

A New Approach for Quorum Sensing System in Several Halophilic Bacteria Isolated from Salt Lake in Central Anatolia

İç Anadolu'daki Tuz Gölü'nden İzole Edilen Çeşitli Halofilik Bakterilerdeki Quorum Sensing Sistemine Yeni Bir Yaklaşım

Research Article

Demet Erdönmez^{1*}, Kübra Erkan Türkmen² and Nilüfer Aksöz²

¹Department of Biology, Aksaray University, Aksaray, Turkey.

²Department of Biology, Division of Biotechnology, Hacettepe University, Ankara, Turkey.

ABSTRACT

Quorum sensing (qs) is the system that microorganisms use to improve common behavior with another population member in their environment. Salt Lake in Turkey that is considered as an extreme environment due to its salt concentration, teems in plankton and bacteria. Especially, it is an important field for halophilic bacteria because it provides suitable growth conditions. In this study, it was determined that 20 halophilic bacteria strain isolated from Salt Lake could produce qs signal molecules which are N-acyl homoserine lactone (HSL) derivatives. For detection of these bacterial signal molecules, thin layer chromatography was utilized by using *Chromobacterium violaceum* cv026 and *Agrobacterium tumefaciens* A136 that are biosensor bacteria strains. Genomic analysis of strains that produce signal molecules was carried out via 16s rRNA. *Halobacterium salinarium*, *Salicola* sp., *Halovibrio*, and *Halomonas* were found to be common in Salt Lake. It was also observed that these strains could produce C6-HSL and C8-HSL signaling molecules and produce biofilms.

Key Words

Homoserine lactone, quorum sensing, halophilic bacteria, Salt Lake.

ÖZ

Quorum sensing (qs), bir mikroorganizmanın yaşadığı ortamdaki diğer popülasyon üyeleri ile ortak davranışları geliştirmek için kullandığı bir sistemdir. İçeriğindeki tuz yoğunluğundan dolayı ekstrem ortamlardan sayılan Tuz Gölü, bünyesinde barındırdığı planktonik canlılar ve bakteriler yönünden zengindir. Özellikle tuz gölü uygun üreme koşullarını sağladığı halofilik bakteriler için çok önemli bir alandır. Tuz Gölü'nden izole edilen Halofilik karakterli 20 bakteri türünün qs sinyal moleküllerinden N-asil homoserin lakton (AHL) türevlerini üretebildikleri saptanmıştır. Bu bakteriyel sinyal moleküllerini tespit etmek için biyosensör bakteriler *Chromobacterium violaceum* cv026 ve *Agrobacterium tumefaciens* A136 kullanılarak ince tabaka kromatografisinden yararlanılmıştır. Sinyal molekülü üretebilen suşların 16sRNA ile genomik analizleri gerçekleştirilmiştir. *Halobacterium salinarium*, *Salicola* türleri, *Halovibrio* ve *Halomonas* türlerinin Tuz Gölü içerisinde yaygın olduğu görüldü. Ayrıca bu suşların C6-AHL ve C8-AHL sinyal moleküllerini üretebildikleri ve biyofilm oluşturabildikleri gözlenmiştir.

Anahtar Kelimeler

Homoserin lakton, quorum sensing, halofilik bakteriler, Tuz Gölü.

Article History: Received: January 16, 2018; Revised: Mar 15, 2018; Accepted: Mar 26, 2018.

DOI: 10.15671/HJBC.2018.227

Correspondence to: D. Erdönmez, Department of Biology, Aksaray University, Aksaray, Turkey.

