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REVIEW

Thermostable marine microbial proteases for industrial applications: scopes and risks

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Abstract
Thermostable proteases are important in biotechnological and industrial sectors, due to their stability against denaturing agents and chemicals. The feature that gives them such unique applicability is their reaction at high temperatures, which affords a high concentration of substrate, and less risk of microbial contamination. Nearly 65% of industrial proteases are isolated from marine microbial source, and they can significantly resist a wide range of organic solvents at high temperatures. The most important trait of marine organisms is their adaptability, which allows them to grow optimally in harsh environments such as high salt, temperatures, and pressure—the characteristics of deep-sea hot springs and geothermal sediments. However, proteases are immunogenic, and they can trigger inflammatory conditions in human; so their safety assessment prior to industrial usage is of paramount importance. This review focusses on marine-origin thermophilic proteases, their thermal resistance, scopes of their industrial applications, and risks.

Keywords Marine microorganisms · Thermostable proteases · Tailor-made enzymes · Industrial applications · Immunogenic risks

Introduction

Enzymes are the catalytic keystones of the metabolic activities in living organisms (Dadshahi et al. 2016; Beygmoradi and Homaei 2017). Classified into six groups, all enzymes are vital for survival, and their malfunction leads to diseases (Homaei 2015b; c). Proteolytic enzymes (EC 3.4), or proteases, the members of hydrolase class, are the most fundamental and most flexible family of enzymes involved in all aspects of a living organism's actions (Sookkheo et al. 2000; Beg et al. 2003). Although protease enzymes were once thought to be a vital element in leather processing, silver recovery, medical purposes, food processing, feeds, chemical and waste treatment (Homaei et al. 2010, 2014; Homaei 2015a; Homaei and Etemadipour 2015), they are currently deemed pivotal to different physiologic processes, including development, apoptosis, regulatory mechanisms, infection, fecundation, allergic responses, blood clotting, tumor growth, and bone remodeling, as well as essential to therapeutic targets (e.g., anti-inflammation, digestion, wound healing) (Anwar and Saleemuddin 1998; Gupta et al. 2002; Barrett et al. 2012; Turk et al. 2012; Sanman and Bogyo 2014). Proteases are complex groups of enzymes distinguishable by their site of cleavage, catalytic active site, and optimal pH. However, the classification of proteases remains challenging, given the variety of their mechanisms of action and structures. As per the peptide bond cleavage site and their functions, protease enzymes are divided into two categories, the exopeptidases and endopeptidases. Whereas exopeptidases (exoproteases) cleave the terminal amino acid residues and break peptide bonds near the N- or C-terminus of the substrate, endoproteases (EC 3.4.21-99) hydrolyze

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peptide bonds in the polypeptide chains (Souza et al. 2015). Further, they can be classified according to their location of cleavage, for example, aminoprotease acts on the free amino-terminal of the polypeptide chain, or carboxyprotease acts on the carboxyl-terminal of the polypeptide chain (Souza et al. 2015). Proteases comprise the global sales of more than 65 percent of all enzymes (Shanmugavel et al. 2016).

Proteases are classifiable into four groups according to the character of their catalytic active site, such as serine proteases (Patel 2017a, b; Patel et al. 2017), cysteine proteases (Dadshahi et al. 2016), aspartic proteases, and metalloproteases (Hartley 1960; Rao et al. 1998a; Abebe et al. 2014). Also, proteases may be classified by the optimal pH as neutral, acidic or alkaline (Homaei et al. 2016b). For instance, if the protease pH range is between 2.0 and 6.5, it will be known as an acid protease, while pH ranging from 6.5–8.0 to 8.0–14.0 is identified as neutral and basic proteases, respectively (Abebe et al. 2014).

Proteases are widely produced by organisms, including plants, animals, and microorganisms. Among the proteases from diverse sources, the bacterial proteases have been exploited for commercial purposes, due to the ease of culturing bacteria (Wang et al. 2007; Haddar et al. 2010). As our knowledge about the sources and characteristics of thermostable enzymes expands, there are more opportunities to improve their usability in different industrial applications. As mentioned earlier, marine organisms have high resistance in harsh environments (Potumarthi et al. 2007). Such durability is the result of these organisms' metabolism adapted to the saline habitat. Marine source-derived enzymes have different features than terrestrial organisms, such as high salt toleration, thermostability, barophilicity etc., among others. Some enzymes in the marine organisms might be absent in the terrestrial organisms, or they might be known as enzymes from terrestrial sources with novel characteristics and potential applications (Debashish et al. 2005; Zeinali et al. 2015; Dadshahi et al. 2016; Homaei et al. 2016a; b). The unique traits in the marine microorganisms are due to physiological adaptations, unique genetic structures, and metabolic peculiarities as compared to their terrestrial homologues (Marrs et al. 1999; Jackson and Young 2001). The purpose of this literature review is to critically analyze existing literature and present it in a condensed way about marine thermophiles, their thermostable proteases, and industrial prospects of these enzymes. Finally, the risks of excess usage of this enzyme have been hypothesized, as these enzymes might be immunogenic and exercising caution before adopting a new technology is extremely important to safeguard human health.

