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ORIGINAL PAPER

The effects of environmental conditions on growths of halophilic archaea isolated from Lake Tuz

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Abstract
Recent studies indicate that the microbial ecosystem is not limited to specific areas but may also be found in extreme temperature, extreme salt, extreme pH, extreme pressure, etc. Haloarchaea enzymes are very resistant to salinity stress and also have thermotolerant properties according to environmental conditions. Very few studies have been published about Archaea till date. Firstly, Archaea were isolated from Tuz Lake by traditional methods. Thereafter, the antibiotic resistance of the organisms was investigated. The antibiotic resistance of Archaea was determined by disk diffusion method. The aim of this work was to investigate the effects of environmental conditions on growths of halophilic archaea isolated from Lake Tuz in Turkey. In this study, halophilic archaea isolated from Lake Tuz were investigated under different salt, nitrogen and carbon sources. The best NaCl tolerance of isolated strains is 10% for B8 isolate. The highest nitrogen tolerance is 1% protease peptone for B7 isolate. The best strain for the use of different carbon sources is B7 isolate, and this rate is 1% starch. The study showed that Archaea have tolerance to different environmental conditions.

Keywords Archaea · Environmental conditions · Growth · Tolerance

Introduction

Today, extreme conditions are increasing in the world, and microorganisms have adapted to extreme environments. These environments are salinity, acidity, alkalinity, temperature and pressure (Woese et al. 1990; Madigan and Marrs 1997; Rothschild and Manicinelli 2001).

Tuz Lake is the one from extreme conditions of Turkey and is the largest salt lake in Central Turkey. This lake at northeast of Konya province is a dry lowland. The area of lake is about 1500 km^2, and salt concentration of lake is 33% (Birbir and Sesal 2003).

Extreme saline environments contain two groups of halophilic archaea. These are aerobic haloarchaea and anaerobic halophilic methanoarchaea (Kamekura 1998). The aerobic haloarchaea belong to the family Halobacteriaceae. These are chemoorganotrophic organisms that need at least 1.5 M NaCl for growth. In addition, these organisms show optimal growth at 3.5–4.5 M NaCl (Grant et al. 2001; Aponte et al. 2010; Lee 2013; Tapingkae et al. 2010; Yeannes et al. 2011).

Halophilic organisms have different mechanisms to get rid of from extracellular osmotic pressure. In addition, some halophilic organisms produce organic compatible solutes (Borowitzka and Brown 1974). These archaebacteria accumulate too much inorganic ions within the cell. Furthermore, the biochemical system of halophilic organisms must be adapted to work at high salt concentrations (Eisenberg et al. 1992). Halophilic archaebacteria protect an osmotic level of their cytoplasm by accumulating high salt concentrations. This mechanism requires intracellular enzymes working in the presence of high salt. In contrast, halophilic or halotolerant eubacteria have low intracellular salt concentration. They protect the osmotic level of their cytoplasm with various compatible solutes (Margesin and Schinner 2001).

In recent years, the number of biotechnological uses of halophilic microorganisms has increased, and additional

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applications are under development. The uses of halophiles in biotechnology can be divided into a number of categories. First, the halotolerance of many enzymes derived from halophilic microorganisms can be exploited wherever enzymatic transformations are required to function at low water activities, such as in the presence of high salt concentrations. A few extracellular proteases from the haloalkaliphilic group have been characterized (Studdert et al. 1997; Gimenez et al. 2000). Second, some organic osmotic stabilizers produced by halophiles have found interesting applications. Third, some halophilic microorganisms may produce valuable compounds that can also be found in non-halophiles, often without any direct connection with their halophilic properties, but halophiles may present distinct advantages for the development of biotechnological production processes (Oren 2002).

