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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Determination of important enzymes and antimicrobial resistances of gram-positive haloalkaliphilic bacteria isolated from Salda Lake

Salda Gölünden izole edilen gram-pozitif haloalkalifik bakterilerin önemli enzimlerinin ve antibiyotik dirençlerinin belirlenmesi

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Abstract: As an extreme environment, soda lakes harbor various haloalkaliphilic microorganisms. Salda Lake is one of the natural soda lake (pH>9) in Turkey. Haloalkaliphiles are unique microorganisms in their ability to live in high alkaline and high saline conditions, and play an important role in biodegradation and bioremediation of hydrocarbons. Hence, the aims of this study were to isolate haloalkaliphilic bacteria from water sample of Salda Lake, to identify these isolates by both conventional and molecular methods, to screen their industrially important enzymes, and to investigate their antimicrobial resistance profiles. Six isolates were identified as *Bacillus horneckiae*, *Bacillus subtilis*, *Bacillus paramycoides*, *Bacillus pumilus*, *Staphylococcus epidermidis*, *Bacillus haynesii* according to 16S rRNA gene sequencing analysis. The industrially important enzymes (amylase, cellulase, pullulanase, lipase, urease, protease, caseinase, oxidase, catalase) were produced by haloalkaliphilic isolates. These enzymes maybe used in alkaline and saline industrial processes. Although *Bacillus subtilis* was susceptible to all antibiotics, other isolates showed resistance to at least one antibiotic. The resistance against antibiotics were found as ampicillin/sulbactam 83%, amoxycillin/clavulanic acid 83%, ampicillin 67%, mupirocin 67%, chloramphenicol 50%, tetracycline 50%, imipenem 50%, meropenem 50%, cefadroxil 17%. These bacteria may have develope resistance to antibiotics that entering their natural environment in different ways.

Keywords: Microbial enzymes, antibiotic resistance, soda lake, haloalkaliphiles, biodiversity, gram positive bacteria

Öz: Aşırı ortamlar olarak soda gölleri, çeşitli haloalkalifilik mikroorganizmaları barındırır. Salda Gölü, Türkiye'deki doğal soda göllerinden (pH>9) biridir. Haloalkalifiller yüksek alkali ve yüksek tuzlu koşullarda yaşama kabiliyetleri bakımından benzersiz mikroorganizmalardır ve hidrokarbonların biyolojik olarak parçalanması ve biyoremediasyonunda önemli bir rol oynarlar. Bu nedenle, bu çalışmanın amacı haloalkalifilik bakterileri Salda Gölü'nden izole etmek, geleneksel ve moleküler metotlarla tanımlamak, ürettikleri önemli endüstriyel enzimleri ve antibiyotik direnç profillerini araştırmaktır. 16S rRNA gen dizi analizine göre altı adet haloalkalifilik izolat (*Bacillus horneckiae*, *Bacillus subtilis*, *Bacillus paramycoides*, *Bacillus pumilus*, *Staphylococcus epidermidis*, *Bacillus haynesii*) tanımlanmıştır. Endüstriyel öneme sahip amilaz, selülaz, pullulanaz, lipaz, üreaz, proteaz, kazeinaz, oksidaz, katalaz enzimleri haloalkalifilik izolatlar tarafından üretilmiştir. Bu enzimler alkali ve tuzlu endüstriyel işlemlerde kullanılabilir. *Bacillus subtilis* tüm antibiyotiklere duyarlı olmasına rağmen, diğer izolatlar en az bir antibiyotiğe direnç göstermiştir. Antibiyotiklere direnç; ampisilin/sulbaktam %83, amoksisilin/klavulanik asit %83ampisilin %67, mupirosin %67, kloramfenikol %50, tetrasiklin %50, imipenem %50, meropenem %50, sefadroksil %17 olarak bulunmuştur. Bu bakteriler doğal ortamlarına farklı yollardan giren antibiyotiklere karşı direnç geliştirebilirler.

Anahtar kelimeler: Mikrobiyal enzimler, antibiyotik direnci, soda gölü, haloalkalifiller, biyoçeşitlilik, gram pozitif bakteri

INTRODUCTION

Extreme environments harbor extremophile microorganisms such as alkaliphiles, halophiles, thermophiles, psychrophiles and acidophiles. These environments have enormous potential for novel enzymes which are salt tolerant, thermostable, active at high pH. Microorganisms that can survive under extreme pH values are referred as alkaliphiles (Raval et al., 2015). The enzymes of alkaliphiles are used in different industries for various applications (Table 1).

