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Short Note on Biotechnological Application of Halophilic Enzymes

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Abstract

Evolution of life in extreme environments entails wide adaptations. Amongst these environments, those with high salt concentration are abodes to so-called halophilic microorganisms. The unique key properties of halophilic proteins not only enable them to survive in hostile and precluding environments, but also make their enzymes as precious biocatalysts in pharmaceutical, fine chemical processes, industries, food processing, detergents, waste treatments, etc., where harsh conditions prevent favorable reactions by normal enzymes. Unfortunately, due to lack of deep knowledge and proper equipment, up to know only a limited number of extremozymes have characterized and applied in appropriate industries. Discovery of new extremophiles or genetically engineering of mesophilic enzymes will open new horizons to novel applications of these invaluable enzymes in biotechnology and biotransformation.

Keywords: Halophilic Proteins; Extremozymes; Biotechnological Applications

Introduction

Environments with high salt concentration are among most extreme and strongly discordant habitats to support life. The interests in studying halophilic proteins and enzymes have risen in many laboratories, in order to understand their enigmatic strategies, by which they are capable to thrive, tolerate and colonized in such extreme habitats. It has well documented that proteins from a large variety of microorganisms are useful as biocatalysts for various industrial purposes. Among these proteins, extremozymes are in the center of attention. The main character of these extremozymes and proteins is their stability and function under very precluding conditions where other proteins cannot withstand. Due to these outstanding properties, extremozymes have considered as excellent biocatalysts in some biotechnological and industrial processes, which demand harsh conditions, e.g., high or low temperature, extreme pH and high ionic [1]. Table 1 summarized some halophilic proteins and related producing microorganisms.

Alpha amylases are biotechnologically important extra cellular enzymes that can perform hydrolysis of starch to products with different lengths e.g., glucose and dextrin. These enzymes are active in a wide range of salt concentrations and pH (7-10) and maintain their activity at temperatures higher than 50˚C. These characteristics make these enzymes very precious for application in biotechnological processes such as starch hydrolysis, food bioprocessing, baking and textile industries and detergents [16]. Neoagaro-oligosaccharides as products of agar hydrolysis have applications as additives in pharmaceutical industries e.g., probiotics, food ingredients and cosmetics [17]. One way to produce neoagaro-oligosaccharides is acid hydrolysis of agar, but a drawback of this method is uncontrollable reaction, so the production of heterogeneous unwanted products, which are unfavorable for industrial scale production.

Extremozymes	Microorganisms and reference	Salt stability	Possible applications
α-Amylase	*Halobacterium salinarium* [2]	3-4 M	Hydrolysis of starch, food bioprocessing, confectionery, baking, textile industries and detergents.
α-Amylase	*Halothermothrix orenii* [3]		
α-Amylase	*Haloferax mediterranei* [4]		
Amylase	*Halobacillus sp. strain* [5]	1-2 M	
Amylase	*Psychromonas antracticus* [6]		
α-Amylase	*Bacillus dipsosauri* [7]		
Xylanase 1, 2	*Halophilic bacterium, CL8* [8]	3-4 M	Pulp and paper production, Biobleaching and detergents.
β-Xylanase, β-Xylosidase	*Halorhabdus utahensis* [9]	2-3 M	
β-Agarase	*Pseudomonas sp. strain W7* [10]		Production of neoagaro-oligosaccharides used as probiotics, foods and cosmetics.
Agarase	*Alteromonas sp.* [11]	2-3 M	
Haloprotease CP1	*Pseudomonas sp.* [12]	0-4 M	Peptides and amino acid production, detergents and food processing.
Serine protease	*Halobacterium halobium* [11,13]	3-4 M	
Lipase	*Halococcus dombrowskii* [14]	4 M	Waste treatment, pharmaceuticals, detergents and food taste and aroma improvement.
β-Galactosidase	*Haloferax alicantei* [15]	3-4 M	Production of low lactose dairy foods and oligosaccharides.

Table 1: Summarization of halophilic enzymes, their microbial sources and applications.

Alteromonas sp., a halotolerant bacterium has found to produce agarase enzyme, which can produce neoagaro-oligosaccharides e.g., neoagarohexaose, neoagarobiose and neoagarotetraose [11]. β-Agarase is another halophilic enzyme that hydrolyzes agar and agarose into neoagarotetraose. A marine bacterium, Zobellia galactanivorans possesses two β-agarase genes (agaA and agaB) and the products of both genes are able to cleave the β1→4 linkages of agarose [18]. β-Galactosidase as a member of glycosyl hydrolase family, known to be able to break glycosic bonds. Halophilic β-galactosidase has been isolated from haloarchaeon *Haloferax alicantei*. This halophilic enzyme retains its activity and stability in high salt concentrations and has optimal activity in 4M NaCl [19]. The main biotechnological applications of β-Galactosidase enzyme are production of low lactose dairies e.g., low lactose milk and cheese for lactose-intolerant individuals, industrial scale production of oligosaccharides for further use in pharmaceuticals and bioprocessing of salty whey for galactooligosaccharide production probiotic applications to prevent allergic and gastrointestinal diseases by reducing adherence of enteropathogenic [20].