Thermophiles and hyperthermophiles diversity

The thermophiles thrive in temperatures above 50 °C (Giddings and Newman 2015). Most thermophiles are prokaryotes, although there are few thermophilic eukaryotes. Generally, the thermophilic marine microorganisms consist of bacterial genera such as *Actinobacteria* (Dalmaso et al. 2015), *Bacillus* (Gey and Unger 1995), *Clostridium* (Slobodkina et al. 2008), *Desulfotomaculum* (Sievert and Kuever 2000), *Thermus* (Pantazaki et al. 2002), *Thiobacillus* (Dalmaso et al. 2015)), and the archaeal organisms *Pyrococcus* (Antranikian et al. 1995), *Sulfolobus*, *Thermococcus* (Godfroy et al. 1997), and *Thermoplasma* (Dalmaso et al. 2015). Thermophiles are divided into moderate or facultative thermophiles, extreme or obligate thermophiles, and hyperthermophiles, according to their temperature tolerance ranges (Segerer et al. 1993). Moderate thermophiles can grow in a temperature range of 40–60 °C; extreme thermophiles can grow in a range from 60 to 85 °C; and the growth temperature of hyperthermophiles is above 85 °C.

The highest temperature that hyperthermophiles can grow is limited to 121 °C (Vieille and Zeikus 2001). Thermophilic microorganisms thrive in shallow, deep-sea hot springs and heated beach sediments. Hot springs beneath the sea are hydrothermal vents, of which high temperature, low oxygen, extreme pH, and high pressure are characteristic features (Christie et al. 2006). Therefore, the majority of thermophiles are categorized as obligate anaerobes; however, some aerobic species are also well known. The microbial population in hydrothermal vents encompasses microorganisms of both the bacteria and archaea domains. Thermophilic archaea include *Methanopyrus kandleri* (Kurr et al. 1991), a rod-shaped hyperthermophilic methanogen, characterized by an optimal temperature at 110 °C, and the limiting temperature of 121 °C. *Pyrococcus* is a genus of *Thermococcus* archaea, an anaerobic hyperthermophile. *Pyrococcus abyssi* (Erauso et al. 1993) has maximum growth at a temperature of 92 °C, and it can survive 1 h at a temperature of 121 °C. *Pyrolobus fumarii* (Blochl 1997) is a thermophilic archaeon lithotroph, which has optimum growth at 106 °C, and tolerance up to 113 °C. *Archaeoglobus profundus* (Burggraf et al. 1990), an obligate heterotroph archaea member, has optimum growth at a temperature of 92 °C. Other types of thermophilic bacteria have earned fewer attentions compared to the members of archaea. The thermophilic bacteria encompass *Thermosipho melanesiensis* (Antoine et al. 1997), a thermophilic bacteria in shallow-water habitats (Huber et al. 1989), which shows optimum growth at a temperature of 70 °C; *Desulfurobacterium thermoautotrophum* (L'haridon

et al. 1998), a mandatory anaerobic bacterium with an optimal activity at 70 °C and the highest temperature limiting growth at 75 °C; and *Marinithermus hydrothermalis* (Sako et al. 2003), a rod-shaped gram-negative bacterium with an optimum growth temperature of 67.5 °C. Some of the thermophilic bacteria and thermophilic archaea from hydrothermal vents are mentioned in Table 1.

Structural stability of enzymes and heat resistance strategies in thermophile

A common property of all thermophilic microorganism-derived enzymes is their high thermal stability and heat resistance (Kumar 2001). Large proteins known as chaperones enable the folding of the enzymes into their native state, thereby helping these enzymes retain their functionality in high temperature (de Macario and Macario 2000). Hyperthermophilic archaea produce specific molecular chaperones that react only at very high temperatures. Thermosome (30) is a chaperonin, isolated from thermophilic *Methanopyrus kandleri*, *Pyrococcus abyssi*, and *Pyrodictium occultum*. This complex seems to bind to heat denaturation proteins, and hinder them, which leads to the folding of the proteins to their active state (Andrä et al. 1998). The cell membrane of thermophilic organisms is a monolayer lipid, and is constructed of saturated fatty acids, which provide a hydrophobic environment for the cell, maintaining the cell stiffness to survive at high temperatures (Herbert and Sharp 1992). Generally, membrane fluidity increases with the increment in temperature. To maintain and to keep the optimum fluidity of the membrane, the cell requires a different lipid composition, so that saturated straight-chain fatty acid content in the membrane lipid of thermophiles is higher than that of mesophiles. This allows the thermophiles to stay active in high temperature conditions, by providing the degree of fluidity necessary for membrane functions. The DNA of thermophiles undergo positive supercoiling to survive the high temperatures (López-García 1999). Another strategy of thermophilic organisms is the use of disulfide bridge that stabilizes proteins by reducing the entropy of the protein's unfolded structure often via entropic effects (Kumar 2001). Another factor that causes DNA stability is the parliament molecules. There are some polyamines like putrescine and spermidine, which help to stabilize DNA by Mg^{2+} action. In *Sulfolobus*, a thermophilic archaeon, polyamines stabilize ribosomes by simplifying