There are less reports on optimization of environmental conditions for growths of haloarchaeal culture. Till date, only few attempts have been made to study the effect of environmental conditions on growths of halophilic archaea. Samples have been collected from Lake Tuz in February 2017. Experimental studies have been conducted at Microbial Biotechnology Laboratory from Mugla Sitki Kocman University. Here researchers describe three haloarchaeal species that were isolated from Tuz Lake. This study reports here the optimum conditions for halophilic archaea growths isolated from Tuz Lake. These studies can help in increasing the yield of halophilic enzymes. In this study, salt, soil and water samples collected from different locations of Tuz Lake were analyzed to identify and characterize the halophilic microorganisms. This report describe the effects of environmental conditions on the growth of three halophiles.

Materials and methods

Organisms

Yavşan Saltern in Saglik Village of Cihanbeyli district of Konya province was chosen as a study area with the aim of supplying the sources of organism. For the isolation of the organisms used in this study, salt, soil and water samples were collected from this Saltern and used as organism source. The study was conducted in February 2017.

Isolation and identification

In this study, salt, soil and water samples taken from Yavşan Saltern in Health Village of Cihanbeyli district of Konya Province were used as organism source. Primarily, halophile isolates were selected from medium containing high salt concentrations. For this purpose, inoculation was made by dilution method from Sehgal and Gibbons broth (SGB) medium containing 5, 10 and 20% NaCl. Isolates were taken randomly from the different morphological features; then, serial passaging was made. In this study, 14 isolates were purified, and three of these isolates used at studies. These halophilic isolates were B7, B8 and A22. All isolates were grown on solid media (Sehgal and Gibbons Agar, SGA). Then, the isolates were re-inoculated to SGA/SGB medium. The growth of isolates was provided at 37 °C for 7 days (Sehgal and Gibbons 1960). The pure cultures are stocked under refrigerator conditions. Identification of isolated cultures has been performed traditionally using morphological and biochemical tests (Oren et al. 1997). These tests are Gram stain, antibiotic susceptibility test (novobiocin 5 µg, erythromycin 15 µg, streptomycin 10 µg, bacitracin 0.04 U, penicillin G 10 U, ampicillin 10 µg and tetracycline 30 µg), colony morphology, pigmentation and salinity tolerance. Additionally, indole test, nitrate reduction test, oxidase test, catalase test were also applied (Birbir and Sesal 2003; Birbir et al. 2004; Birbir et al. 2007).

Antibiotic susceptibility tests

The pure cultures were activated for 7 days at 37 °C in liquid medium (SGB); then, these cultures were inoculated (100 µL) to plates with SGA, and antibiotic disks were placed aseptically onto agar plates. These plates were incubated for 7 days at 37 °C by incubator. At the end of the incubation period, the inhibition zone diameters were recorded as mm (Birbir and Sesal 2003; Birbir et al. 2004).

The effect of environmental factors on the growth of halophiles microorganisms

In these studies with the aim of determining the effects of different environmental factors (temperature, pH, carbon, nitrogen, salt, yeast extract and magnesium) on growth, the growths of all cultures were recorded as dry weight (mg/mL).

Effect of temperature on the growth of halophilic microorganisms

To determine the effects of different temperatures on growth, SGB media were inoculated with cultures (100 µL) and incubated at 20, 37 and 45 °C, and at a shaking speed of 100 rpm in a shaking incubator. The growths of halophile cultures were monitored for 7–14 days, and the optimum temperature values were determined after recording the growth of the cultures (mg/mL) (Stan-Lotter et al. 2002; Gruber et al. 2004).

Effect of pH on the growth of halophilic microorganisms

In this study, the pH values of the SGB medium were adjusted to 5, 7, 9 and 10, and the cultures (100 µL) were inoculated to medium, and these cultures were incubated at 100 rpm and 40 °C in a shaking incubator. The growths of halophile cultures were monitored for 7 days, and the optimum pH values were determined after recording the growths of the cultures (mg/mL) (Montalvo-Rodriguez et al. 1997; Montalvo-Rodriguez et al. 1998; Stan-Lotter et al. 2002).