Xylanase is responsible to degrade xylan, but despite the importance of these enzymes, up to now, only a few xylanase with favorable properties have purified. These enzymes are derived mainly from marine habitats and halotolerant bacteria e.g., *Glaciecola mesophila*, *Chromohalobacter sp*. and *Nesterenkonia halobia*. Some of these enzymes show good activity at temperatures higher than 60˚C and with strict requirement for NaCl. In addition, they are stable in wide pH ranges, 6-11 [6]. The most important applications of xylanase are in the pulp and paper production, where high pH and temperature is necessary for high efficient biobleaching; however, in most of these applications, xylanase should be haloalkaliphile and thermostable. Alkaliphilic xylanase used in detergents, which work in high temperature and alkaline conditions. Some less well documented applications of xylanase is in coffee extraction, production of soluble coffee, protoplasting of plant cells, production of active polysaccharides as antimicrobial agents and antioxidants and production of alkyl glycosidase for use as surfactant. Xylanase is useful in conjugation with other enzymes e.g., proteases, oxidases and isomerases [21].

Many halophilic bacteria produce lipase enzymes, e.g, *Marinobacter flavomaris* with C_{14} lipase activity can thrive optimally at 2-6% NaCl, *Marinobacter lipolyticum* thrives at 7.5% NaCl, *Psychrofexus tropicus* shows C_4/C_6 esterase activity and *Sinococcus qinghaiensis* grows in range of 1-25% NaCl. Among haloarchaeons, *Halococcus dombrowski*, with C_8 esterase activity and *Halobacterium* sp. with optimal growth at 2-4 M NaCl and maximum lipase production at 4 M NaCl and 45˚C are recognized as the two best lipase producers [14]. Biotechnological and industrial applications of lipases include hydrolysis of fats in wastes, production of chiral intermediates of important pharmaceuticals and detergents. These applications normally required harsh conditions, e.g., high salt contents of factories waste output and use in organic media with low water activity. Biocatalysis with lipases mostly demands organic solvents to carry out reactions at low water activity [22]. Therefore, due to low water activity requirement of halophilic lipases, they are highly stable in a mixture of water-organic solvents. It has been shown that low molecular weight volatile fatty acids (VFA) are associated to taste and aroma of foods. Halophilic lipases also are used to improve the taste and the aroma of foods such as salted cheeses and butters, sausages, beverages, bakery products and salted fish sauces [23].

Proteases are enzymes with the ability to hydrolyze peptide bonds to produce simple and shorter peptides and amino acids. These enzymes are one of the largest groups of hydrolytic enzymes used in industries (approx. 60% of world enzyme sale) with wide applications, e.g., food processing, pharmaceuticals, laundry and dish washing, diagnostic reagents and waste treatments [24]. Many halophilic proteases are detected in a wide variety of halophilic archaea; enable them to in-situ degradation of proteins. These archaea include *Natronomonas pharaonis*, *Haloferax mediterranei*, *Natronococcus occultus* and *Natrienema sp*. J7 [25]. *Halobacterium halobium* has shown to be an excellent protease producer with the requirement of 4 M NaCl for optimal activity and stability. This enzyme has used successfully for peptide synthesis especially for glycine-containing oligopeptides in the aqueous / organic media [11]. Detergent compatible proteases have a unique characteristic to be stable and active in high temperature (50-70°C) and alkaline conditions (pH 8-12) in laundry [26]. Using of halophilic and halotolerant microorganisms with the ability to produce protease enzymes are more favorable in food processing under highly saline conditions e.g., processing of protein rich foods, e.g., fish, meat and soy based products [6].

One of the interesting properties of haloarchaea is their ability to harvest light energy through a photo-reactive protein, bacteriorhodopsin [27]. Since the discovery of this protein's function in 1970s, many patents for its application have issued including ATP and electricity production from sunlight, development of bioelectronics elements of computer memories and data processing units, ultra-fast light and motion detectors. Applications in nanotechnology, e.g., molecular sensors, motors and transistors also have being developed [28]. Recently this protein is used to develop military devices e.g., spatial light modulators and hybrid biomolecular. For instance, spatial light modulators have used as non-destructive system to scrutinize artillery and tank ammunition; possibly, a thin film of bacteriorhodopsin has used to obtain high resolution in real-time measurements. Hybrid biomolecular opens new horizons for developing of telecommunication, radar-signal processing, conventional transistors, semiconductor lattices, broad-band and fiber-optic converters, and multiplexers [29].

Conclusion

Very diverse hypersaline environments exist on our planet and so are the living forms inhabited them. These organisms manifest different protein adaptations acquired during million years of evolution to remain both active and stable in such precluding habitats. It is very interesting to know that, in theory, it is possible to convert a non-halophilic protein into a halophilic one, but it requires detailed biophysical and biochemical studies as well as a deeper understanding in folding, stability, solubility and translocation of halophilic proteins which has not been really addresses in literatures. In this regard, however, many basic questions have been answered and much more yet to be learnt. Therefore, expanded understanding and optimization of aforementioned parameters can lead us to more effective, economically potential and novel enzymatic processes, which once were restricted or have not used until now. This will help to gain new and valuable knowledge on extremozymes and will open new biotechnological applications. Finally, scientists strongly believe that discovery of new extremozymes or manipulating of currently available extremophiles will offer new opportunities for developing biocatalysis and biotransformation systems [30].

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