Table 1 List of thermophilic bacteria and thermophilic archaea isolated from deep-sea hydrothermal vents

Domain	Species	Optimum temparature (°C)	References
Bacteria	*Desulfothermus okinawensis*	50	Nunoura et al. (2007)
	Marinotoga camini	55	Wery et al. (2001)
	Caloranaerobacter azorensis	65	Wery et al. (2001)
	Thermosipho melanensis	70	Antoine et al. (1997)
	Carboxydobrachium pacificum	70	Sokolova et al. (2001)
	Caminicella sporogenes	55–60	Alain et al. (2002)
	Thermovibrio ruber	75	Huber et al. (2002)
	Thermovibrio ammonificans	75	Vertriani et al. (2003)
	Marinithermus hydrothermalis	60–70	Sako et al. (2003)
	Thermosipho globiformans	68	Kuwabara et al. (2011)
	Thermodesulfatator atlanticus	65–70	Alain et al. (2010)
Archaea	*Thermococcus hydrothermalis*	85	Godfroy et al. (1997)
	Thermococcus profundus	90	Kobayashi et al. (1994)
	Archaeoglobus profundus	92	Burggraf et al. (1990)
	Pyrococcus abyssi	96	Erauso et al. (1993)
	Methanocaldococcus jannaschii	86	Jones et al. (1983), Zhao et al. (1988)
	Methanopyrus kandleri	110	Kurr et al. (1991)
	Thermococcus guaymasensis	80–90	Canganella et al. (1997)
	Thermococcus barophilus	95	Marteinsson (1999)
	Methanothermococcus okinawensis	60–65	Takai et al. (2000)
	Thermococcus gammatolerans	88	Jolivet et al. (2003)
	Geogemma barossii	105–107	Kashefi and Lovley (2003)
	Pyrococcus yayanosii	98	Birrien et al. (2011)
	Thermococcus coalescens	87	Kuwabara et al. (2005)

protein synthesis at high temperature. The hydrophobic interactions could be another factor for thermostability of proteins (Goodenough and Jenkins 1991). Also, there are major differences in thermophile proteins in comparison with mesophile proteins, despite the high sequence similarities between the protein structure pairs (Zuber 1988; Russell et al. 1998). In thermophile proteins, the proportion of thermolabile residues like cysteine and serine is decreased remarkably, whereas the proportion of arginine and tyrosine (the charged and aromatic amino acids, respectively) significantly increases, compared to their mesophilic homologs (Frappier and Najmanovich 2015). The amino acids asparagine, glutamine, methionine, and cysteine are thermolabile, which are easily inactivated at high temperature by the effect of deamidation (asparagine and glutamine) and oxidation (methionine and cysteine) (Kumar et al. 2000). However, this pattern of amino acid distribution is not definite, and immense number of deviations can occur (McDonald 2010). Thermophilic adaptations of protein have been reviewed in details, which can be referred to for further insights on this aspect (Sterner and Liebl 2001). The thermophile proteins possess substituted amino acids. As an instance, they can possess arginine instead of lysine. The event of amino acid pair interchanges like lysine to arginine; serine, and glycine to alanine; serine to threonine; and valine to isoleucine, which occur in thermophile proteins are considered as the most important reason of determinative thermostability of protein (Menéndez-Arias and Argosf 1989). One of the reasons for increasing the thermostabilization of thermophilic protease is enzyme immobilization on the surface of a carrier that makes the enzyme capable of resisting artificial solvents, high temperature and pressure (Cowan and Daniel 1982; Kumakura et al. 1984). There are other several factors that might cause increased thermostabilization such as covalent cross-linking (Rao et al. 1998b; Grazú et al. 2005), entrapment (Eggers and Valentine 2001; Lee et al. 2005), and physical adsorption (Norde and Zoungrana 1998; Ladero et al. 2006), which are methods for immobilization of enzymes. Generally, immobilization increases the thermostabilization of thermophilic proteases (Simpson et al. 1991; Unsworth et al. 2007). Research has shown that there is a positive correlation between the higher growth temperature of a thermophilic microorganisms and stabilization of extracellular protease. Also, another reason for the stability of thermophilic proteases is the presence of metal ions to enhance molecular stability. For instance, the higher thermostability for caldolysin