The effect of carbon source on the growth of halophilic microorganisms

Glucose, starch and sucrose were used as the carbon source in this study, which was aimed at determining the effects of different carbon sources on growth. Glucose, starch and sucrose at 1% concentration were added aseptically to the prepared SGB medium, and then, the cultures (100 µL) were inoculated to this media and incubated by a shaking incubator at 40 °C and 100 rpm. The growth of the halophile cultures was monitored for 7 days, and the growth of the cultures (mg/mL) was recorded; then, the optimum carbon source was determined for each culture (Montalvo-Rodriguez et al. 1997; Montalvo-Rodriguez et al. 1998; Stan-Lotter et al. 2002).

The effect of nitrogen source on the growth of halophilic microorganisms

Protease peptone, meat extract and NH_4Cl were used as a nitrogen source to determine the effects of different nitrogen sources on growth in this study. Protease peptone, meat extract and NH_4Cl at 1% concentration were added aseptically to the SGB medium, and then, cultures (100 µL) were inoculated to medium, and cultures were shaken by shaking incubator at 100 rpm and 40 °C. The growth of the halophile cultures was monitored for 7 days, and the growth of the cultures (mg/mL) was recorded; then, the optimum nitrogen source was determined for each culture (Montalvo-Rodriguez et al. 1997; Montalvo-Rodriguez et al. 1998; Stan-Lotter et al. 2002; Gruber et al. 2004).

The effect of salt on growth of halophilic microorganisms

NaCl was used as salt source in this study to determine the effects of different concentrations of salt on growth. NaCl at 0, 5, 10, 20 and 30% concentrations were added aseptically to the SGB medium, and then, cultures (100 µL) were inoculated to this medium and were shaken by the shaking incubator at 100 rpm and 40 °C. The growth of the halophile cultures was monitored for 7 days, and the growth of the cultures (mg/mL) was recorded; then, the optimum salt source was determined for each culture (Montalvo-Rodriguez et al. 1997; Montalvo-Rodriguez et al. 1998; Gruber et al. 2004).

Effect of yeast extract on the growth of halophilic microorganisms

In this study, which aimed to determine the effects of different concentrations of yeast extract on growth, yeast extract was added to SGB medium at 0, 0.01, 0.1 and 0.5% concentrations. The cultures (100 µL) were inoculated to the medium; then, cultures were shaken in a shaking incubator at 100 rpm and 40 °C. The incubation time was 7 days. At the end of this period, the growth of the cultures (mg/mL) was recorded, and then, the optimum yeast extract concentration was determined (Montalvo-Rodriguez et al. 1998; Gruber et al. 2004).

The effect of magnesium on the growth of halophilic microorganisms

$MgCl_2{\cdot}6H_2O$ was used as a salt source in this study to determine the effects of different concentrations of magnesium on growth. Different concentrations of $MgCl_2{\cdot}6H_2O$ (0, 0.1, 0.2, 0.4, 0.6, 0.8 and 3%) were added to the prepared SGB medium. The cultures (100 µL) were inoculated to the medium, and then, cultures were incubated in a shaking incubator at 100 rpm and 40 °C. Incubation period of halophil cultures is 7 days. The growth of the cultures was monitored for 7 days, and after the growth of the cultures (mg/mL) was recorded, the optimum $MgCl_2{\cdot}6H_2O$ concentration was determined for each culture (Montalvo-Rodriguez et al. 1997; Stan-Lotter et al. 2002; Gruber et al. 2004).

Results and discussion

This study provides that the response to environmental conditions of archaea isolated from Lake Tuz. Biochemical and morphologic properties of halophilic microorganisms are shown in Table 1. All isolates are Gram negative. The pigment color of isolates was cream. These isolates include the following properties: oxidase positive, catalase positive, nitrate reduction positive, casein hydrolysis positive and motility test negative. Other biochemical test results are tabulated in Table 1. Asha et al. (2004) reported that biochemical properties of halophilic Archaea were pleomorphic, catalase positive, nitrate reduction positive and casein positive. This study supports the results.