The most widely studied enzymatic group is alkaline proteases (Chand and Mishra, 2003). The researchers reported that *Bacillus licheniformis*, *B. subtilis*, *B. amyloliquefaciens* and *B. majovensis* produce alkaline protease enzymes (Gupta et al., 2002). Alkaliphilic bacterial species were reported by other researchers. Alkaliphilic *Bacillus cohnii*, *B. pseudofirmus*, and *B. clarkii* were isolated from bauxite residue in the southern region of Minas Gerais (Brazil) (Nogueira et al., 2017). Haloalkaliphilic bacterial strains belonging to the genera *Bacillus*, *Staphylococcus*, *Halobacillus*, *Virgibacillus*, *Oceanobacillus* were isolated from saline desert of Little Rann of Kutch in India (Bhatt et al., 2018). These isolates were able to produce protease, cellulase, carboxymethyl cellulase (CMCase) and amylase enzymes at high salt concentration and high pH (Bhatt et al., 2018). The researchers also reported that these isolates showed resistance against ampicillin, amikacin, augmentin, cefaclor, colistin, cefoperazone, cefuroxime, cefotaxime, cefixime, erythromycin, azithromycin, co-norfloxacin, trimoxazole, amoxycillin, cefadroxil, cefpodoxime, penicillin and gentamycin (Bhatt et al., 2018).

Table 1. Microbial enzymes and their industrial applications

Microbial enzyme	Industries using enzymes	The use of enzymes	References
Cellulase	Textile	Softening and shining of clothes	Aygan and Arikan, 2008
	Detergent	Polishing fabrics	Wang et al., 2009
	Agriculture	Production of biofuel from cellulosic material	
		Conversion of agricultural biomass into useful products	
Protease	Detergent	Laundry additives	Chand and Mishra, 2003
	Food	Formulations of detergents	Mitra and Chakrabartty, 2005
	Animal feed	Peptide sythesis	
	Baking	Fish sauce preparation	
	Biomedical	Dehairing goak skin	
	Brewing		
	Cheese		
	Chemistry		
	Tanning		
	Leather		
	Pharmaceutical		
Lipase	Detergent	Detergent additives	Jaeger and Holliger, 2010
	Food	Enantioselective biocatalyst for the Production of fine chemicals	Babu et al., 2008
	Paper	Esterification	
		Trans-esterification	
		Amynolysis	
Xylanase	Food	Production of coffee, feed and flour	Ratnakar, 2013
	Paper	Removal of lignin from pulp	Khandeparker and Numan, 2008
	Pulp	Increasing loaf volume	
	Baking	Production of biofuel	
		Starch production from lignocellulose	
Pullulanase	Food	Biocatalysis in organic solvents and super critic fluids	Delgado-García et al., 2015
Amylase	Textile	Starch saccharification	Ratnakar, 2013
	Food	Strach hydrolysis	Ammar et al., 2002
	Brewing	Saccharification of marine microalgae	Kikani et al., 2010
	Distilling	Removal of starch from clothes	
	Detergent	Desizing process	
		Production of syrups	
		Reduction of turbidity of fruit juice	
DNase	Food	Acid 5'-guanilic and acid 5'-inosinic as flavor agents	Delgado-García et al., 2015
Urease	Beverage	Removal of urea from wine	Liu et al., 2012
Caseinase	Food	Degrading casein in milk	Johnson and Case, 2010

Salda Lake, which is an extremely alkaline, slightly saline and closed system, is located in the lakes district of Turkey (Balci et al., 2018). Salda Lake is close to Yesilova, located in 44 km^2 area and nearly 200 m deep. In Salda Lake region, agriculture, animal husbandry, livestock, tourism and fishing activities are carried out. The pollutants caused by these activities are mixed into the water of the Salda Lake. The contamination of lake water with antibiotic resistant bacteria may be resulted from the livestock-related or agricultural activities. The researchers reported that the water of the Salda Lake may be used as drinking water in the future (Varol et al., 2021). It should be known which microbial populations live in Salda Lake so that the lake water can be decontaminated with the correct chemicals or methods. Therefore, it is important to isolate and identify haloalkaliphilic bacteria found in Salda Lake water resistant to antibiotics used in human and veterinary medicine.