Tel: +90 382 288 21 69

Fax: +90 382 288 21 00

E-Mail: demet.erdonmez@gmail.com

INTRODUCTION

The technological and economic developments initiated by the industrial revolution have made it possible to reach many points of the world through different trade lines. The most common of these lines is the method of shipping method. By shipping, it became ordinary to carry heavy cargos among different points of the world. It is observed that costs of this common trading way have increased with the increase of fuel consumption caused by biofilm on the ship hull formed by interaction between bacteria in the salty environment and ship hull. There is some info about biofilm layer formed by bacteria living in salty environments like sea salt. It is needed to carry out biotechnological and microbial-based studies to prevent biofilm layer formation. A specific part of these studies is carried out for having enough knowledge about how bacteria orient environment, biofilm formation abilities or if bacteria can communicate with each other and if possible, how they communicate? Studies based on usage of bacteria living in a salty environment in biotechnologically different areas are common. Quorum sensing defined as bacterial communication, arise with gene regulation arranged by bacteria population that reach specific majority depending on population [1-5]. Although quorum sensing system is spotted in Gram-negative bacteria first, it is a microbial communication system used for Gram-positive bacteria and some fungi [6-8]. Reproduction and continuity of metabolic activities of microorganisms in different environments show that both structural and cellular specifications of these organisms should be confirmed.

In this study, biofilm formation abilities and detection of quorum sensing signals of different bacteria species isolated from water and soil samples, which are taken from Salt Lake that is a halophilic and extreme environment, are targeted.

MATERIALS and METHODS

Soil and water samples were taken aseptically from parts of the Salt Lake near Şereflikoçhisar were brought to the laboratory under sterile conditions. 20 of these isolates were used for these study. The isolates which were grown in HS

medium were kept Hacettepe University Faculty of Science, Department of Biology, Division of Biotechnology and ASÜBTAM Laboratory of Microbiology at -20°C.

Isolation of Halophilic Bacteria

Soil and water samples were diluted with a sterile saline solution with the ratio of 1:10 (g/ml). 1 ml of each sample were inoculated to the Sea Water medium (Sea Water medium (NaCl 202.5 g, MgCl₂ 17.5 g, MgSO₄ 24 g, CaCl₂ 0.9 g, KCl 5 g, NaHCO₃ 0.15 g, NaBr 0.065 g, Yeast extract 5 g/L⁻¹ pH 7.2) and HS medium (250 g of NaCl, 20 g of MgSO₄ ·7H₂O, 3 g of trisodium citrate·2H₂O, 2 g of KCl and 10 g of Oxoid bacteriological peptone L-34 , pH 6.5-7.4) and incubated for 7 days at 37-39°C , 150 rpm (Figure 1). 100 µl of each culture were spread on HS agar plaques. After 7-10 days incubation, selected colonies and control microorganism were inoculated to HS medium again. Third days of incubation colorless colonies and after 10-15 days pink-red colonies were observed at plaques (Figure 2). Gram-negative isolates were selected for this research [1-3].



Figure 1. Halophilic bacterial cultures obtained from water and soil samples.

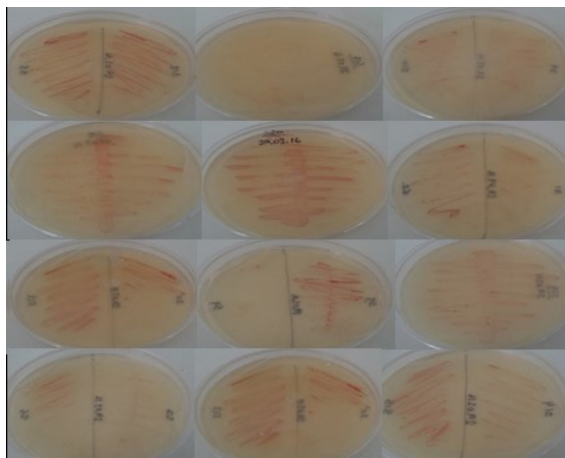


Figure 2. All isolated pure halophilic bacteria.

Bacterial DNA Isolation

Biospeedy Bacterial DNA isolation kit was used for DNA isolation and DNA was obtained according to the procedure. The obtained DNA's were stored at -20°C until running for 16s rRNA analysis.

Real-Time PCR (Q-PCR)

Biospeedy Bacterial Diversity Kit was used for identifying bacterial diversity. Primers in which was used during the procedure, were special to 16s rRNA side of bacterial genomic DNA (forward primer AGA GTT TGA TCC TGG CTC AG and reverse primer AAG GAG GTG ATC CAG CCG CA) [4,5]. BIORAD CFX Connect (Bio-Rad Laboratories, USA) Real-time PCR instrument was used for all reactions. The reaction contained 1.5 mM MgCl₂, 0.2 mM dNTP mix, 1x Reaction Buffer, 0.1 U Fast Start Taq DNA Polymerase, 1x Eva Green, 4 ng/µl template cDNA and 0.5 µM of each primer. In the device, the following heat cycle program has been applied with an optimized primer pair (Table 1). During Q-PCR, melting curve analysis was per-

formed at 65°C to 95°C to determine that only the desired product was replicated. Then, it was analyzed by Q-PCR Biorad CFX Connect Software 3.0.