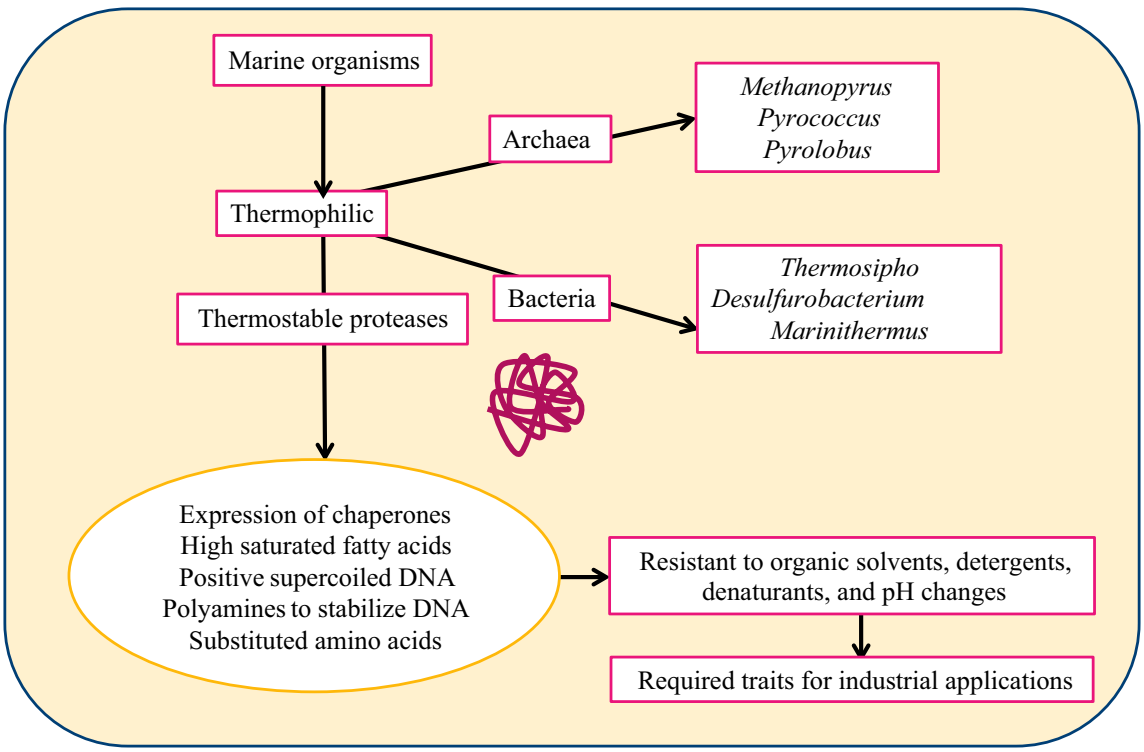

Fig. 1 Marine organisms encompass thermophilic bacteria and archaea. Thy elaborate thermostable proteases to coppe with stressors of high temperature, high pressure, high salinity marine environments. The high stability of the proteases are due to the expression of chaperones, high level of saturated fatty acids, positive supercoiled DNA, polyamines, and substituted amino acids. These traits render the proteases resistant to alkali, detergent, pH, and enhance their industrial applicability

or aqualysin 1 (extracellular metal-chelator-sensitive lytic protease from *Thermus aquaticus*) than thermolysin (thermostable neutral metalloproteinase) can be ascribed to its binding to 6 Ca^{2+} (Khoo et al. 1984; Simpson et al. 1991), as opposed to 4 Ca^{2+} of the latter (Roche et al. 1978), apart from the presence of tyrosine (Ohta 1967) in thermolysin. Caldolysin is alkaline serine protease, which shows optimal caseinolytic activity at 80 °C.

Resources of marine thermostable proteases

Proteases, like a gamut of other enzymes, are secreted by microbial organisms to the extracellular medium. Among them, thermophilic bacteria and archaea are commercially popular compared to other microbial organisms (Lasa and Berenguer 1993; Fujiwara 2002), as they can grow in optimum temperature conditions between 50 °C and 121 °C (Antranikian et al. 1995). Thermozymes, isolated from thermophile organisms, demonstrate features of thermal stability which could be the reason that they are allocated between hyperthermophilic and mesophilic enzymes. As was pointed out earlier, thermophile enzymes have a high optimum temperature. For this reason, they are poorly effective when the temperature is below 40 °C. However, when the temperature is raised, the enzymes are activated (Fig. 1). Thermophile protease are more resistant to organic solvents, detergents, pH changes and other chemical denaturants, and they also have stiffer proteins (Cowan et al. 1985; Cowan 1997; Gupta and Ramnani 2006).

