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Comparison of Enzyme Production by Halophilic Bacteria in Sterilized and Non-Sterilized Conditions

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Abstract

In present study, we aimed to determine the enzymes of moderately halophilic bacteria isolated from Sereflikochisar Lake the largest salt lake in Turkey and compared their enzymes in sterilized and non-sterilized conditions to obtain any contamination from non-sterilized growth condition. The enzymes of moderately halophilic bacteria isolated from Şereflikoçhisar Lake were determined by SDS-PAGE. In addition of zymogram analysis, the enzymes from the moderately halophilic bacteria cultured in sterilized and non-sterilized growth condition were compared on SDS-PAGE to determine any contamination in non-sterilized growth condition. The enzyme bands in both conditions were similar and therefore the investigation suggests that the moderately halophilic isolates can be easily cultured in non-sterilized growth conditions without sterilization of growth medium and thereby energy cost can be reduce to produce such enzymes for industrial applications.

Key words: Halophilic bacteria, enzyme production, zymogram analysis

INTRODUCTION

Halophiles can be loosely classified as slightly, moderately or extremely halophilic, depending on their requirement for NaCl. Slight halophiles grow optimally at 0.2–0.85 mol L^{-1} (2–5%) NaCl; moderate halophiles grow optimally at 0.85–3.4 mol L^{-1} (5–20%) NaCl; and extreme halophiles grow optimally above 3.4–5.1 mol L^{-1} (20–30%) NaCl. In contrast, nonhalophiles grow optimally at less than 0.2 mol L^{-1} NaCl. Halotolerant organisms can grow both in high salinity and in the absence of a high concentration of salts. Many halophiles and halotolerant microorganisms can grow over a wide range of salt concentrations with requirement or tolerance for salts sometimes depending on environmental and nutritional factors [1].

Moderately halophilic bacteria constitute a complex group of micro-organisms adapted to thrive in hypersaline environments. Apart from their ecological importance, moderately halophilic bacteria have great potential for its use in biotechnology. They accumulate high cytoplasmic concentrations of compatible solutes that may be used as osmoprotectants and stabilizers of enzymes and cells [2], and some of them are used for the degradation of polluting industrial residues or toxic chemicals and for enhanced oil-recovery processes [3, 4]. Moreover, moderately halophilic bacteria produce extracellular salt-tolerant enzymes of great interest for biotechnological processes [4, 5]. Extracellular proteins, such as those secreted into the medium and probably those in the periplasmic space, are exposed to external saline environments and must adapt to variable salinities.

In the study, we aimed to determine the enzymes from moderately halophilic bacteria isolated from Sereflikochisar Lake in Turkey by SDS-PAGE. Furthermore, the enzymes from the moderately halophilic bacteria cultured in sterilized were compared with non-sterilized growth condition on SDS-PAGE to determine any contamination from non-sterilized growth condition.

MATERIALS and METHODS

Microorganism Strains and Growth Media

Ten moderately halophilic and halotolerant *Bacillus* sp. isolates (Isolate 1-10) isolated from Sereflikochisar Lake in Turkey in previous study were used in this study [6]. Identification and characterization of the isolates were done in the former study [6]. Because of the isolates could grow in both LB medium with 10% and 1% w/v salts, they were determined as halophilic and halotolerant. In the study, these halophiles were cultured in LB medium (Luria Bertani, pH 7.5) with final concentration of 10% w/v total salts as moderately halophiles at 37ºC to create selective growth condition.

After the sterilization of growth medium (LB with 10% w/v salts) with autoclaving, the isolates were inoculated fresh sterilized medium under sterilized growth condition for sterilized growth condition. To grow the isolates in non-sterilized growth condition, the medium wasn't sterilized (without autoclaving) and the inoculation of the bacteria was done under non-sterilized room conditions.

Protein Extraction and SDS-PAGE Electrophoresis

Total protein of isolated strains was obtained by growing cells in liquid medium for 2 days with shaking. The culture was centrifugation at 15.000 rpm for 15 minutes and the supernatant was then mixed with an equal volume of tricholoroacetic acid (TCA, 20% w/v). Total proteins were collected by re-centrifugation. The total protein analysis was performed by using a denaturating polyacrylamide gel electrophoresis (SDS-PAGE, 12% w/v) [7].