There are a few reports on the microbial populations found in Salke Lake. *Lyngbya majuscula*, *Gloeocapsa aeruginosa*, *Synechococcus* sp., *Microcystis aeruginosa*, *Chroococcus turgidus* and *Oscillatoria limnosa* were found in Salda Lake by the researchers (Braithwaite and Zedef, 1996; Kazanci et al., 2004; Shirokova et al., 2013). In another study, new generation sequence analysis showed that 97.3% of the lake belongs to *Bacteria* domain (*Gammaprotobacteria* 39.6%, *Alphaprotobacteria* 25.6%, *Bacilli* 23.7%, *Cyanobacteria* 5.3%, *Betaproteobacteria* 2%, *Actinobacteria* 1.7%) (Balci et al., 2018).

Antibiotics are used in food animals to treat, control and prevent diseases (Mirzaagha et al., 2011). Although antibiotics are commonly used in animal husbandry to promote growth, in human and veterinary medicine to treat infections caused by bacteria, antibiotic-resistant bacteria increased worldwide because of the misuse and overuse of antibiotics. While the researchers focused on the antibiotic resistance in human, agriculture and animal, much less information is available about antibiotic-resistant bacteria in natural environments. The natural environments contain various reservoir of antimicrobial resistance genes and genetic elements (Berkner et al., 2014). These genes constitute the resistome of environment which are an important part of resistome of human pathogens (Gillings, 2013).

Carbapenems (imipenem, meropenem); cephalosporins 1st generation (cefadroxil); penicillins-β-lactam/β-lactamase inhibitor combinations (amoxicillin-clavulanic acid, ampicillin, ampicillin/sulbactam); amphenicols (chloramphenicol); tetracyclines (tetracycline) are among the antibiotics which are used in both human and veterinary medicine (WHO, 2017). Hence, chloramphenicol, cefadroxil, ampicillin, tetracycline, mupirocin, imipenem, meropenem, ampicillin/sulbactam and amoxycillin/clavulanic acid were selected as test antibiotics.

According to the literature review, there are no studies in which haloalkaliphilic bacteria were isolated from Salda Lake and identified, and their enzymatic functions and antibiotic susceptibilities were determined. In this respect, this study provided original results. Hence, this study focused on the haloalkaliphilic bacterial diversity of Salda Lake, their enzymatic fuctions in alkaline ecosystem and their antibiotic resistance profiles. Therefore, haloalkaliphilic bacterial species were isolated from water sample collected from Salda Lake; these bacterial isolates were identified at the species level using conventional and molecular methods; their industrially important enzymes were determined; and antibiotic resistance profiles of haloalkaliphilic wild-type bacteria were investigated in the present study.

MATERIALS AND METHODS

Isolation of haloalkaliphilic bacteria

Haloalkaliphilic medium containing 5 g glucose, 4 g $Na_2B_4O_7.10{\times}H_2O$, 3 g $NaNO_3$, 1 g NH_4Cl, 0.5 g KH_2PO_4, 1 g yeast extract, 0.5 g casamino acids, 100 g NaCl, 1000 ml distilled water was used for isolation from water sample of Salda Lake (Burdur/Turkey). The final pH was adjusted to 9. After overnight incubation at 35°C, different bacterial colonies were selected and restreaked several times to obtain pure cultures, then subjected to phenotypic and genotypic analysis (Gonzales et al., 1978; Rodríguez-Montalvo et al., 1998; Harley and Prescott, 2002).

Amplification and sequencing of 16S rRNA genes

Chromosomal DNA were extracted and PCR products were purified as described by the manufacturer's instructions (QIAamp DNA Mini Kit, QIAquick PCR Purification Kit, Qiagen). The 16S rRNA genes were amplified using specific universal 1492R (5'TACGGYTACCTTGTTACGACTT3') and 27F (5'AGAGTTTGATCMTGGCTCAG 3') primers (Sambrook and Russell, 2001). The 16S ribosomal RNA gene sequences were determined by IONTEK Laboratory (Istanbul/Turkey). 16S rRNA gene similarities were further determined between isolates and closely related species by using ChromasPro and EzTaxon-e tool (Kim et al., 2012).

