The Bioscan 5(1) : 97 - 99, 2010
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

SCREENING OF EXTRACELLULAR HYDROLYTIC ENZYMES FROM *MARINOBACTER HYDROCARBONOCLASTICUS* STRAIN AK5

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KEY WORDS
Hydrolytic enzymes
Extreme halotolerant
Marinobacter hydrocarbonoclasticus

Received on :
30.11.2009
Accepted on :
17.01.2010

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ABSTRACT
The aim of this study was to screen the extracellular hydrolytic enzymes from extreme halotolerant *Marinobacter hydrocarbonoclasticus* strain AK5, isolated from Arabian Sea Mumbai, Maharashtra, India. Identification of the bacterium was done based upon biochemical tests and 16S rRNA sequence. It is a gram-negative, aerobic, non-spore forming, motile, rod-shaped bacterium. The strain AK5 grows at optimum temperature 35°C and pH 8.5. It grows at NaCl concentrations of 0.08 to 4.1 M. The novel extremozymes such as proteases, amylases, xylanases, lipases, esterases, glutaminases asparaginases and inulinases were screened. The strain AK5 able produce three hydrolytic enzymes such as esterase, asparaginase and glutaminase which have potential applications in various biotechnological processes.

INTRODUCTION

Microbial life is not limited to specific environments since the past few decades it was known that the microbial communities can be found in the most diverse conditions, including extremes of temperature, pressure, salinity and pH. These microorganisms, called extremophiles, produce biocatalysts that are functional under extreme conditions. Consequently, the unique properties of these biocatalysts have resulted in several applications of enzymes in industrial processes (Bertus van den Burg, 2003).

The extracellular hydrolytic enzymes such as amylases, proteases, lipases, DNases, pullulanases and xylanases have quite diverse potential usage in different areas such as food industry, feed additive, biomedical sciences and chemical industries (Rao *et al.,* 1998; Kulkarni *et al.,* 1999; Niehaus *et al.,* 1999; Pandey *et al.,* 1999). Industrial processes are carried out under specific physical and chemical conditions which cannot always be adjusted to the optimal values required for the activity of the available enzymes. The present study was carried out to screen extracellular hydrolytic enzymes from extreme halotolerant bacterium *Marinobacter hydrocarbonoclasticus* strain AK5, which serve as a novel source of extremozymes.

MATERIALS AND METHODS

Microorganism and growth conditions

Extreme halotolerant bacterium *Marinobacter hydrocarbonoclasticus* strain AK5 was isolated from marine water Gate way of India located in Mumbai, Maharastra, India. The isolate was identified as *Marinobacter hydrocarbonoclasticus* by biochemical tests and 16S rRNA analysis and the sequence is deposited in NCBI GenBank with accession number EU878305. The organism was grown on agar plates composed of 10 g/L of yeast extract, 10 g/L of peptone, 3 g/L trisodium citrate, 20 g/L of $MgSO_4.7H_2O$, 2 g/L of KCl, 0.023 g/L of $FeCl_2$, 100 g/L of NaCl, 20 g/L of agar. After 48 hrs of incubation at 37°C the culture was stored at 4°C. In order to detect the production of extracellular hydrolases such as proteases, amylases, xylanases, lipases, esterases, asparaginases, glutaminases and inulinases, different enzymatic agar plate assays were carried out.

Screening of strain AK5 for extracellular hydrolytic activities

Extracellular protease activity

Proteolytic activity of the culture was screened qualitatively in a saline medium containing milk (50%) plus 10% (w/v) NaCl (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. Zones of precipitation of paracasein around the colonies appearing over the next 48 hrs were taken as evidence of proteolytic activity.

Extracellular amylase activity

The presence of amylolytic activity on plate was determined following the method described by Amoozegar *et al.,* (2003), using starch agar medium containing 10% (w/v) NaCl. After incubation at 37°C for 48 hrs, the plates were flooded with 0.3% I_2–0.6% KI solution: a clear zone around the growth indicated hydrolysis of starch.

Extracellular lipase activity

To observe lipase production, the strain was cultured on nutrient agar plate containing olive oil (2.5%), victoria blue (0.4 mg/L) and 10% salt with an initial pH of 7.2 - 7.4. The plates were incubated at 37°C for 48 hrs and the colonies with blue color zones were identified as lipase producing strain (Samad *et al.,* 1989; Martin *et al.,* 2003).

Extracellular esterase activity

Esterase activity of the isolate was detected by screening for zone of hydrolysis around the colony growing on plate containing 10 g/L of yeast extract, 20 g/L of $MgSO_4.7H_2O$, 2 g/L of KCl, 0.1 g/L of $CaCl_2.H_2O$, 10% of NaCl, 20 g/L of agar, supplemented with 0.1% Tween-80.

Extracellular xylanase activity

Xylanase activity was detected according to using a saline medium containing 1% xylan and 10% of NaCl. After incubation at 37°C for 48 hrs, the plates were flooded with 0.1% congo red solution. The clear zones around colonies indicated qualitative xylanase activity (Wejse and Ingvorsen, 2003).

Extracellular L-asparaginase activity

The presence of L-asparaginase activity on plate was determined following the method described by Gulati *et al.,* (1997), using 0.5% of L-asparagine in medium containing 10% NaCl. Phenol red was used as pH indicator. Pink zone formed around growth indicated the production of L-asparaginase.

Extracellular L-glutaminase activity

The presence of L-glutaminase activity on plate was determined using 0.5% of L-glutamine in medium containing 10% NaCl. Phenol red was used as pH indicator. Pink zone formed around growth indicated the production of L-glutaminase.