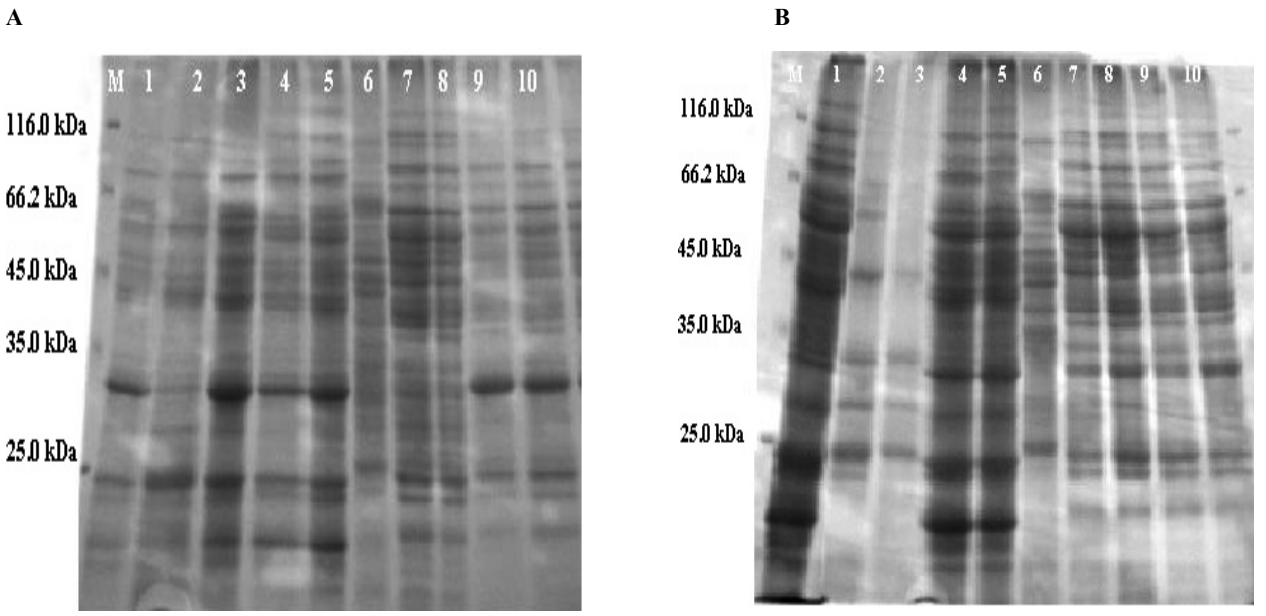

Figure 1. Total proteins of the isolates (1-10) on SDS-PAGE, **A**: sterilized growth condition, **B:** non-sterilized growth condition

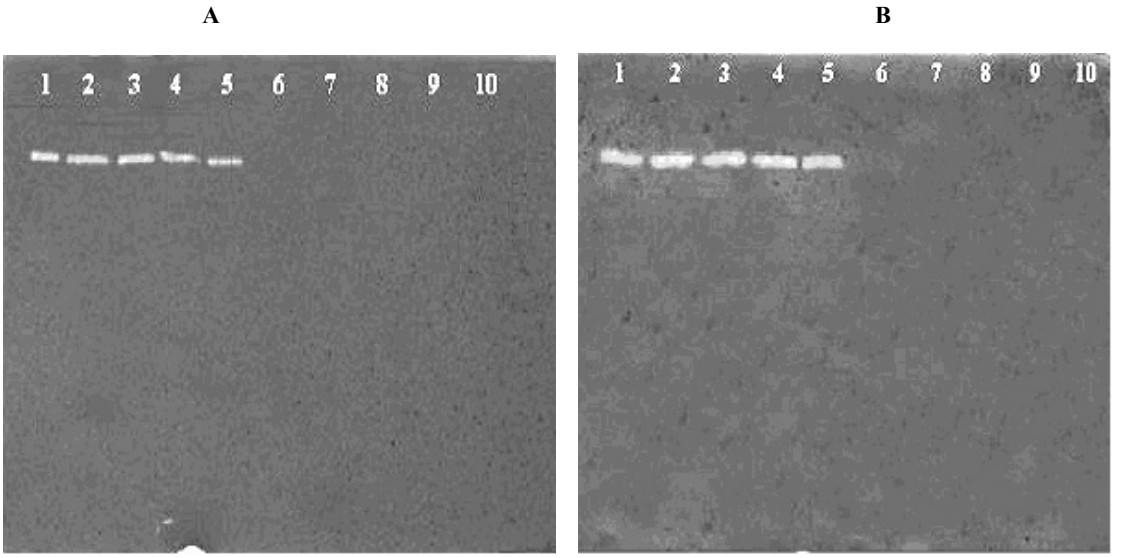

Figure 2. α-Amylase activity of the isolates (1-10) on SDS-Starch-PAGE, **A**: sterilized growth condition, **B:** non-sterilized growth condition