16s RNA Sequencing

Obtained bacterial amplicon sequence, were determined through Sanger Method with ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit and ABI Prism 377 DNA Sequencer (Applied Biosystems, USA).

Phylogenetic Analysis

Obtained sequences from each bacteria were analyzed by Chromas software package version 1.45 (<http://www.technelysium.com.au/chromas.html>). Sequences were compared with known bacteria species in NCBI DNA databank (BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>)) and were determined most similar species, similarity rates, and accession number.

Table 1. Q-PCR reaction contents.

Detection Format		Reaction volume		
SYBR Green		10 µl		
Programmes				
Programme name	Cycle	Analyse mode		
Pre-incubation	1			
Enhancement	45	Enumeration		
Melting curve	1	Meeting curve		
Cooling	1			
Temperature				
Target(°C)	Mood	Duration (hh:mm:ss)	Speed (°C/s)	Reading (per °C)
Pre-incubation				
95		00:10:00	4,8	-
Enhancement				
95		00:00:15	4,8	-
53		00:00:20	2,5	-
72	Single	00:00:40	4,8	-
Melting curve				
95		00:00:02	-	-
65		00:01:00	-	-
98	Continuous	-	0.2	10
Cooling				
35	Single	00:00:10	2,5	-

Extraction and Purification of N-Acyl Homoserine Lactone from Halophilic Bacteria Culture Medium

For signal molecule extraction, halophilic Gram-negative bacteria culture were inoculated loop-full to 10 ml HS liquid medium and incubated at 37°C until stationary phase [6,7]. Obtained cultures were centrifuged at 4°C 4000 rpm 10 minutes. The supernatant was transferred into a new tube and dichloromethane was added equal volume. Organic phase that was transferred new tube was filtered by 0.2 µm pore size sterile syringe filter (non-pyrogenic, Sartorius). Dichloromethane was removed via evaporator [2]. The residue in the tube that included the completely evaporated organic phase was dissolved in 20 µL of methanol and stored at -20°C until use [8,9].

Signal Molecules Analysis by Thin Layer Chromatography

Signal molecules analysis were conducted by Reverse-phase C18 thin layer chromatography (RP-C18 TLC). N-butanoyl homoserine lactone (C4-HSL), N-hexanoyl homoserine lactone (C6-HSL), N-octanoyl homoserine lactone (C8-HSL) and N-dodecanoyl homoserine lactone (C12-HSL) (Sigma, Germany) molecules were used as standard signal molecules. The signal molecules dissolved in methanol were applied to the reversed phase TLC plates (RP-18 F254; Merck) with a 1.5 cm interval for each sample loading area up to the starting point. Chloroform- Methanol (95:5, v/v) was prepared as the mobile phase. Mobile phase ran up to 2 cm below the top of TLC plate. After the separation process was completed, TLC plates were dried in fume hood during 2 h. *Chromobacterium violaceum* cv026 and *Agrobacterium tumefaciens* A136 (pCF218)(pCF372) were used as biosensor strains to detect molecules. The dried TLC plates were spread onto 50ml of AB mineral soft agar medium (50°C) + 0.9ml agar containing 10% *Agrobacterium tumefaciens* A136 (pCF218) (pCF372) (100 ml for 48 h) and X-gal (60 µg/ml). Besides, other TLC plates were spread onto LB soft agar medium (%0.9 agar contained) + 10ml *Chromobacterium violaceum* CV026 (24 h culture) culture. When medium solidified, TLC plates were incubated at 30°C 24-48 h [10-12]. During the experiment, methanol was used as negative

control. The spots where AHL molecules located were determined with violaceous or greenish spot occurrence.

