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Development of marine biotechnology as a resource for novel proteases and their role in modern biotechnology

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ABSTRACT

Marine environment consists of the largest sources diversified genetic pool of material with an enormous potential for a wide variety of enzymes including proteases. A protease hydrolyzes the peptide bond and most of proteases possess many industrial applications. Marine proteases differ considerably from those found in internal or external organs of invertebrates and vertebrates. In common with all enzymes, external factors such as temperature, pH and type of media are important for the activity, catalytic efficiency, stability and proper functioning of proteases. In this review valuable characteristics of proteases in marine organisms and their applications are gathered from a wide literature survey. Considering their biochemical significance and their increasing importance in biotechnology, a thorough understanding of marine proteases functioning could be of prime importance.

1. Introduction

The history of proteolytic enzymes or peptidases can be traced back at least to the late eighteenth century. However, in recent times the research work in this area has accelerated greatly, fuelled by numerous practical applications in biotechnology, and the realization of the fact that they are among major therapeutic targets [1]. The basic definition of a protease states that they are enzymes that hydrolyze peptide bonds in proteins. Classification of proteases is a hierarchical one built on the concepts of catalytic type, clan, family and peptidase. Proteases break the long chained molecules of proteins into shorter parts as demonstrated in Fig. 1 [2]. Proteases are divided into two groups according to their mode of action: endopeptidases and exopeptidases (Fig. 2). Endopeptidases hydrolyze peptide bonds in the middle of polypeptide chains while exopeptidases remove terminal amino acid residues from polypeptide chains [3].

Alternatively, proteases may be classified by the optimal pH as neutral, acidic or alkaline as presented in Fig. 3 [4]. With respect to their active center they could be classified into cysteine, serine, metallo, and aspartyl proteses (Fig. 4) [5]. About 75% of industrial enzymes are hydrolytic enzymes, representing one of the three largest groups of industrial enzymes allocated about 60% of the total worldwide sale of enzymes (Fig. 5). A number of industries that use proteases include detergents, leather processing, silver recovery, medical purposes, food processing, feeds, chemical and waste treatment [6,7]. For industrial application of proteases different temperature, salt concentration, optimal pH, type of media and incubation period are needed to be taken into account [4]. Proteases contribute for use in products that require the enzyme-aided or digestion of proteins from different sources [8]. Proteases that are comsumed in the pharmaceutical industry differ from those used in food and detergent industries. In the pharmaceutical industry, proteases are produced in small amounts and extensive purification is required before they can be introduce to the market. On the other hand, they are prepared in bulk quantities and used as crude preparations for other industries [5]. A number of sources are available to produce the enzymes including microorganisms, fungi, plants and various animals. However, much more efforts are directed towards extremophiles and symbiotic microorganisms. Fishes, prawns, crabs, snakes, plants and algae possess a wide range of biodiversity, although the most current bioprospecting activity is founded in microbial products [9]. It is a fact that optimal activities of enzymes in different organisms are related to salt concentration, pH and temperature as well as the interactive effects of these environmental factors. Moreover, a marine derived enzyme may carry novel chemical and stereochemical properties as compared to other

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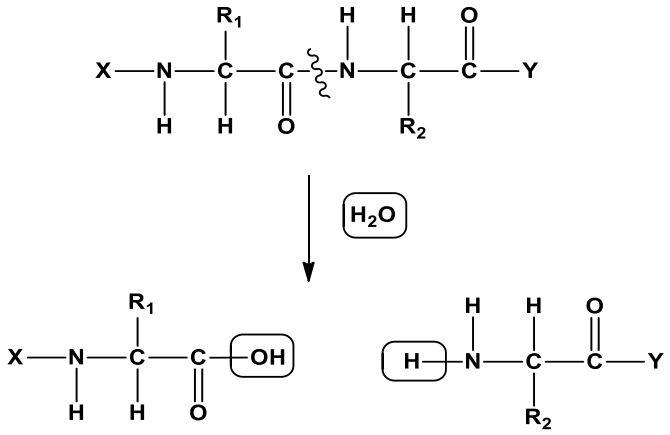

Fig. 1. The general mechanism of protease enzymes action. The Proteases cleave proteins by a hydrolysis reaction the addition of a molecule of water to a peptide bond.

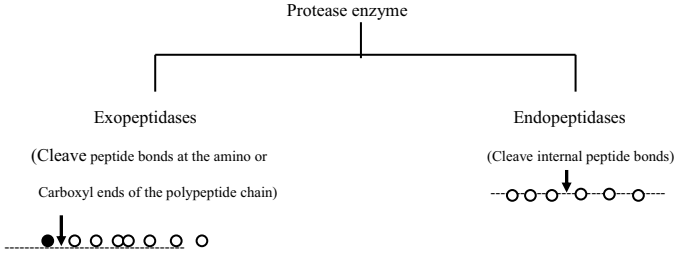

Fig. 2. Classification of proteases, in terms the site they react. They are divided into *exo*- and endopeptidases according to their action at or away from the termini, respectively.

