Research Paper

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Extremozyme Production by Extremely Halophilic Bacterial Strains isolated from Saline/Hypersaline Environments

Biotechnology

KEYWORDS : Extremophiles, Casein, Gelatin, Lipase, Xylanase.

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ABSTRACT

The extracellular hydrolytic enzyme production profile of the 16 isolated bacterial strains was achieved by testing their ability to hydrolyze various substrates such as casein, gelatin, starch, Tween-80, and RBB-xylan. Casein and gelatin hydrolyzing enzyme activity was observed in 4 strains (MJS1, JS-4, BB1 and BB2). Strains MJS2 and BC2 showed amylase activity. Lipolytic activity was exhibited by 3 halophilic bacterial isolates (JS-1, JS-5, and BC2). Three isolates including MJS4, BC1 and JS-3 hydrolyzed xylan. Majority of the isolates (68%) showed their potential to hydrolyze atleast one of the above mentioned substrates while some strains (32%) failed to hydrolyze none of the substrates.

Introduction

Extensive global research efforts have revealed the novel diversity of extremophilic microbes. They grow under extreme environmental conditions that are hostile to most organisms. Extremophilic microorganisms are adapted to thrive in such hostile environments (Vijayanand *et al.*, 2010). As a result of adaptations to extreme environment, extremophiles have evolved unique properties which can be of biotechnological and commercial significance. The organisms living in such dual extreme environment possess special adaptation strategies that make them interesting not only for fundamental research but also towards exploration for industrial applications (Margesin and Schinner, 2001). Extremophiles have been proven to be a rich source of biological information (Vijayanand *et al.*, 2010). Extremozymes, the enzymes isolated from extremophiles are now replacing the harsh chemical catalysts in many industries, including manufacturing of chemicals, textiles, pharmaceuticals, detergents, food, paper and agricultural chemicals (Mehta *et al.*, 2006). They include protease, amylase, cellulase, xylanase, keratinase and other enzymes that have numerous applications in many industrial processes. These enzymes are adapted to extreme environments and as a result, they are unusually stable. They are therefore suitable candidates for applications in industrial processes that are performed under harsh conditions, such as high temperatures or in the presence of organic solvents or high ionic strength. For the same reasons, they are also ideal molecules for investigations aimed at elucidating the mechanisms of chemico-physical stability of proteins (Schiraldi *et al.*, 2002).

With increasing emphasis on environmental protection the use of enzymes particularly from extremophiles has gained considerable attention during last several years. The increasing industrial demands for biocatalysts that can cope with industrial process conditions, has led to the considerable efforts for the search for such enzymes. Despite the fact that to date more than 3000 different enzymes have been identified and many of these have found their way into biotechnological and commercial applications, the present enzyme tool box is still not sufficient to meet the complete demands. A major reason for this is that many available enzymes do not withstand industrial reaction conditions (Burg, 2003). As a result, the characterization of microorganisms that are able to thrive in extreme environments has received a great deal of attention. Such extremophiles act as a valuable source of novel enzymes (Sanchez-Porro *et al.*, 2003; Cojoc *et al.*, 2009). The present study aims to investigate the potential of extremely halophilic bacterial strains to produce extracellular halophilic hydrolytic enzymes.

Materials and Methods

16 extremely halophilic bacterial strains isolated from various saline and hyper saline environments were evaluated for their potential to produce extracellular halophilic hydrolytic enzymes.

Extracellular hydrolytic enzyme production by the isolates

Extracellular protease activity

Proteolytic activity (caseinolytic) of the isolates were screened quantitatively in a saline medium containing milk (50%) plus 10-20% total salts (Ventosa *et al.*, 1982) supplemented with 0.5% (w/v) yeast extract and 1% peptone. The medium was solidified by adding 20 g/l of agar-agar. Zones of precipitation of paracasein around the colonies appearing over the next 15 days were taken as evidence of proteolytic activity.

Assay of gelantinolytic activity

The medium contained 2% (w/v) agar, 1% (w/v) gelatin in 50mM tris buffer of pH 7.2 with 20% NaCl and was sterilized at 120°C for 15 min. About 15 ml of the medium was poured in a petridish under aseptic conditions. Using a sterilized cork borer, two 6mm diameter cups were made in each of the agar plate. The culture filtrate of the isolated halophilic bacterium was added carefully into each well. The petridishes were incubated at 37°C for 48 h. After incubation the plates were developed with 15% (w/v) mercuric chloride in 20% HCl. After 10 min., a clear transparent zone indicated the hydrolysis of the gelatin by extracellular proteases. Where as the rest of the plate became opaque due to the coagulation of gelatin by $HgCl_2$. The diameter of the clear zone was used as a measure of protease activity. (Vidyasagar *et al.*, 2006).

Extracellular amylase activity

1% starch and 2% agar were taken and mixed with 100 ml of above mentioned media containing 20% NaCl (w/v) and autoclaved. After solidification, the agar plates were inoculated with the isolates and incubated for 15 days, after incubation the plates were developed with 2% KI in 0.2 g iodine solution. The blue colour developed in the petriplates and clear zones seen around the colonies indicated the hydrolysis of the starch by the enzyme.

Extracellular Lipolytic activity

Lipolytic activity of the isolates was detected by screening for zones of hydrolysis around colonies growing on above mentioned salt medium containing 1% Tween-80, after incubation for 15 days (Gonzalez *et al.*, 1978).

Extracellular xylanase activity

Bacterial isolates were grown in plates containing 10-20% (w/v) total salt, with substrate 0.1% (w/v) Remazol Brilliant Blue (RRB)-xylan (Sigma-Aldrich) and incubated for 15 days at 37°C. The xylanolytic activity was detected by the presence of a halo under UV light (Farkas *et al.*, 1985).