Determination of Biofilm Formation Capabilities of Halophilic Bacteria

Biofilm formation was determined with crystal violet method which is a classical method for biofilm formation [13]. Halophilic isolates were incubated at 37-39°C during 7 days in HS medium. Then all isolates were diluted with HS medium at 0.05 O.D. value at 600 nm and inoculation of isolates was performed with 50 ml culture to 100 ml HS medium. All medium which was inoculated before were incubated 200 rpm for 7 days. After incubation, 900µl HS medium + 100µl halophilic bacteria were added to 24 well polystyrene plate. Two different experimental setups were designed for 37-39°C and 7-10 days produced cultures. The medium was discharged from the plate in which incubated cultures. Each well was washed with 1xPBS buffer three times. Washed plate well was dried at 65°C. Wells were stained with 1% crystal violet for 2 minutes. After, stained wells were washed at three times and were dried at room temperature. Crystal violet in the well was solubilized with 30% Acetic acid solution and was measured by spectrophotometer at 595 nm [14,15].

RESULTS and DISCUSSION

When the test results were examined, it was observed that the bacterial isolates from soil and water samples could not be grown on medium with higher salt content but they could be grown on medium containing 4-5 M NaCl, so preliminary definitions were made in accordance with the characteristics of halophilic bacteria. In addition, as gram features, the selection was made among Gram-negative bacillus or coccobacillus morphology isolates [16]. After DNA isolations of the isolates were carried out in the microbiology laboratory of ASUBTAM, their genomic identification was done by Bioeksen [17,18]. In this context, according to 16S rRNA results for species differentiation, *Halobacterium salinarium*, *Halovibrio*, and *Halomonaras* species were determined to be widespread in Salt Lake (Table 2).

Table 2. Types of strains isolated by 16sRNA analysis.

Isolate Number	Identity	Similarity Rate	Accession No
HS1	<i>Halobacterium salinarum</i>	355/364(98%)	F J236084.1
HS2	<i>Salicola sp.</i>	663/669(99%)	KR611161.1
HS3	<i>Halovibrio sp.</i>	470/585(80%)	AM774418.1
HS4	<i>Salicola sp.</i>	691/719(96%)	EU931298.1
	<i>Halomonas sp.</i>	690/719(96%)	KF321909.1
HS5	<i>Salicola sp.</i>	715/763(94%)	KR611161.1
HS6	<i>Salicola sp.</i>	328/358(92%)	KF511768.1
HS7	<i>Salicola sp.</i>	915/955(96%)	KR611161.1
HS8	<i>Salicola sp.</i>	529/549(96%)	KR611161.1
HS9	<i>Salicola sp.</i>	468/490(96%)	KR611161.1
	<i>Halomonas sp.</i>	468/490(96%)	KF321909.1
HS10	<i>Salicola sp.</i>	621/653(95%)	KR611161.1
HS11	<i>Halomonas sp.</i>	663/688(96%)	KF321909.1
HS12	<i>Halophilic bacterium (Halobacterium salinarum)</i>	959/1016(94%)	F J236084.1
HS13	<i>Salicola sp.</i>	641/656(98%)	KR611161.1
HS14	<i>Salicola sp.</i>	558/568(98%)	CP003412.1
HS15	<i>Salicola sp.</i>	921/945(97%)	KR611161.1
HS15	<i>Halophilic bacterium</i>	921/945(97%)	KC142106.1
HS16	<i>Salicola sp.</i>	538/628(86%)	KR611161.1
	<i>Halomonas sp.</i>	538/628(86%)	KF321909.1
HS17	<i>Salicola sp.</i>	503/507(99%)	EU931298.1
HS18	<i>Halophilic bacterium</i>	721/752(96%)	F J236084.1
HS19	<i>Salicola sp.</i>	537/572(94%)	KF511768.1
	<i>Halomonas sp.</i>	537/573(94%)	KF321909.1
HS20	<i>Salicola sp.</i>	911/953(96%)	KR611161.1

As a reason for the condensation of genotypically and phenotypically identified strains among certain species in our study, it can be suggested that *Dunaliella salina* species algae, which is common in Salt Lake during the sampling period, affects the bacterial population. This is supported by the study of Le Chevanton et al. , in which some metabolites that produce *Dunaliella* algae in the saline environment cause inhibition of the population of bacteria in the environment [19]. Liu et al. found that some gram-negative and positive bacteria, in the study conducted, carried out the excessive proliferation of the algae population, which was expressed as algae bloom [20]. In this context, halophilic bacteria are considered to play an active role in hyperproliferation of *Dunaliella* in *Halobacterium*, *Salicola*, *Halovibrio* and *Halomonas* species of bacteria.