Zymogram analysis was performed on SDS-PAGE with Lichenan, Xylan, CMC (Carboxymethylcellulose) and Starch as substrates. The substrates were added to the 12% SDS-PAGE gel (resolving gel) to a final concentration of 0.2% (w/v). After electrophoresis, the gel was gently rocking in 50 mM sodium phosphate (pH 7.0) and 20% (v/v) isopropanol solution at room temperature for an hour to remove denaturated agents. The gel was then transferred into renaturation solution (50 mM sodium phosphate, 5 mM β-mercaptoethanol, 1 mM EDTA (Ethylenediamiltetraacetic acid)) and incubated overnight at 4°C. After renaturation of the protein, the gel was soaked in 50 mM sodium phosphate buffer at 4°C for an hour. It was then covered with parafilm and incubated at 37°C for four hours. After staining of the gel with 0.2% (w/v) Congo-Red and 5 mM NaOH for an hour, it was washed with 1 M NaCl and 5 mM NaOH overnight to remove excess stain from the active bands [8] to detect of Lichenase, Xylanase and CMCase (Carboxymethylcellulase) activity. For α-amylase activity, proteins on a gel were stained with Coomassie Brilliant Blue.

RESULTS

In the present study, enzyme activities of moderately halophilic bacteria were shown on SDS-PAGE by using zymogram analysis. On the other hand, halophilic enzymes extracted from non-sterilized growth condition were compared with the enzymes of the same organisms grown on sterilized growth conditions to check any bacterial and thereby any enzyme contamination in non-sterilized growth condition by SDS-PAGE.

Figure 1A shows total proteins of the moderately halophilic isolates that were grown on sterilized growth condition. The total protein bands on SDS-PAGE seem to be similar for ten isolates in non-sterilized growth medium (Figure 1B).

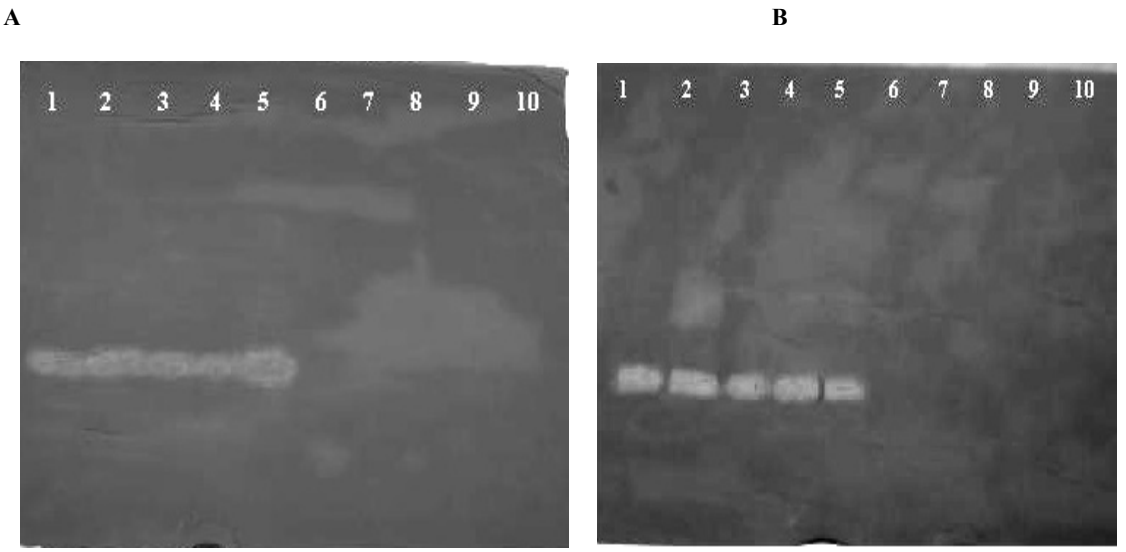

Figure 3. Lichenase activity of the isolates (1-10) on SDS-Lichenan-PAGE, **A**: sterilized growth condition, **B:** non-sterilized growth condition