Extracellular inulinase activity

The production of inulinase by AK5 was detected by preparing medium containing 2 g/L of inulin, 10 g/L of yeast extract, 20 g/L of $MgSO_4.7H_2O$, 2 g/L of KCl, 10% of NaCl, 20 g/L of agar. Inulin was used as the sole source of carbon in this medium; thus, bacterial growth after 48 hrs of incubation at 37°C, shows the presence of inulinase activity.

RESULTS

Phenotypically, the *Marinobacter hydrocarbonoclasticus* strain AK5 is a gram-negative, motile, non-spore forming, rod-shaped, aerobic, oxidase and catalase-positive. Strain AK5 utilized acetate, butyrate, lactate, citrate, L-asparagine, and L-glutamate, and was unable to utilize L-arabinose, ribose, lactose, α-ketoglutarate, D-sorbitol, L-tryptophan, glycine, L-lysine, as single source of carbon and energy. The strain was also unable to utilize DL-β-hydroxybutyrate and did not accumulate its polymer.

This bacterium exhibited extreme halotolerance, since it was able to grow in a medium containing NaCl at concentrations ranging from 0.08 to 4.1 M. The optimal NaCl concentration for growth was about 0.6 M. The strain AK5 is, an extremely halotolerant and slightly halophilic bacterium. The strain AK5 able to grow at temperature ranging from 10 to 45ºC with optimum temperature at 35°C and pH ranging from 4–10

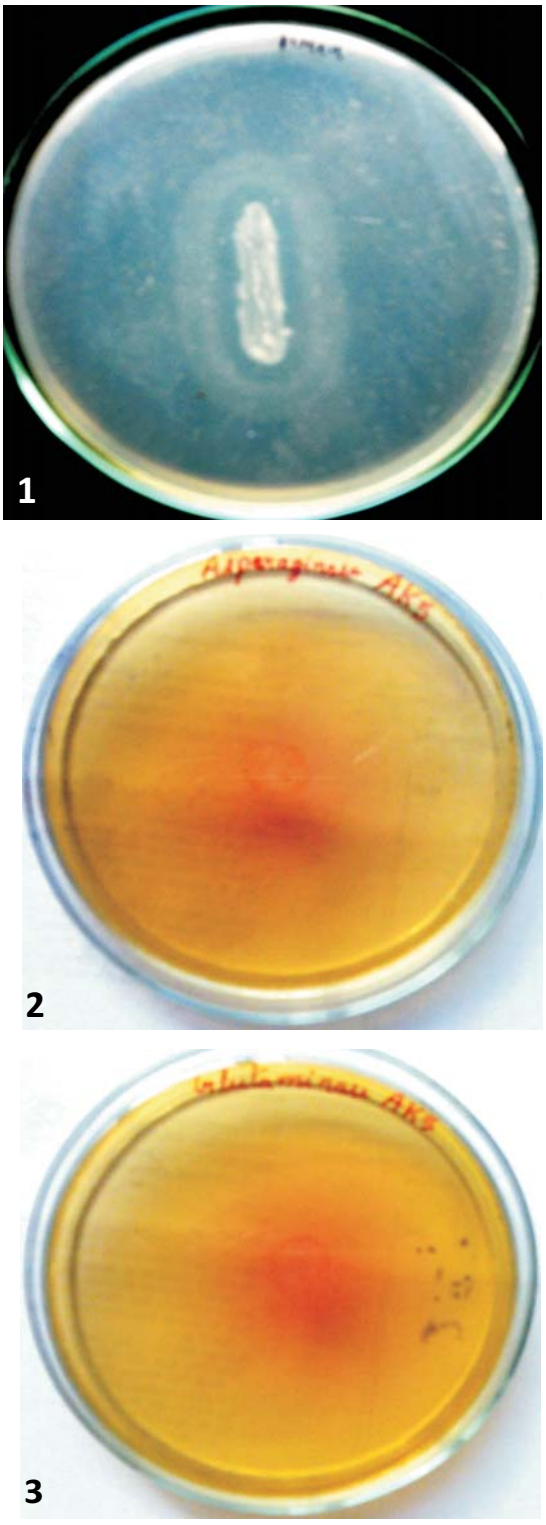

Figure 1 to 3: Hydrolysis of tween 80, L-asparagine and L-glutamine respectively by *Marinobacter hydrocarbonoclasticus* strain AK5 after 24 hrs at 37ºC

with optimum pH 8.5. Different extracellular hydrolytic enzymes were screened from *Marinobacter hydrocarbonoclasticus* strain AK5. The strain AK5 able to produce three enzymes such as esterase, asparaginase and

glutaminase with zone diameter of 24 mm, 12mm and 15mm respectively as shown in Figs. 1to3.

DISCUSSION

Extremozymes have a great economic potential in many industrial processes, including agricultural, chemical and pharmaceutical applications. Many consumer products will increasingly benefit from the addition or exploitation of extremozymes. Sanchez-Porro *et al.,* (2003), showed the abundance of five hydrolytic enzymes including amylase, protease, lipase, DNase and pullulanase by moderately halophilic bacteria from salterns in Spain. Zavaleta and Cardenas-Fernandez (2007), determined the amylase, lipase and protease production among halophilic bacteria isolated from Pilluana brines, Peru. In the present study, the ability of the strain AK5 to produce three different extracellular hydrolases has been investigated. We isolated the strain with significant ability to produce esterase, asparaginase and glutaminase. These enzymes produced by *M. hydrocarbonoclasticus* strain AK5 is being reported here for the first time. The salt-tolerant esterase detected in strain AK5 provides the possibility to use for hydrolysis and esterification in lipid modification under saline conditions.

Further studies are currently in progress in order to select the best hydrolytic enzymes and investigations should be directed towards the in-depth characterization of these extremozymes and the cloning and characterization of the corresponding encoding genes.

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