Disclosure of the biofilm mechanism regulated by the quorum sensing system, a bacterial communication system, and the detection of signaling molecules that play an active role in the formation of the biofilm mechanism has an important role in explaining the metabolism of bacteria. According to the data obtained from the study; it has been found that HS2, HS5, HS7, HS8, HS9, HS10, HS14, HS15, HS18 and HS19 from halophilic isolates can also produce the C6-AHL signaling molecule in thin layer chromatography in proportion to cross-validation. On the other hand, it has been found with cross-validation with *Agrobacterium sp* species that each strain is able to produce signal molecule in the detection of strains which are able to produce signal molecule but signal molecules have not been detected in thin layer chromatography (C8-AHL). It

has been shown that HS1, HS3, HS5, HS6, HS7, HS8, HS9, HS11, HS13, HS14, HS15, HS16 and HS18 from the isolated strains can produce C8-AHL signaling molecules. The isolates HS5, H7, HS8, HS9, HS14, HS15, and HS18 were able to produce both C6-AHL and C8-AHL signaling molecules (Table 3).

Enzymes to be used in industrial areas are also required to exhibit high activity in extreme conditions. For this reason, it is necessary to know what kind of signal molecules are produced by many bacterial strains in the extreme conditions, which features are expressed with these signal molecules (enzyme production, antibiotic production, biofilm, exopolysaccharide production) [2,21,22]. *Agrobacterium tumefaciens* NTL4 (pZRL4) and

Chromobacterium violaceum CVO26 have been identified in N-acyl homoserine lactones produced by these bacteria in a study showing that a sufficient number of perceptions also play an important role in hyperphosphorous environments [2]. Since the biofilm formation ability was regulated by the quorum sensing system, the biofilm production characteristics of the isolates producing signal molecules were examined and it was found that HS1, HS5, HS17 strains had higher biofilm forming abilities than the other isolates. 16 sRNA analysis showed that these strains are HS1; *Halobacterium salinarium*, HS2, and HS17; *Salicola* sp. It has been found that other isolates have biofilm producing properties even though they are few (Figure 3).

Table 3. The Rf values of the signal molecules of the halophilic isolates in TLC result.

Strain Number	AHL detection with CVO26	AHL detection with A136	RF1	RF2	RF3	RF4	C4-AHL	C6-AHL	C8-AHL	C12-AHL
							Average Rf values			
							0,8	0,42	0,36	0,12
HS1	-	++	-	-	0,35	-	-	X	-	
HS2	+	+	-	0,40	-	-	-	X	-	
HS3	-	++	-	-	0,34	-	-	-	X	
HS4	-	+	-	0,42	-	-	-	X	-	
HS5	+	++	-	0,40	0,34	-	-	X	X	
HS6	-	++	-	-	0,30	-	-	-	X	
HS7	+	++	-	0,40	0,31	-	-	X	X	
HS8	+	+++	-	0,41	0,32	-	-	X	X	
HS9	+	++	-	0,42	0,28	-	-	X	X	
HS10	+	+	-	0,40	-	-	-	X	-	
HS11	-	++	-	-	0,33	-	-	-	X	
HS12	-	+	-	-	-	-	-	-	-	
HS13	-	++	-	-	0,34	-	-	-	X	
HS14	+	++	-	0,41	0,29	-	-	X	X	
HS15	+	++	-	0,42	0,31	-	-	X	X	
HS16	-	++	-	-	0,33	-	-	-	X	
HS17	-	+	-	-	-	-	-	-	-	
HS18	+	+++	-	0,41	0,30	-	-	X	X	
HS19	+	+	-	0,39	-	-	-	X	-	
HS20	-	+	-	-	-	-	-	-	-	

C4-AHL ; 0,7-0,84 , C6-AHL; 0,39-0,42 , C8-AHL;0,28-0,36 , C12-AHL; 0,18-0,22.