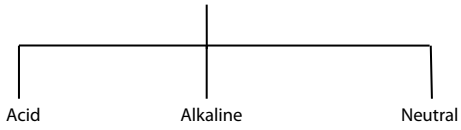

Fig. 3. Classification of proteolytic enzymes on the basis of their sensitivity to pH.

rich sources. Biocatalytically oriented studies (suitable substrates, appropriator conditions, stereochemical assessment of catalysis) should be performed to reveal this "chemical biodiversity" which, in turn, increases interest for these enzymes [9]. In the last few decades, marine enzymes have been suggested for many industrial applications i.e. in pharmaceuticals, cosmetics, nutritional supplements, molecular probes, food additives, fine chemicals and agrichemicals [10,11]. In particular, the use of sea-derived enzymes in food technology is becoming a promising application for the development of new processes and new products. Some examples are substitution of rennet in cheese manufacture; removal of the oxidized flavor from milk; ripening and fermentation of fish products; and preparation of fish protein hydrolysates and concentrates [12,13]. The ability to maintain enzymatic activity at low temperature has made the digestive enzymes from marine organisms to be useful in food processing, As they can resist bacterial contamination and unwanted chemical reactions [14,15].

In the present review an attempt is made to mainly compile an inclusive report covering classification, characterization and properties of proteases in different marine organisms such as, fishes, crustaceans, algaes, acidians, bacteria and fungi.

2. Protease enzymes

2.1. Protease in marine bacteria and fungi

The sources of proteases in microorganisms are more than plant or animal kingdoms [16]. It has been reported that microorganism comprise of nearly 60% of the total worldwide microbial protease market [17]. Their rapid growth, wide range of biochemical diversity, limited space required for cell cultivation and their various applications, are all contributed to the advantages of microbial sources as suitable pathways for large scale production of proteases [4]. Some divalent cations such as Mg^{2+}, Ca^{+2} are required for enhancing the activity of bacterial proteases, while other ions such as Hg^{2+}, Zn^{2+}, Fe^{2+}, Ag^{2+} perform inhibitory effect on the activity of microbial proteases [18–23]. However, a wide range of fungi such as genus *Aspergillus*, *Mucor* and *Rhizopus* and bacteria including genus *Clostridium*, *Bacillus* and *Pseudomonas* are potential sources to produce proteases [24,25]. A mainly nutrient alkaline extracellular protease, present in genus *Bacillus*, has been recognized as sources of commercial protease and used in detergent, surfactants, builders, bleaching agent, bleach activators, fillers, fabric softeners and various other formulation aids due to increased production capacities, high catalytic activity and high degree of substrate specificity [8,26]. Alkaline proteases possess many applications in food complementary of beasts and poultries, bakery, leathering, oil manufacturing, alcohol production, and beer production industries [4]. Some of bacterial alkaline proteases with major application as detergens are subtilisin Carlsberg, subtilisin BPN′ and savinase which are also available commercially in market [27]. Another marine bacterium, *Pseudo alteromonas*sp strain A28, has been reported to produce an extracellular serine protease with the ability to destroy the diatom *Skeletonema costatum* strain NIES-324 cells due to its potent algicidal activity [28]. A facultative alkalophile *Bacillus clausii* could produce a kind of alkaline serine protease used as detergent additive to remove protein-containing spots from laundry [29]. *B. clausii* protease can exhibit suitable stability towards both surfactants and oxidizing agents, retaining its activity of 73 and 116% after incubation for 72 h with 5% SDS and 5% H_2O_2. It can, therefore, be useful as additive in industrial applications including detergent preparations [30]. Hwang et al., have reported two *Escherichia coli* bacterial ATP-dependent proteases: while protease Ti is absolutely specific for ATP or dATP with two subunits of P and A, protease La can cleave and function with other nucleotides [31]. It has been reported that the specific activity of protease Ti against casein is quite high; for component P, the activity appears to be about l0 fold higher compared to protease La [32]. The optimum pH and temperature of marine bacterium *P. alteromonas* sp. strain A^{28} have been reported to be 8.8 and 30 °C [28]. The optimum pH and temperature for growth and protease productions of *Staphylococcus* are 8–10 and 37–45 °C respectively [4]. However, the optimum temperature for proteolytic activity of protease producing bacteria is reported to be 37–50 °C [33]. The optimum conditions for *Bacillus cereus* enzymatic maximum activity are at 50 °C and pH 10 [29]. Joo et al. have found a new strain of *B. clausii* that produce high levels of an extracellular alkaline protease with optimal pH 11and temperature of around 60 °C [30]. *Arthrobacter ramosus* and *Bacillus alcalophilus* is used for removing blood stains from cotton fabric due to their high protease activity. The enzyme is thermostable, ie., could remain stable at pH 12 and active in the presence of commercial detergent, suitable for use in detergent formulations [34]. The type of microorganism, together with chemical and physical parameters can affect proteolytic activity of enzymes. Thus, for good proteolytic activity identification and selection of potential microorganisms and optimization of physical and chemical conditions for protease producing isolates is definitely needed [4,33]. In this regard the temperature, pH and the type of media used for potential protease