Susceptibility to antibiotics is a criterion which is often used in taxonomical studies in which strains are described or compared. Antibiotic resistance of isolates is given in Table 2. In this study, seven antibiotics were used against test organisms. Many of the isolates were not affected from

Table 1 Biochemical and morphological characteristics of halophilic microorganisms

Microorganisms tests	B7	B8	A22
Gram stain	–	–	–
Colony morphology	Pleomorphic (triangle, square)	Basil	Pleomorphic (round, square)
Pigment	Cream	Cream	cream
Oxidase	+	+	+
Catalase	+	+	+
Nitrate reduction	+	+	+
Nitrite reduction	+	+	–
Indole	–	–	–
Casein hydrolysis	+	+	+
Motility test	–	–	–
Glucose	–	–	–
Sucrose	–	–	–
Lactose	–	––	–
H_2S production	–	–	–
Acid production	–	–	–

Table 2 Antibiotic resistance profiles of halophilic microorganisms

Microorganisms antibiotics	Inhibition zone diameters (mm)		
	B7	B8	A22
Ampicillin	–	20	10
Streptomycin	–	40	40
Erythromycin	–	50	50
Penicillin	–	0	40
Bacitracin	0	0	0
Novobiocin	16	16	14
Tetracycline	–	0	0

(0) no inhibition and (–) not tested

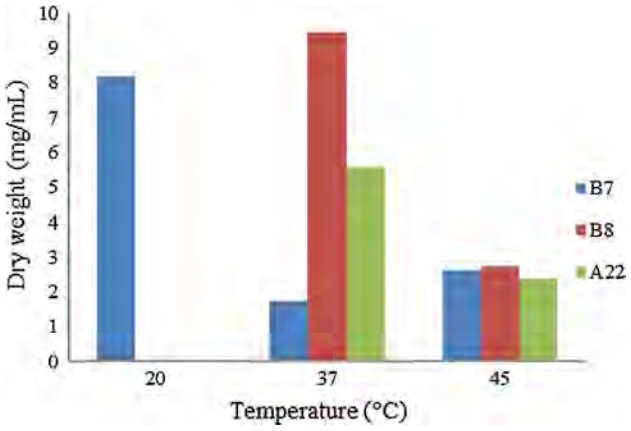

Fig. 1 The effects of different temperature values on the growth of cultures

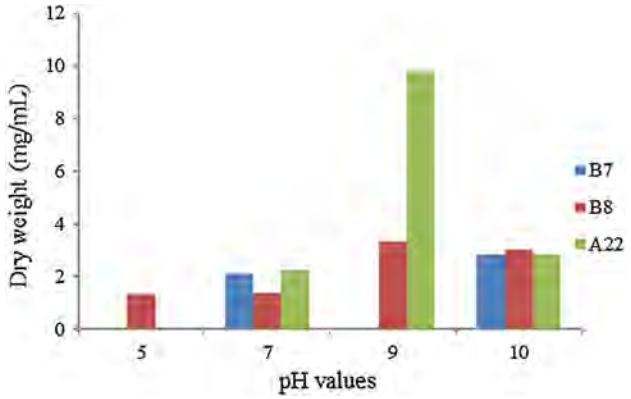

Fig. 2 The effects of different pH values on the growth of cultures

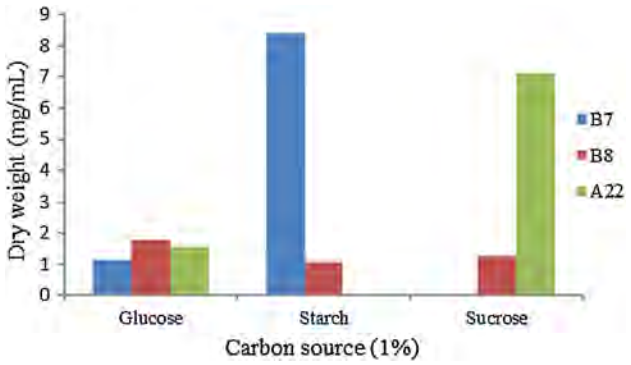

Fig. 3 The effects of different carbon sources on the growth of cultures