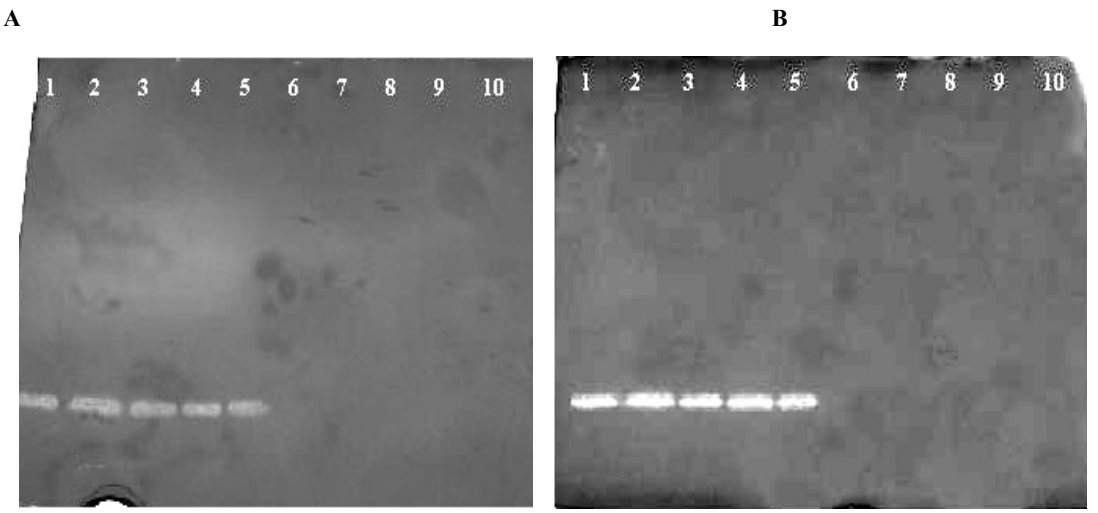

Figure 4. Xylanase activity of the isolates (1-10) on SDS-Xylan-PAGE, **A**: sterilized growth condition, **B:** non-sterilized growth condition

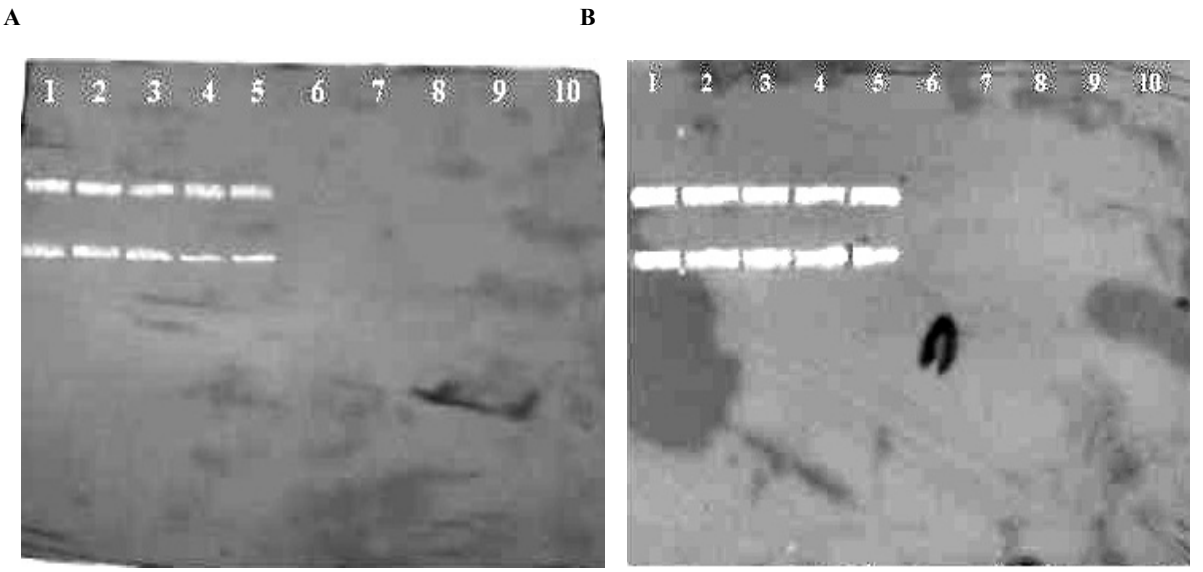

Figure 5. CMCase activity of the isolates (1-10) on SDS-CMC-PAGE, **A**: sterilized growth condition, **B:** non-sterilized growth condition

To visualize α-amylase enzyme activity of the isolates, proteins were electrophoretically separated in an SDS-PAGE (12% w/v) with starch as a substrate. Isolates 1-5 showed α-amylase activity out of the ten isolates. Zymogram analysis showed that protein bands responsible for the α-amylase were determined as approximately 85-90 kDa on SDS-PAGE. Similar enzyme activities were detected for the same bacteria grown in either sterilized or non-sterilized growth media (Figure 2A and B).

