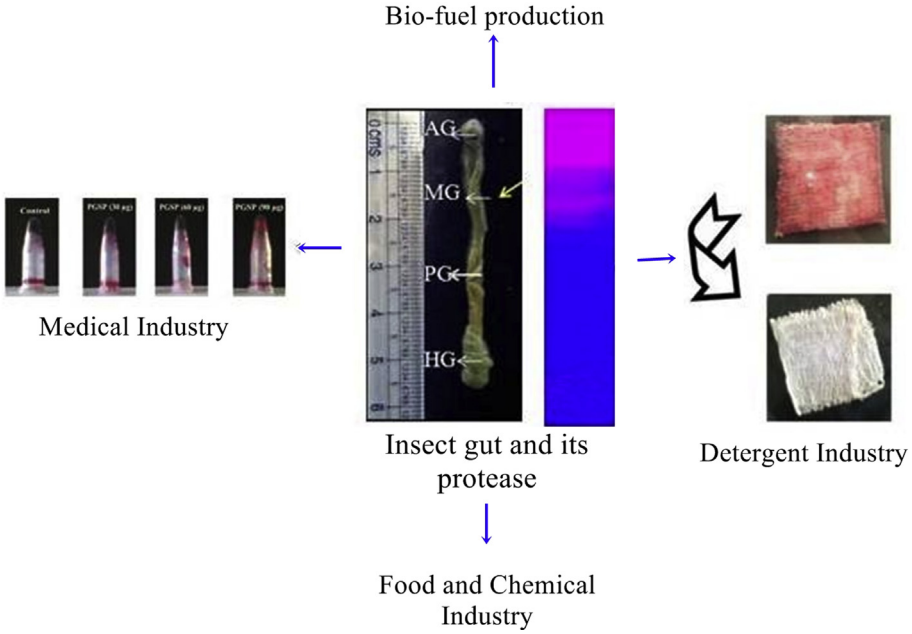

Fig. 3. Applications of insect gut protease in various industries.

powder.

3.2. Chemical industry

The organic solvent resistant protease is a feature which is highly desired in applications involving bio-catalysis in non-aqueous medium for organic synthesis, peptide synthesis and enzymatic esterification of oligosaccharides in anhydrous dimethylformamide (DMF) (Riva et al., 1998; Klibanov, 2001; Filippova and Lysogorskaya, 2003; Singhal et al., 2012). Previously, solvent stable alkaline proteases from insect gut were characterized by some researchers (Gupta et al., 1999; Sanatan et al., 2013). They also suggested that it has great opportunity in pharmaceutical industry for synthetic reaction and peptide synthesis. Recently, Kannan et al. (2017) reported that pupal gut serine protease from *B. mori* exhibited chemo-resistance towards a wide range of solvents like xylene, ethanol, isopropanol, DMSO, toluene and hexane (at 25% concentration).

3.3. Food and pharmaceutical application

The hydrolyzed plant based proteins using insect gut protease may be explored in food industry for human consumption and it may be helpful to improve the digestion of plant based products in the human intestine (Mika et al., 2015). The enzyme laccase identified from insect may have possible role in the clarification of juices (Osma et al., 2010). The insect gut protease has shown a significant role in the treatment of human celiac disease which is caused by abnormalities in metabolism of wheat grains and their products (prolamines or gluten) in the gut (auto-immune entopathy of small intestine) due to lack of cysteine digestive protease and prolyl endopeptidase in humans (Brandt et al., 2007). Since, *Tenebrio molitor* gut protease have natural ability to digest the gluten rich plant products and possess stabile activity at acidic pH, it may presumably work in the human stomach. So research is underway to use the insect gut protease from *Tenebrio molitor* as a food supplement or oral administration to treat Celiac disease (Elpidina and Goptar, 2007). Kannan et al. (2017) also reported that the pupal gut serine protease from silkworm has fibrinolytic activity (degradation of fibrin) and others are suggesting that the fibrinolytic enzyme has future application in therapeutics especially as anti-cancer drugs (Simkhada et al., 2010; Mukherjee and Rai, 2011).

3.4. Bio-ethanol production

For bio-ethanol production, cellulose must be converted into the fermentable sugar or glucose in a complex process which requires synergistic action of three different enzymes such as endoglucanases, cellobiohydrolases and β-glucosidase (Beguin and Aubert, 1994). Considering that β-glucosidase is essential for cellulose degradation, several studies have been done on β-glucosidase producing bacteria and fungi for biomass utilization which may facilitate the production of ethanol (Kotaka et al., 2008; Nascimento et al., 2010). The termites are efficient decomposers of cellulosic material (74–99%), so it was termed as Microscale bioconversion system (Watanabe and Tokuda, 2010; Ni et al., 2007; Uchima et al., 2011). The β-glucosidase from the gut of termite plays a major role in the digestion of cellulosic materials (Cellulose, hemicelluloses, pectin, and the non-carbohydrate polymer lignin) (Scharf et al., 2010). The termite gut proteases have activity but the production level is less or not enough for commercial exploitation. Therefore, the β-glucosidase of termite needs to be produced in greater quantity through molecular techniques for bio-ethanol production. The laccase enzyme from the termite guts (*R. flavipes*) play a role in lignocellulose digestion and same can be explored for bio-fuel production (Coy et al., 2010).

3.5. Other industry

The insect laccase is a multi-copper oxidase enzyme playing an important role in cuticle sclerotization by catalyzing the catechols into quinones, which then undergo polymerization with proteins to form the insect cuticle (Yatsu and Asano, 2009; Dittmer et al., 2009). The insect laccases have broad potential applications in production of middle-density fiber boards and decolorization of dyes (Jensen, 1983; Xu, 2005; Euring et al., 2011).

4. Conclusions

The applications of proteases in industry have grown rapidly in the last two and half decades. The properties and application of insect gut protease in various studies have been highlighted. Insect gut proteases were characterized by different researchers and their potential represent a new way to explore the insect protease for various industrial applications. However, the purification of protease from insect gut leads to less production quantity and is often time consuming due to their small body size. Therefore, production of industrially important protease from insect gut through heterologous expression may provide higher enzyme yield with desired properties like different substrate specificities and improved stabilities for industrial application.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.01.048.

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