Result and Discussion

The extracellular hydrolytic enzyme production profile of the 16 isolated bacterial strains was achieved by testing their ability to hydrolyze various substrates such as casein, gelatin, starch, Tween-80 and RBB-xylan (Table.1). Majority of the isolates

(68%) showed their potential to hydrolyze atleast one of the above mentioned substrates while some strains (32%) failed to hydrolyze none of the substrates (Fig.1).

Protease activity
About 25% of the isolates (MJS_1, JS-4, BB_1 and BB_2) hydrolyzed gelatin and casein, showing their extracellular protease activity. These halophilic proteases can be widely exploited in laundry and detergent industries, where they help in the removal of protein based stains from clothings (Lanyi, 1974). Halophilic proteases have been produced by a variety of halophilic bacteria such as, *Halobacterium salinarum* (Norberg and Hofsten, 1969), *Natrialba magadii* (Gimenez *et al.*, 2000) and *Halogeometricum borinquense* TSS 101 (Vidyasagar *et al.*, 2006) . Interestingly, the gelantinolytic activity of these isolates can break down hide proteins, if the unprocessed salt in directly used in the brine curing of hides, these strains will digest the collagen of hide, lowering the quality of leather. Therefore, before using the salt in hide preservation, the halophilic bacterial content and their gelantinoytic activity should be restricted to prevent hide damage (Birbir *et al*., 1996).

Amylase activity
Only two isolates (MJS_2 and BC_2) showed positive amylase activity. Similar to other microbial amylases, halophilic amylases produced by the above mentioned halophilic bacterial strains can be exploited in different biotechnological applications including the food industry, in detergents to promote stains removal and in the paper and pulp industry for the modification of starches for coated paper (Ellaiah *et al.*, 2002; Setati, 2010). Halophilic amylases, commonly cyclomalto-dextrinases (EC: 3.21.54), have been produced from a variety of halophilic bacteria such as *Halomonas meridiana* (Coronado *et al.*, 2000), *Halobacillus* sp. (Amoozegar *et al.*, 2003) and *Chromohalobacter* sp.TVSP.101 (Prakash *et al.*, 2009).

Xylanase activity
Nearly 19% of the isolates (MJS_4, BC_1 and JS-3) hydrolyzed RBB-xylan effectively, showing their potential to produce extra cellular xylanases. These halophilic xylanases can be widely used in the baking industry to improve the dough properties and in biobleach of paper and pulp (Collins *et al.*, 2005; Mamo *et al.*, 2009) Pulp pre-bleaching by amylases reduces the amount of chlorine required for conventional chemical bleaching and minimizes the release of toxic chloroorganic wastes into environment (Annamalai *et al.*, 2009). Recently, halophilic amylases have been purified from halophilic microorganisms, such as *Halorhabdus utahensis* (Waino and Ingvorsen, 2003), a halophilic bacterium, CL8 (Gomes and Steiner, 2004) and *Glaciecola mesophila* (Guo *et al.*, 2009).

Lipase activity
Three isolates showed positive lipase activity by hydrolyzing Tween 80. Halophilic lipases from the above mentioned halophilic bacterial strains, show a promising role in laundry and automatic dish washing detergents to improve the oil cleaning capabilities (Birbir and Sesal, 2003; Sanchez-Porro *et al.*, 2003). The presence of lipolytic activity has been reported in many halophilic bacteria such as, *Chromohalobacter candensis* ATCC 43984, *Salinivibrio costicola* DSM 8285, and *Salibacillus marismourtui* DSM 12325 (Sanchez-Porro *et al.*, 2003) and *Salinivibrio* sp.strain SA-2 (Amoozegar *et al.*, 2008).

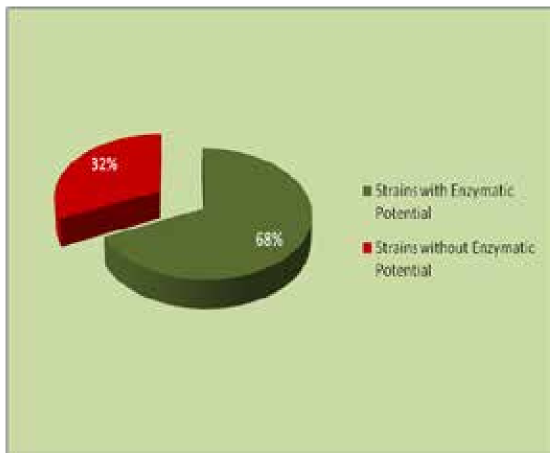
Table.1 Extracellular hydrolytic enzymes of the isolated halophilic bacterial strains

Strain	Hydrolysis of				
	Starch	Gelatin	Casein	Tween 80	RRB – Xylan
MJS_1	-	++	++	-	-
MJS_2	+	-	-	-	-
MJS_3	-	-	-	-	-
MJS_4	-	-	-	-	+
JS-1	-	-	-	+	-
JS-2	-	-	-	-	-
JS-3	-	-	-	-	+
JS-4	-	++	++	-	-
JS-5	-	-	-	+	-
BB_1	-	+	+	-	-
BB_2	-	+	+	-	-
BB_3	-	-	-	-	-
BC_1	-	-	-	-	+
BC_2	+	-	-	+	-
BC_3	-	-	-	-	-
BC_4	-	-	-	-	-

RBB-Xylan – Remazol Brilliant Blue xylan;

(+)□ presence of enzyme activity; (-) □ absence of enzyme activity

Fig. 1 Enzymatic potential of the isolated halophilic bacterial strains



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