Characterization of the haloalkaliphilic isolates

Exponentially growing broth cultures of six isolates were examined for cell morphology on prepared wet mounts using light microscopy. Colony pigmentation was observed by growing colonies on haloalkaliphilic agar medium. Gram staining, catalase and oxidase activities were carried out according to the standard procedures (Arahal et al., 1996; Johnson and Case, 2010; Sánchez-Porro et al., 2011). Salt requirement and salt tolerance of the isolates were investigated on plates containing haloalkaliphilic agar medium in which the NaCl concentration was varied (0%, 1%, 1.5%, 2%, 5%, 7.5%, 10%, 12.5%, 15%, 17.5%, 20% NaCl). The pH tolerance of the isolates was tested on haloalkaliphilic agar

medium adjusted to pH values of 5-13. To determine optimum growth temperature of the isolates, the plates inoculated with each isolate were incubated at different temperatures (4°C-60°C).

Enzymatic activities of the haloalkaliphilic isolates

Amylase activity was detected using haloalkaliphilic agar medium supplemented with 0.5% (w/v) soluble starch. After incubation, the plate was flooded with 0.3% I_2-0.6% KI solution. Clear halos around the colonies indicated starch hydrolysis. The DNase test agar was used to determine DNase activity. After incubation, the plate was flooded with 1N HCl. Clear zones around the colonies showed hydrolysis of DNA (Sánchez-Porro et al., 2003). The cellulose medium agar plate containing 0.2% (w/v) carboxymethyl cellulose was used to detect production of cellulase. After incubation, 0.1% congo red test reagent was flooded on the colonies and left for 30 min. Then, the colonies were washed with 1 M NaCl solution. Clear zones around the colonies showed cellulase activity (Birbir et al., 2007). Hydrolysis of casein was tested with the Plate Count Agar medium containing 2% skim milk. After incubation, clear zones around the colonies were interpreted as caseinase production (Sánchez-Porro et al., 2011). Lipase activity was screened on Tween 80 agar medium containing 1% (w/v) Tween 80. After incubation, opaque zones around the colonies were accepted as evidence of lipase activity (Caglayan et al., 2018). Protease activity was screened on gelatin agar medium containing 2% gelatin (w/v). After incubation, the plate was flooded with Frazier solution. Clear zones around the colonies were interpreted as positive protease activities (Sánchez-Porro et al., 2003). Urease activity was detected on Christensen Urea Agar. After growth was obtained, the tube was examined for pink or red color changes (Johnson and Case, 2010). To detect pullulolytic and xylanolytic activities of the isolates, plates containing the chromogenic substrates such as azurine-cross-linked (AZCL)-pullulan and AZCL-xylan were respectively used. Clear zones around the colonies were accepted as positive pullulolytic and xylanolytic activities (Sánchez-Porro et al., 2003; Caglayan et al., 2017). The pH of all media was adjusted to 9.

Nucleotide accesion number

16S rRNA sequence data of the haloalkaliphilic water isolates S10, S4A, S6, S2, S1, S4, reported in this article have been deposited in NCBI and GenBank nucleotide sequence database under the respective accession numbers: MH752438, MH748635, MH748638, MH748643, MH748647, MH748673.

Antimicrobial susceptibility tests

The haloalkaliphilic isolates were grown in Mueller Hinton Broth containing 1% (w/v) NaCl. After the overnight incubation at 35°C, the optical densities of the bacterial suspensions were adjusted to McFarland Standard No 0.5 (1×10^8 CFU/mL) with sterile saline solution (1% NaCl). The Kirby-Bauer disc diffusion method was used to determine the resistance of isolates to antibiotics such as chloramphenicol (30 µg), cefadroxil (30 µg), ampicillin (10 µg), tetracycline (30 µg), mupirocin (20 µg), imipenem (10 µg), meropenem (10 µg), ampicillin/sulbactam (10/10 µg; 20 µg) and amoxycillin/clavulanic acid (20/10 µg; 30 µg). The surface of the Mueller Hinton Agar plates were inoculated with bacterial suspension of each test isolate and antibiotic discs (Oxoid, UK) were placed on the surface. After 24 hours incubation of plates at 35°C, the inhibition zone diameters were mesured and evaluated according to zone diameter distributions of wildtype microorganisms and ECOFF information explained by European Committee on Antimicrobial Susceptibility Testing as resistant and susceptible (EUCAST, 2014).