Using thermophile proteases is beneficial because its reaction at high temperatures result in higher concentrations of substrate, and the higher rate of reactions (Bruins et al. 2001; Eichler 2001). This could be the reason that for the global demand for thermostable proteases being higher than mesophilic proteases. Thermostable enzymes isolated from marine microbial are reported frequently from thermophilic family members of *Thermotoga*, *Thermus*, *Thermococcus*, *Pyrococcus*, *Bacillus*, and *Sulfolobus*. Figure 2 illustrates the maximum activity of thermostable proteases in the optimal situation of pH and temperature in a few marine microorganisms. The industrially important thermophile proteases are generated by thermophile bacteria, which belongs to the genus *Bacillus* (Haki and Rakshit 2003). The first thermostable protease isolated from *Bacillus* belongs to *Bacillus stearothermophilus,* which become stable at 60 °C (Salleh et al. 1977). The main difference in the thermostability of protease between strains isolated from *Bacillus stearothermophilus* TP 32 and protease from *Bacillus stearothermophilus* TP 26 is their optimum activity, which is 85 °C for the former, and 75 °C for the latter (Gey and Unger 1995). *Bacillus* sp. strains produce extracellular subtilisin proteases, which have been extensively exploited for laundry

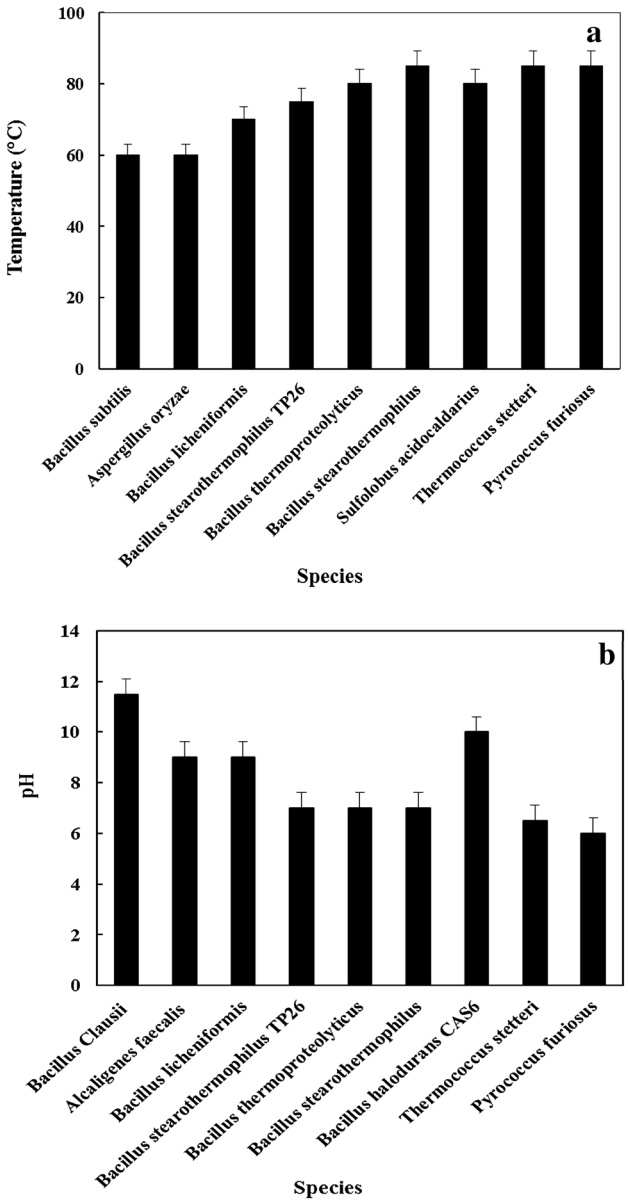

Fig. 2 The maximum activity of thermostable proteases in optimum conditions of **a** pH and **b** temperature in some of marine microorganisms

detergents and other industrial applications. Thermolysin is a thermostable neutral metalloproteinase enzyme that is isolated and characterized from *Bacillus stearothermophilus* and *Bacillus thermoproteolyticus* with the optimal activity at the temperature of 70 °C, and with 1-h half-life at the temperature of 80 °C, respectively. Pyrolysin is a thermostable subtilisin-like serine protease derived from *Pyrococcus furiosus*, that acts at the optimum temperature of 100 °C (Antranikian et al. 1995). Aqualysin is a subtilisin thermostable serine protease isolated from *Thermus aquaticus* and *Thermus thermophiles* (Pantazaki et al. 2002). Streptolysin isolated from hyperthermophile *Thermos stature* has high

activity at 85 °C. Another strain of *Thermus* produces ATP-dependent metalloprotease, which accelerates the degradation of small peptides (Pantazaki et al. 2002). Thermophilic proteases have been reported in thermophilic archaea, such as *Pyrococcus, Thermococcus, Staphylothermus*, *Desulfurococcus*, and *Sulfolobus*. Some thermal stability properties of thermostable proteases are mentioned in Table 2. Thermostable serine proteases are isolated from marine bacteria strain *Desulfurococcus* (HANZAWA et al. 1996). Additionally, a novel thermophilic protease is isolated from *Penaeus vannamei* (Dadshahi et al. 2016), which demonstrates high catalytic activity at neutral pH at 80 °C.