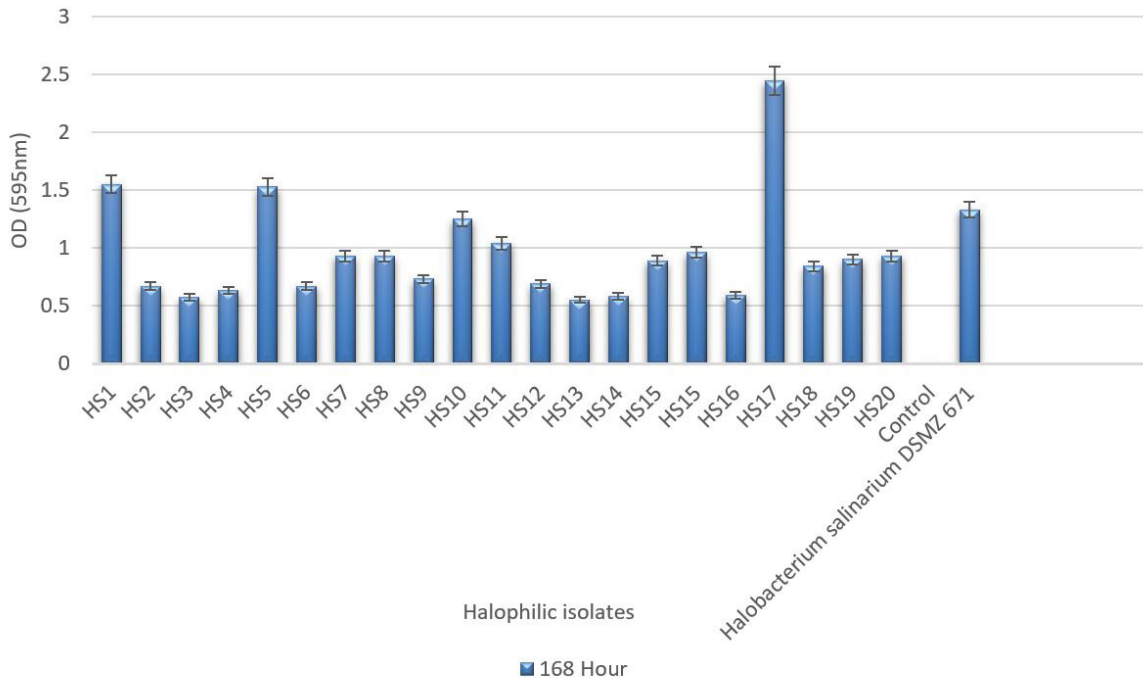


Figure 3. The ability of halophilic isolates to biofilm formation.

The HS12, HS17 and HS20 strains were observed not to produce a quorum sensing signal molecule but were able to generate biofilms. Then these bacteria use different signaling molecules in biofilm formation and it is likely that there is a signal molecule except from the one used as a standard. Figure 3 and Table 3 show that bacteria can produce biofilms regardless of producing quorum sensing signal molecule. In many studies, it is stated that these two systems are coordinated.

In the species of *Halomonas anticariensis*, In the species *Halomonas anticariensis*, the quorum sensing system regulates the ability to form biofilms with *hanR* (the default transcription regulator) and *hanI* (the autoinducer synthase gene), which are the *luxR/luxI* homologs [21]. In extreme conditions, it is emphasized by many scientists that this system works more actively [23,24]. Formations and densities of biofilms are a result of microbial evolution [25]. Halophilic bacteria in Salt Lake-like environment have developed some features that can give them an advantage over time. Some of them are capable of producing biofilms and being protected from other negativities in the environment. For instance, Salt Lake is exposed to the pollution of some factories and domestic wastes even though it is in the natural conservation areas [26]. Therefore, this