RESULTS

Phylogenetic analysis of haloalkaliphilic bacteria

The 16S ribosomal RNA gene sequences and the 16S rRNA the pairwise sequence similarities of the haloalkaliphilic isolates were respectively found as 1155-1299 bp and 98.1-100%. Two different genera, *Staphylococcus* (1 isolate) and *Bacillus* (5 isolates), were determined in *Firmicutes* (Table 2). The colonies of haloalkaliphilic bacterial isolates on haloalkaliphilic medium agar plates were yellow, cream and white. All of the isolates were Gram-positive. Cell morphologies of the isolates were coccus and rod-shaped. All isolates grew at 0-7.5% salt concentrations and pH range 7-12. Four and two isolates respectively showed optimum growth at 1.5% and 1% salt concentrations. All isolates showed optimum growth at pH 9. While five isolates grew at 10-55°C, only one isolate grew at 10-60°C. Four isolates, one isolate and one isolate were respectively exhibited optimum growth at 35°C, 37°C and 35-37°C (Table 2).

Catalase (100%), oxidase (67%), protease (50%), cellulase (50%), lipase (33%), caseinase (33%), urease (17%), amylase (17%), and pullulanase (17%) were produced by the isolates (Table 2). Xylanase and deoxyribonuclease were not produced. The isolates showed combined enzymatic activities. *Bacillus haynesii* (catalase, protease) and *Bacillus horneckiae* (catalase, cellulase) produced two enzymes. *Bacillus subtilis* produced oxidase, catalase and cellulase. *Bacillus paramycoides*, *Bacillus pumilus*, and *Staphylococcus epidermidis* respectively produced seven (oxidase, catalase, amylase, cellulase, urease, protease, caseinase), five (oxidase, catalase, lipase, protease, caseinase) and four (oxidase, catalase, pullulanase, lipase) enzymes. Among the isolates *Bacillus paramycoides* produced all enzymes except pullulanase, xylanase, lipase and deoxyribonuclease. It is known that the haloalkaliphilic bacteria and their enzymes are active and stable under harsh conditions. Therefore, the test isolates may have potential for novel enzymes due to their ability to be stable under saline conditions and high pH. The enzymes of haloalkaliphiles such as oxidase, catalase amylase, cellulase, pullulanase, lipase, urease, protease,

caseinase may be used in several industrial applications. In order to characterize the properties of these enzymes and determine their biochemical reactions, more detailed studies are needed to be performed.

Table 2. Characteristics of haloalkaliphilic bacteria isolated from water sample in Salda Lake

Characteristics	*Staphylococcus epidermidis*	*Bacillus subtilis*	*Bacillus pumilus*	*Bacillus paramycoides*	*Bacillus haynesii*	*Bacillus horneckiae*
Isolate code	S10	S4A	S6	S2	S1	S4
Gram staining	+	+	+	+	+	+
Colony pigmentation	yellow	cream	cream	white	cream	yellow
Cell morphology	coccus	rod-shaped	rod shaped	rod-shaped	rod-shaped	rod-shaped
NaCl range (%)	0-10	0-10	0-7.5	0-10	0-10	0-12.5
Optimum NaCl (%)	1	1.5	1.5	1	1.5	1.5
Temperature range (°C)	10-55	10-55	10-55	10-55	10-60	10-55
Optimum temperature (°C)	37	35	35	35-37	35	35
pH range	7-12	7-12	7-12	7-12	7-12	7-12
Optimum pH	9	9	9	9	9	9
Oxidase	+	+	+	+	-	-
Catalase	+	+	+	+	+	+
Amylase	-	-	-	+	-	-
Cellulase	-	+	-	+	-	+
Pullulanase	+	-	-	-	-	-
Xylanase	-	-	-	-	-	-
Lipase	+	-	+	-	-	-
Urease	-	-	-	+	-	-
Protease	-	-	+	+	+	-
Caseinase	-	-	+	+	-	-
Deoxyribonuclease	-	-	-	-	-	-

Antibiotic resistance profiles of haloalkaliphilic bacteria

The zone diameter breakpoints were determined for chloramphenicol (30 µg ≥18mm susceptible), cefadroxil (30 µg ≥12mm susceptible), ampicillin (10 µg ≥12mm susceptible), tetracycline (30 µg ≥22mm susceptible), mupirocin (20 µg ≥30mm susceptible), imipenem (10 µg ≥24mm susceptible), meropenem (10 µg ≥25mm susceptible), ampicillin/sulbactam (20 µg ≥17mm susceptible) and amoxycillin/clavulanic acid (30 µg ≥19mm susceptible) according to zone diameter distributions of wildtype microorganisms and ECOFF information (EUCAST, 2014).