Furthermore, the thermostable endopeptidase, a cysteine peptidase, isolated from marine *Pyrococcus horikoshii,* is the first allosteric enzyme that has negative cooperativity with Cl^- ions that highlight its importance for pharmaceutical developments (Zhan et al. 2014). *Alcaligenes faecalis* APCMST-MKW6 isolated from marine sediment, produces an alkaline protease with maximum activity at pH 9 and at an optimum of 60 °C. It is stable between 50 and 60 °C, even after 1.30 h (Maruthiah et al. 2016). Another alkaline that is isolated from marine bacterium *Bacillus alveayuensis* CAS 5 has a high stability at 80°C, pH 12, and in the presence of ionic, non-ionic and commercial detergents (Annamalai et al. 2014). The optimum temperature of *Bacillus subtilis* TKU007 protease is 50 °C and its pH is between 5 and 11 (Wang and Yeh 2006). Also, a thermostable protease extracted from the marine bacterium *Bacillus halodurans* CAS6 which retains its original activity, even at 70 °C, pH 10.0 and 30% NaCl for 1 h (Annamalai et al. 2013). A marine bacterium SD11 isolated from sea muds produced a thermostable alkaline serine protease, with optimal temperature of 60 °C (Cui et al. 2015). Additionally, an extracellular alkaline protease was isolated from marine *Streptomyces sp*. D1 has maximum activity at 45 °C and a pH value of 10. The enzyme stabilizes in the pH range of 8–10 and temperatures between 45 and 60 °C (MANE et al. 2013). A thermostable alkaline protease that is detached from marine *Bacillus clausii* shows that 11.5 is an optimal pH range and 80 °C is the ideal activity, the temperature of an enzyme, which is partially purified (Kumar et al. 2004). Also, *Marinobacter sp*. MBRI 7 produces a thermostable extracellular protease which is steady at 60 °C(Fulzele et al. 2011. Marine fungi as well as marine bacteria are sources of thermophilic proteases. The thermophilic proteases-producing thermophilic fungus species included *Achaetomium, Chaetomium, Penicillium, Rhizopus, Torula,* etc. For instance, thermophilic acid protease that is isolated from marine *Penicillium duptonti* K1014 (Emi et al. 1976) and also alkaline thermophilic proteases are isolated from marine fungi *Malbranchea pulchella* and *Humicola lanuginose* (Ong and Gaucher 1976) and they are active at high temperatures. The extracellular proteases from origin bacterial are alkaline serine proteases or metalloproteases. In contrast, cysteine protease enzymes are limited to thermophilic fungi origin. Some of the properties of thermostable proteases are shown in Fig. 3.

Industrial applications of marine thermostable proteases

The benefits of using thermophilic enzymes include the reduction of losses during purification, preparation, storage and transportation. This factor may favorably affect the economics of industrial production and application. Proteolytic enzymes account for almost 65% of total global enzyme sales (Shanmugavel et al. 2016). The optimum activities of common proteases are between 25 and 40 °C, which is not suitable for some industrial processes. Most industrial processes that act at a higher temperature cause the denaturation of common proteases. To solve this problem, investigators are still looking for an enzyme that stays active at high

Table 2 Thermal stability in some of marine thermophilic proteases

Source	Thermal stability (°C)	High temperature: half-life	References
Pyrococcus sp. KOD1	> 90	100 °C: 1 h	Morikawa et al. (1994)
Bacillus brevis	> 25	25 °C: 288 h 50 °C: 60 h 60 °C: 7 h	Banerjee et al. (1999)
Bacillus spp.	> 50	50 °C: 50 min	Kumar et al. (1999)
Streptomyces fungicidicus MML1614	> 60	–	Ramesh et al. (2009)
γ-Proteobacterium	> 70	70 °C: 1 h	Sana et al. (2006)
Pyrococcus furiosus	> 95	103 °C: 0.5 h	Fiala and Stetter (1986)
Bacillus halodurans CAS6	> 70	70 °C: 1 h	Annamalai et al. (2013)
Marinobacter sp. MBRI 7	> 70	70 °C: 4 h 80 °C: 1 h	Fulzele et al. (2011)
Strain of SD11 marine bacteria	> 60	60 °C: 1 h 70 °C: 1 h	Cui et al. (2015)