antibiotics. However, ampicillin and novobiocin were shown low inhibition against microorganisms. Novobiocin inhibit the activity of DNA gyrase and in sensitive bacteria and archaea acts in the same place (Holmes and Dyall-Smith 1991). Asha et al. (2004) reported that *Haloarcula quadrata* was resistance against all the tested antibiotics, whereas *Haloarcula vallismortis* was sensitive to all the antibiotics tested except ampicillin. In addition, the isolates were found to have ether-bound membrane lipids and were resistant to antibiotics that target the bacterial peptidoglycan (Bonelo et al. 1984). These results provided further evidence that the isolates are members of Archaea. The literature studies are consistent with the results.

In this study, different environmental conditions were tested with the aim of optimizing the growths of isolated halophilic cultures. The effects of different environmental conditions on growths of halophilic microorganisms are summarized in Figs. 1, 2, 3, 4, 5, 6 and 7. The isolates were shown different tolerance to these conditions. One of them is temperature, and 20, 37 and 45 °C temperature values were

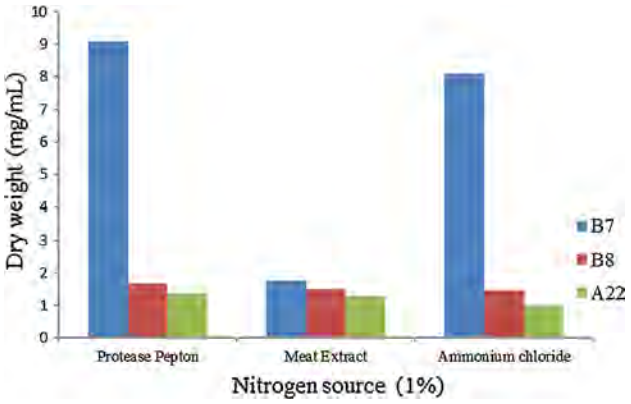

Fig. 4 The effects of different nitrogen sources on the growth of cultures

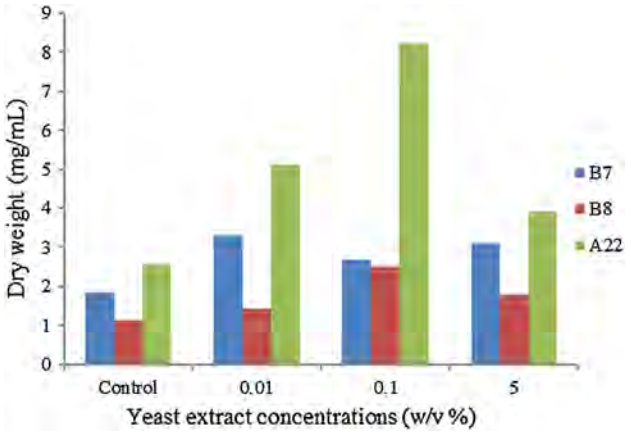

Fig. 5 The effects of different yeast concentrations on the growth of cultures

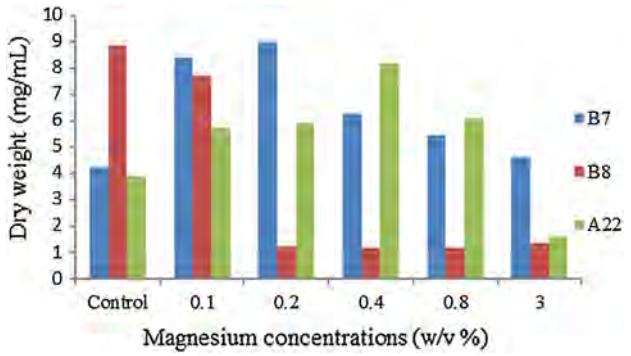

Fig. 6 The effects of different magnesium concentrations on the growth of cultures

worked in this study. The optimum growth of the isolated cultures was at 37 and 20 °C (8.2 mg/mL for B7, 9.4 mg/mL for B8 and 5.6 mg/mL for A22) (Fig. 1). When looking at the