The diameter of inhibition zones around chloramphenicol with the highest inhibition zone observed against *Bacillus horneckiae* (23 mm), followed by *Staphylococcus epidermidis* (22 mm), *Bacillus subtilis* (21 mm); around cefadroxil with the highest inhibition zone observed against *Bacillus paramycoides* and *Bacillus haynesii* (20 mm), followed by *Bacillus horneckiae* (19 mm), *Bacillus pumilus* (18 mm), *Bacillus subtilis* (17 mm); around ampicillin with the highest inhibition zone observed against *Bacillus haynesii* (22 mm), followed by *Bacillus subtilis* (21 mm); around tetracycline with the highest inhibition zone observed against *Staphylococcus epidermidis* and *Bacillus horneckiae* (25 mm), followed by *Bacillus subtilis* (23 mm); around mupirocin with the highest inhibition zone observed against *Bacillus haynesii* (34 mm), followed by *Bacillus subtilis* (32 mm); around imipenem with the highest inhibition zone observed against *Bacillus paramycoides* (26 mm), followed by *Bacillus subtilis* and *Bacillus horneckiae* (25 mm); around meropenem with the highest inhibition zone observed against *Bacillus pumilus* (30 mm), followed by *Bacillus subtilis* (28 mm) and *Bacillus haynesii* (27 mm). The diameter of inhibition zones around ampicillin/sulbactam and amoxycillin/clavulanic acid against *Bacillus subtilis* were respectively measured as 18 mm and 22 mm (Table 3).

Moreover, *Staphylococcus epidermidis*, *Bacillus pumilus*, *Bacillus paramycoides*, *Bacillus haynesii*, *Bacillus horneckiae* showed no zone of inhibition around cefadroxil, ampicillin, mupirocin, imipenem, meropenem, ampicillin/sulbactam, amoxycillin/clavulanic acid; chloramphenicol, ampicillin, tetracycline, mupirocin, imipenem, ampicillin/sulbactam, amoxycillin/clavulanic acid; chloramphenicol, ampicillin, tetracycline, mupirocin, meropenem, ampicillin/sulbactam, amoxycillin/clavulanic acid; chloramphenicol, tetracycline, imipenem, ampicillin/sulbactam, amoxycillin/clavulanic acid; ampicillin, mupirocin, meropenem, ampicillin/sulbactam, amoxycillin/clavulanic acid (Table 3).

Table 3. Antibiotic resistance profiles of haloalkaliphilic bacteria isolated from water sample in Salda Lake

Antibiotics	*Staphylococcus epidermidis*	*Bacillus subtilis*	*Bacillus pumilus*	*Bacillus paramycoides*	*Bacillus haynesii*	*Bacillus horneckiae*	R%	S%
Chloramphenicol	S (22 mm)	S (21 mm)	R (0)	R (0)	R (0)	S (23 mm)	50	50
Cefadroxil	R (0)	S (17 mm)	S (18 mm)	S (20 mm)	S (20 mm)	S (19 mm)	17	83
Ampicillin	R (0)	S (21 mm)	R (0)	R (0)	S (22 mm)	R (0)	67	33
Tetracycline	S (25 mm)	S (23 mm)	R (0)	R (0)	R (0)	S (25 mm)	50	50
Mupirocin	R (0)	S (32 mm)	R (0)	R (0)	S (34 mm)	R (0)	67	33
Imipenem	R (0)	S (25 mm)	R (0)	S (26 mm)	R (0)	S (25 mm)	50	50
Meropenem	R (0)	S (28 mm)	S (30 mm)	R (0)	S (27 mm)	R (0)	50	50
Ampicillin/sulbactam	R (0)	S (18 mm)	R (0)	R (0)	R (0)	R (0)	83	17
Amoxycillin/clavulanic acid	R (0)	S (22 mm)	R (0)	R (0)	R (0)	R (0)	83	17
Multidrug resistant bacteria	7	0	7	7	5	5		

R%, Percentage of isolates resistant to antibiotics; S%, Percentage of isolates susceptible to antibiotics

All isolates showed resistance to at least one antibiotic tested except *Bacillus subtilis*. This isolate was susceptible to all antibiotics. While more than half of the isolates were resistant to ampicillin/sulbactam (83%), amoxycillin/clavulanic acid (83%), ampicillin (67%), mupirocin (67%), less than half of the isolates were resistant to cefadroxil 17%. Fifty percent of the isolates exhibited resitance to chloramphenicol, tetracycline, imipenem, and meropenem (Table 3). Multidrug resistant bacteria, which are able to survive and grow in the presence of two or more antibiotics, were detected as *Bacillus horneckiae*, *Bacillus paramycoides*, *Bacillus pumilus*, *Staphylococcus epidermidis* and *Bacillus haynesii* (Table 3).