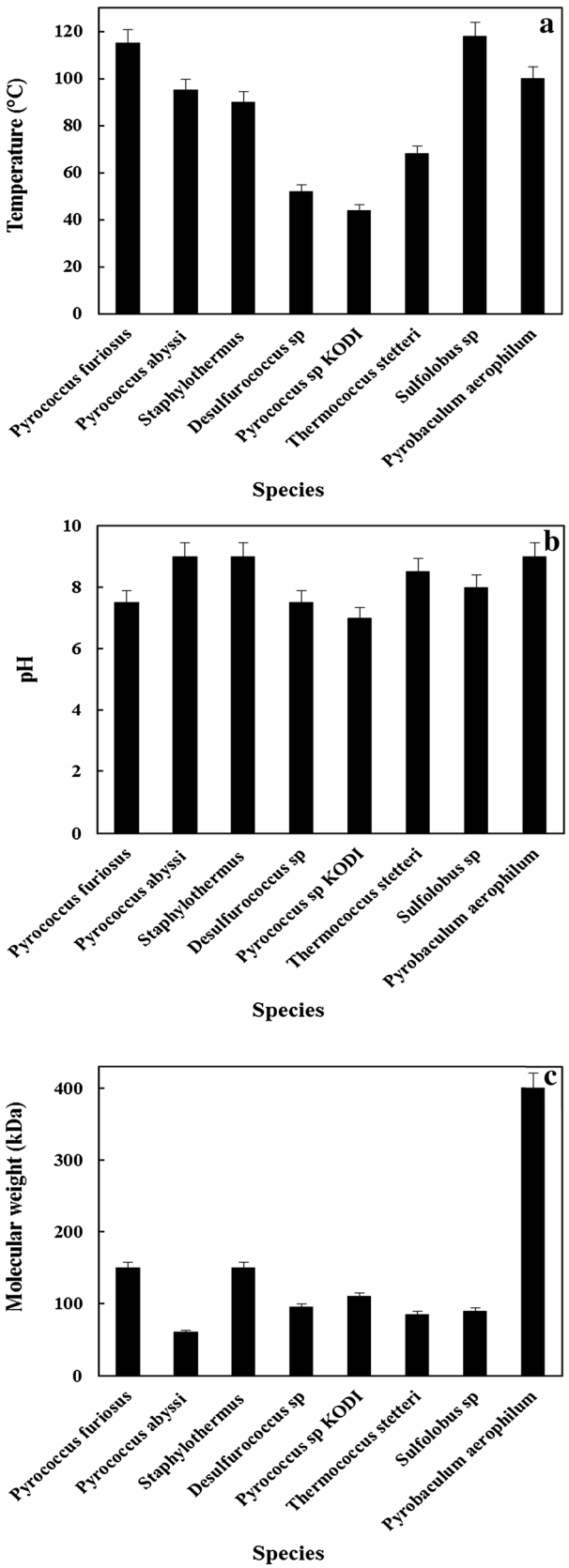

Fig. 3 **a** The optimum temperature of some thermophilic proteases ▶ from marine thermophilic microorganisms. **b** The optimum pH of some thermophilic proteases from marine thermophilic microorganisms. **c** The proteases produced by thermophiles with molecular weight in range of 35–160 kDa

temperatures for a long time. Proteases that are capable of thermal tolerance are chosen for industrial biotechnology processing, which could stay active at a high temperature for a long duration of time. The major benefit of thermostable proteases is that they can survive in a large temperature range of 50–121 °C for a reasonably long time (e.g., 4–5 h at 90 °C). Thermophilic organisms that generate thermostable proteases can stay active for 1 h at 60 °C and about 10 min at 75 °C. They are mainly used in industry for the hydrolysis of proteins, preparation of the organic compounds, and in the areas of food, detergents, silver recovery, pharmaceuticals, leather, and textiles (Srinivasan and Rele 1999). One of the applications of thermostable proteases is organic synthesis, which enables them to synthesize peptide bonds (Glass 1981). The stabilization of thermostable proteases in organic solvents can allow their usage while reducing loss of activity.

Currently, only some commercially significant thermophilic proteases are accessible, and one of them is Alcalase. It is an endopeptidase of the serine protease type that is isolated from *Bacillus licheniformis*, which is active at the temperature of 60 °C, and a pH of 8.5. Alcalase is commonly used in the food industry, for the processing of soy meal, protein-fortified beverages, etc. (Synowiecki et al. 2008). Another application of thermostable proteases in the food industry is meat tenderization, which is due to their activity at high temperatures (above 60 °C) (Homaei et al. 2010, 2016b; Dadshahi et al. 2016). Thermophilic proteases that are stable against surfactants in detergents and heat are widely used in detergents and cleaners (Banerjee et al. 1999; Niehaus et al. 1999).

The resistance of thermostable proteases in aqueous and non-aqueous media results in the correction of the balance reaction and the creation of novel peptide bonds. Proteases can be applied to promote the quality of protein hydrolysates. For example, thermolysin is a thermostable protease that is isolated from *Bacillus thermoproteolyticus* and it contributes to aspartame synthesis (De Martin et al. 2001), which is applied as a sweetener in dietetic foods and drinks. Enzymatic synthesis removes pollution created by bitterness isomers. Some of the thermostable proteases are currently applied in molecular biology and biochemistry processes. Additionally, protease enzymes are used in DNA and RNA purification methods (Homaei et al. 2016b). The thermophilic protease produced by *Thermus* sp. is used in PCR (Bruins et al. 2001).