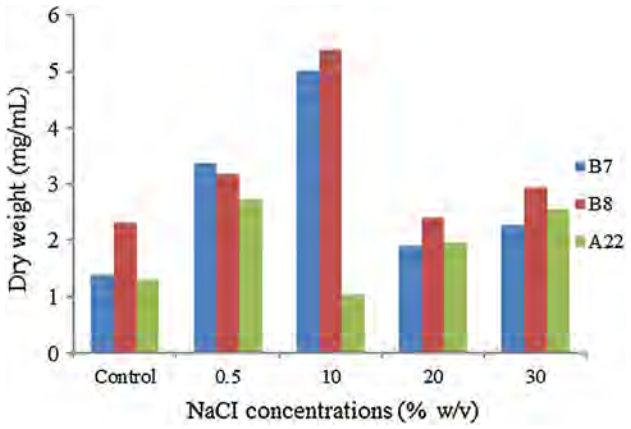

Fig. 7 The effect of different NaCl concentrations on the growth of cultures

results obtained from the literature, Yachai (2009) reported optimal growth temperatures for *Halobacterium piscisalsi* (37–40 °C), *Halobacterium noricense* (28–50 °C) and HPC 1-2T (20–60 °C). Nyakeri (2013) reported that the optimal growth for *Halomonas* X5 isolate was between 25 and 30 °C. Kebbouche-Gana et al. (2009) reported that *Halovivax* isolate grows optimally at 37 °C. Baltaci and Yüksekdag (2014) reported that optimum growth temperatures of *Halobacterium salinarium* and *Halobacterium halobium* cultures are 37 °C. Bilgi (2012) reported that the growths of archaeal cultures were optimum at between 37 and 40 °C. These studies are consistent with the results obtained from the study.

Soil pH will affect the chemical form, concentration and availability of substrates (Kemmitt et al. 2006) and will influence cell growth and activity. In this study, one of the environmental conditions tested on the growths of cultures is different pH values (pH 5, 7, 9, 10). Considering the data obtained from this study, it was determined that cultures had optimum growth at pH 9 and 10 (2.8 mg/mL for B7, 3.3 mg/mL for B8 and 9.8 mg/mL for A22) (Fig. 2). These results could indicate that the organisms are adapted to the special characteristics of the environment on which they are established. Nyakeri (2013) reported that *Halomonas* X5 isolate showed optimum growth at between pH 8–9. The results of Nyakeri's study support data of this study.

Glucose, starch and sucrose as carbon sources were used with the aim of determining the effects of different carbon sources on growth. According to this study, each of the isolates preferred different carbon source for optimum growth (Fig. 3). In this study, starch was used by B7 (8.4 mg/mL), glucose was used by B8 (1.8 mg/mL) and sucrose was used by A22 (7.1 mg/mL) (Fig. 3). Previous studies have shown that some extremely halophilic archaea are able to use several organic compounds, as sole source of carbon and energy (Rodríguez-Valera et al. 1983; Javor 1984). The results show that glucose, starch and sucrose were the main sources used

by the strains in this study. According to the studies, Taran and Amirkhani (2010) reported that the highest growth to *Haloarcula* sp. IRU1 was in 2 g/L glucose. Yachai (2009) reported that the optimal growth of halophilic archaea was in 1% glucose. Jantzer et al. (2011) reported that 0.5% glucose for *Haloferax volcanii* provides optimal growth. Cui et al. (2006) reported that archaeal isolates assimilate glucose and sucrose. Roh et al. (2007) reported that *Halalkalicoccus jeotgali* used glucose and sucrose. Hezayen et al. (2001) reported that archaeal isolates use glucose and starch. Goh et al. (2006) worked with halophilic archaea. They reported that archaea used glucose and sucrose. Coronado et al. (2000) and Koning et al. (2002) reported that archaeal cultures use glucose, sucrose and starch. As shown, these studies are consistent with the results of the study.