DISCUSSION

Bacillus species are widely distributed in water, seawater, soil, marine sediments, and salt. In previous studies, the species isolated and tested in the present study, were isolated from different locations. *Bacillus horneckiae* was isolated from a spacecraft-assembly clean room in the Kennedy Space Center (Vaishampayan et al., 2010). In that study, *Bacillus horneckiae* showed weak protease and lipase activities (Vaishampayan et al., 2010). However, this isolate was protease and lipase negative in the present study. *Bacillus subtilis* was isolated from human gastrointestinal tract (ileum biopsies, faecal samples) (Hong et al., 2009) and dairy effluent (Vijayalakshmi and Murali, 2015). Similar to the results obtained from the study performed by Hong et al. (2009), we observed that *Bacillus subtilis* was amylase negative, and was able to grow at 10-50°C. *Bacillus paramycoides* was isolated from sediment of the South China Sea (Liu et al., 2017) and soil samples collected from rhizospheres of crop plants (Osman and Yin, 2018). *Bacillus paramycoides* was found as amylase and protease positive in the study of Liu et al. (2017) and in the present study. It was reported that this species showed growth at 15-39°C (optimum 30°C), 0-5% NaCl concentration (optimum 0.5% NaCl), 5-9 pH range (optimum pH 7) (Liu et al., 2017). This species was reported as plant growth-promoting rhizobacteria (Osman and Yin, 2018). *Bacillus pumilus* was isolated from coastal environment of Cochin in India (Parvathi et al., 2009) and marine ecosystems (Liu et al., 2013). Similar to the results of this study, *Bacillus pumilus* showed negative deoxyribonuclease activity, and positive lipase and protease activities (Parvathi et al., 2009). Daptomycin-resistant *Staphylococcus epidermidis* was isolated from bovine udders (Brown et al., 1969) and patients (Eladli et al., 2018). *Bacillus haynesii* was isolated from desert soil (Dunlap et al., 2017). According to the results of that study, *Bacillus haynesii* was able to grow at pH 5.5-10 (optimum pH 7), temperatures of 15-60°C (optimum 37°C), and at 0-12% NaCl concentrations (Dunlap et al., 2017).

This is the first study on the isolation and characterization of haloalkaliphilic bacteria from water sample of Salda Lake, screening their industrial enzymes, and testing antibiotic resistance profiles. The haloalkaliphilic *Bacillus horneckiae* *Bacillus subtilis*, *Bacillus paramycoides*, *Bacillus pumilus*, *Staphylococcus epidermidis*, *Bacillus haynesii* species, isolated from Salda Lake, were first reported in the present study. These isolates were found resistant to different antibiotics in the present study. The agricultural, livestock, tourism and fishing activities are considered, these antibiotic resistant bacteria may have harmful impacts on water

sources. Furthermore, amylase, cellulase, pullulanase, lipase, urease, protease, caseinase, oxidase, catalase produced by these isolates would be important candidates for various industrial applications due to enzymatic activity and stability in a broader range of NaCl and pH.

CONCLUSION

Haloalkaliphilic bacteria display different morphological, physiological, molecular and cultural characteristics, Gram staining reaction, biochemical features, antibiotic susceptibility profiles, enzyme and metabolite production. Alkaline environments harbor diverse haloalkaliphilic bacteria producing stable enzymes. Due to the stability and activity of alkaline enzymes in harsh conditions, they have potential to be used in biotechnological applications. Future performed investigations with their metabolites are very important considering their ability to survive under high pH. The presence of multidrug resistant bacteria in Salda Lake was detected. Antibiotic resistant *Bacillus* and *Staphylococcus* species present in the Salda Lake may be transmitted by human, animal, air, soil, or wastewater. These genes are present in natural environments worldwide.

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