The lichenase enzyme activity of the isolates performed on SDS-Lichenan-PAGE. Isolates 1-5 out of the ten isolates showed lichenase activity bands approximately 30-35 kDa on the gel. Zymogram analysis results for lichenase avtivity were similar for the supernatants of bacteria grown in both sterilized and non-sterilized growth media (Figure 3A and B).

To determine xylanase activity of the isolates, proteins were analyzed on SDS-Xylan-PAGE. The five isolates (isolate 1-5) showed xylanase activity. The protein bands responsible for xylanase activity were approximately 20-25 kDa in molecular weight. Zymogram analysis results were also similar for the same bacteria grown in either sterilized or non-sterilized growth media (Figure 4A and B).

The CMCase enzyme activity of the isolates performed on SDS-CMC-PAGE. The five isolates (isolate 1-5) showed CMCase activity. The SDS-CMC-PAGE zymogram showed two bands of approximately 70-75 and 45-50 kDa probably because of CMCase enzyme consisted of two sub-units or the enzyme is a product of different cellulase genes. Similar enzyme activities were detected for the bacteria tested in both sterilized and non-sterilized growth media (Figure 5A and B).

DISCUSSION

Sereflikochisar Lake is the largest salt lake in central Turkey. This lake is a major source of solar salt for food, hide and other industries locally. Due to the economic importance of salt obtained from this lake, a microbial survey has been conducted. In the study, the proteins from ten halophilic isolates (*Bacillus* sp.) were screened with zymogram analysis.

Although moderately halophilic bacteria have a great biotechnological potential, only a few studies have been carried out concerning their production of extracellular enzymes [4]. A considerable amount of effort has been dedicated to the study of extracellular salt-tolerant enzymes of the moderately halophilic bacteria, especially toward the use of such enzymes in biotechnological processes.

The genus *Bacillus* is well known as an enzyme-producer and many industrial processes use species belonging to this genus for commercial production of enzymes [9]. Extracellular hydrolytic enzymes such as amylases, proteases, lipases, DNases, pullulanases, lichenases, cellulases and xylanases have quite diverse potential usages in different areas such as food industry, feed additive, biomedical sciences and chemical industries [10, 11, 12, 13, 14]. *Bacillus* sp. isolates (1-5) producing α-amylase, lichenase, cellulase and xylanase can be important for these industries. Industrial processes are carried out under specific physical and chemical conditions that cannot always be adjusted to the optimal values required for the activity of the available enzymes. For that reason, it would be of great importance to have available enzymes showing optimal activities at different values of salt concentrations.

Halophilic extracellular amylases were characterized from a moderately halophilic *Acinetobacter* [15], *Nesterenkonia halobia* [16], *Micrococcus varians* subsp. *halophilus* [17], *Halomonas meridiana* [18] and other *Micrococcus* isolates [19, 20]. Little information has been published on extracellular proteases from moderately halophilic bacteria. Halophilic nucleases were obtained from *Bacillus halophilus* and *M. varians* subsp. *halophilus* [21, 22].

Because of moderately halophilic bacteria especially *Bacillus* sp. have a great biotechnological potential for production their extracellular enzymes, in the study, the enzyme activities of moderately halophilic bacteria were determined with SDS-PAGE. However, the further studies are currently in progress for characterization of these enzymes from the isolates (Isolates 1-5), cloning and characterization of the corresponding encoding genes in our laboratory.

Moderately halophilic bacteria have the potential for many exciting and promising applications. Not only do many of them produce compounds of industrial interest (enzymes, polymers, and osmoprotectans), but also they possess useful physiological properties that can facilitate their exploitation for commercial purposes. First, most of them can grow at high salt concentrations [23], minimizing the risk of contamination. Second, they are easy to grow, and their nutritional requirements are simple: the majority can use a large range of compounds as their sole carbon and energy source [24]. In the study, we showed that similar enzyme activities were detected from the isolates cultured in sterilized and non-sterilized growth conditions. So, the moderately isolates can be easily cultured in non-sterilized growth conditions. Furthermore, the isolates can be grown large-scale without sterilizing the growth medium to reduce energy cost to obtain their enzymes for using industrial applications.

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