As a result of studies on the effects of different nitrogen sources (protease peptone, meat extract and ammonium chloride) on growth, all cultures were found to exhibit an optimum growth at 1% proteose peptone (9.1 mg/mL for B7, 1.7 mg/mL for B8 and 1.4 mg/mL for A22) (Fig. 4). Hezayen et al. (2002) reported that halophilic archaea used proteose peptone, yeast extract, triptone and casamino acid as nitrogen source. Also this study supports the results.

Another environmental factor in optimization experiments was yeast extract. Considering the data obtained from this study, it was determined that two of the cultures showed the best growth in 0.1% yeast extract (2.5 mg/mL for B8 and 8.2 mg/mL for A22) and the other culture had the optimum growth in 0.01% yeast extract (3.3 mg/mL for B7) (Fig. 5). In Kahraman's (2008) study, while giving 0.1% yeast extracts optimally, Jantzer et al. (2011), 0.01% yeast extract concentrate for *Haloferax volcanii* was optimal. Bilgi (2012) and Gruber et al. (2004) reported optimally the same yeast extract in their study. These works support the results of experiments.

In this work, the effect of $MgCl_2$ different concentrations on growth was examined and it was found that the cultures showed optimum growth at different $MgCl_2$ concentrations (0.1, 0.2 and 0.4) (Fig. 6). Growth of B7 is 8.9 mg/mL at 0.2% $MgCl_2$ concentration, whereas growth of B8 is 7.7 mg/mL at 0.1% $MgCl_2$ concentration. Additionally, growth of A22 is 8.1 mg/mL at 0.4% $MgCl_2$ concentration (Fig. 6). When looking at the results obtained from the literature, concentrations of 0.6–0.9 M for halophilic cultures (Gruber et al. 2004), 0.6–0.9 M for *Halobacterium noricense* (Yachai 2009) and 0.005% and 0.05% concentrations for some archaeal cultures are reported (Montalvo-Rodriguez et al. 1997; Bilgi 2012). As shown, these studies support the results obtained from experiments.

Halophiles are microorganisms that grow in elevated salt concentrations, starting from approximately 10% sodium chloride to saturation, and some of them can even survive in salt crystals (DasSarma and Arora 2002). Sodium ions bind to the outer surface of the *Halobacterium* wall and are absolutely essential for maintaining cellular integrity. When insufficient Na + is present, the cell wall breaks apart and the cell lyses. In response to the salt, all these adapted microorganisms maintain very high concentrations of other solutes in their cytoplasm to keep their insides in osmotic balance with the outside world. Halophilic Archaea keep extremely high concentrations of potassium chloride in their cells (Oren 2004). In salt studies, three different NaCl concentrations were studied on the growth of cultures. According to this study, two halophilic cultures grow optimally at 10% NaCl concentration (5 mg/mL for B7 and 5.4 mg/mL for B8), while the other culture preferred to the 0.5% concentration (2.7 mg/mL for A22) (Fig. 7). Referring to the literature, Nyakeri (2013) reported that 10–15% NaCl concentrations are optimum for the *Halomonas* X5 isolate, whereas Manjula (2014) reported 25% NaCl as optimum concentration for *Natrinema* sp. BTSH10. Baltaci and Yüksekdag (2014) reported that the optimum NaCI concentration for *Halobacterium salinarium* and *Halobacterium halobium* was 20–30%. These results are similar to the values of this study.

Conclusion

In conclusion, this study is the description to taxonomic position of halophilic archaea on Lake Tuz in Turkey. The effects of different environmental conditions on the growth of three halophilic cultures from Tuz Lake have been determined. At the end of study, it was determined that microorganisms were affected differently from environmental conditions. The results of this study supported the hypothesis in the different environmental conditions may well influence the growths of halophilic Archaea. In future studies, more genotaxonomic and chemotaxonomic studies need to be done of interest to elucidate the position of these Archaea. Enzymes of the extreme Archaea are important industrially. Additionally, further research is needed for the purification of the enzymes in optimized archaeal cultures. Studies on the stability of the enzyme must be conducted.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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