In Table 3, several of the industrial applications of thermophilic proteases are summarized. Marine proteases are important research topics for their biotechnological applications (Trincone 2013). Due to the high demand of industrial proteases, microbial proteases have drawn global attention.

Almost 90% of industrial proteases are extracted from marine microbial sources (Inácio et al. 2015). The ideal enzymes for the detergent industry should have high activity and stability at very high temperatures, and high alkaline conditions. The first bacterial protease-containing detergent was found in 1956 (Rao et al. 1998b). Protease enzymes eliminate protein stains like blood, grass, and egg (Hasan et al. 2010). Marine proteases have a wide array of biotechnological purposes (Chandrasekaran and Rajeev Kumar 2010). For example, alkaline proteases isolated from marine *Bacillus cereus* and marine *Streptomyces fungicidicus* are effective in the elimination of blood stains (Ramesh et al. 2009; Abou-Elela et al. 2011), are active at temperatures up to 60 °C, and are resistant to surfactants and bleaching agents (Kumar et al. 2004). The alkaline serine protease produced by *Engyodontium album* BTMF S10 (Chellappan et al. 2006, 2011) with an optimal activity at 60 °C are used to eliminate proteinaceous stains (Sana et al. 2006; Chellappan et al. 2011). Another alkaline serine protease enzyme produced by marine γ-*proteobacterium* has a high resistance against high concentrated salt, organic solvents, dishwashing detergents, and bleaching agents (Sana et al. 2006). A serine protease produced by marine bacterium *Engyodontium album* was reported to have two disulfide bonds and more than two Ca^{2+} binding sites, which are supposed to be responsible for the thermal stability of the enzyme (Jasmin et al. 2010). Alkaline proteases isolated from marine bacterium *Yarrowia lipolytica* can hydrolyze different proteins for producing bioactive peptides, which have varied usages in the medical field for the treatment of high blood pressure and the development of clinical nutrition supplements (Ni et al. 2008). Another example of a protease resource is bacterium *Teredinobacter turner*, enzyme production from which is optimized under solid-state fermentation (SSF) conditions (Elibol and Moreira 2003).

Conclusions and perspectives

One of the main problems in industrial processes is to obtain a balance between harsh conditions and enzyme stability. Enzymes that are isolated from thermophilic organisms offer a potential solution for industrial processes carried out at high temperatures. It is important to be aware of the fact that marine thermophilic organisms are a major source of protease with high heat resistance. They display significant stability against high temperature, solvent, detergent, and whitening agents. Their unique feature makes them a significant ingredient for industrial usage.

However, microbial enzymes are immunogenic. If these enzymes are not deactivated, they can activate human immune system by dermal contact or ingestion. The proteases have evolutionary conserved domains often associated with pathogenicity such as the chitin binding domains (ChtBDs. In such cases, they will behave like allergens, and atopic individuals will develop hypersensitivity. The virulence role of serine protease has been reviewed recently. The pathogenic bacteria *Vibrio*, *Alteromans,* etc., are marine proteobacteria, of which the proteases are various pathogenic factors. Caldolysin belonged to the S8 family of peptidase, which like other families such as M4, M28, M64, and M66, are pathogenically crucial. They contain virulence domains like glycosyl hydrolase, mucin binding, hemagglutinin binding, immunoglobulin-like, heparin binding, and collagen binding, among others. The enzymes are so prone to amino acid substitutions that homology is less, but these crucial domains are fiercely conserved (Patel 2017b).

Table 3 Industrial applications some of marine thermophilic proteases

Extremophiles	Species	Application	References
Thermophiles	*Streptomyces fungicidicus*	Detergent industry	Kim (2013)
	Engyodontium album	Detergent industry	Ali et al. (2014)
	Yarrowia lipolytica	Pharmaceutical field	Harzevili 2014)
	Bacillus cereus	Detergent industry	Prakash et al. (2005)
	Teredinobacter turnirae	Solid-state fermentation	Kim (2015)
	Thermus strain Rt41A	DNA and RNA purifications; cellular structures degradation prior to PCR	Vieille and Zeikus (2001)
	Aureobasidium pullulans HN2–3	Bioactive peptide production	Ni et al. (2009)
	Bacillus alveayuensis CAS 5	Cleansing additive in blood stain remova	Annamalai et al. (2014)
	Bacillus subtilis TKU007	Detergent formulations	Maruthiah et al. (2015)
	Bacillus halodurans CAS6	Bioconversion of marine wastes and antioxidant synthesis	(Maruthiah et al. 2016)
	Strain of SD11 marine bacteria	Synthesis of peptides and detergent formulations	Cui et al. (2015)

So, before the usage of the microbial proteases become rampant, their safety profile must be considered. We are already living amidst inflammatory agents in the form of processed food, personal care products, excessive drug usage, etc. These microbial enzymes will add to the threat, if not restrained.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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