leads to pollution, heavy metal accumulation, and many other factors leading to the resistance of the immune system to infection. Another effect is that these bacteria protect themselves by producing biofilms. While biofilm formation is advantageous for salty bacteria, it causes disadvantages in sectors where commercial losses are high, such as maritime transport. In maritime transport, retention of various sea creatures to the parts of the ships exposed to seawater increases depending on the biofilm created by bacteria. This causes the economy to slow down over time and increase fuel consumption. At the same time, a serious amount of resource transfer is required in the elimination of biofilm formation. There are studies based on the reduction or elimination of this condition, but it has been observed that chemicals to be used on the surface of the ship have toxic effects on marine life. In this context, it is necessary to develop low-toxicity and high-availability chemicals for ship surface coatings [27]. First, the identification of quorum sensing molecules, which form biofilms on the surface of the ship and are effective in the formation of this biofilm, should be a priority in order to avoid such losses. Our work carries a preliminary qualification in order to achieve these results. Further research is needed to for future studies on the biofilm properties of halophilic bacteria. Considering the long

reproduction time of halophilic bacteria, we believe that the signal molecules in the environment may degrade. In addition, these bacteria are thought to have enzymes that can degrade these signaling molecules due to their improved enzyme systems. It has been shown that signal molecules produced by isolated halophilic bacteria change between C6-C8 AHL molecules.

ACKNOWLEDGEMENTS

This work was supported by Aksaray University Department of Scientific Research Projects Coordination (Projects Number:2015-088).

References

- J.J. Nieto, R. Fernandez, Castillo, M.C. Marquez, A. Ventosa, E. Quesada, F. Ruiz-Berraquero, Survey of metal tolerance in moderately halophilic eubacteria, *Appl. Environ. Microbiol.*, 55 (1989) 2385-2390.
- I. Llamas, E. Quesada, M.J. Martinez-Canovas, M. Gronquist, A. Eberhard, J.E. Gonzalez, Quorum sensing in halophilic bacteria: detection of N-acyl-homoserine lactones in the exopolysaccharide-producing species of Halomonas, *Extremophiles*, 9 (2005) 333-341.
- Ö. Çakıcı, Biochemical and genetic characterization of *Halobacterium Salinarium* strain isolated from Tuz Lake in Central Anatolia, 2004, Middle East Technical University.
- K. Ma, R. Conrad, Y. Lu, Responses of methanogen mcrA genes and their transcripts to an alternate dry/wet cycle of paddy field soil, *Appl. Environ. Microbiol.*, 78 (2012) 445-454.
- J. Penger, R. Conrad, M. Blaser, Stable carbon isotope fractionation by methylophilic methanogenic archaea, *Appl. Environ. Microbiol.*, 78 (2012) 7596-7602.
- M.M. Marketon, J.E. Gonzalez, Identification of two quorum-sensing systems in *Sinorhizobium meliloti*, *J. Bacteriol.*, 184 (2002) 3466-3475.
- M.M. Marketon, M.R. Gronquist, A. Eberhard, J.E. Gonzalez, Characterization of the *Sinorhizobium meliloti* sinR/sinI locus and the production of novel N-acyl homoserine lactones, *J. Bacteriol.*, 184(2002) 5686-5695.
- V.A. Blana, G.J. Nychas, Presence of quorum sensing signal molecules in minced beef stored under various temperature and packaging conditions, *Int. J. Food. Microbiol.*, 173 (2014) 1-8.
- H. Lade, D. Paul, J.H. Kweon, Isolation and molecular characterization of biofouling bacteria and profiling of quorum sensing signal molecules from membrane bioreactor activated sludge, *Int. J. Mol. Sci.*, 15 (2014) 2255-2273.
- R.H. González, A. Nusblat, B.C. Nudel, Detection and characterization of quorum sensing signal molecules in *Acinetobacter* strains, *Microbiol. Res.*, 155 (2001) 271-277.
- D. Anbazhagan, M. Mansor, G.O. Yan, M.Y. Md Yusof, H. Hassan, S.D. Sekaran, Detection of quorum sensing signal molecules and identification of an autoinducer synthase gene among biofilm forming clinical isolates of *Acinetobacter* spp, *PLoS one*, 7 (2012)
- S.D. Saroj, P.N. Rather, Streptomycin inhibits quorum sensing in *Acinetobacter baumannii*, *Antimicrob. Agents Chemother.*, 57 (2013) 1926-1929.
- J.H. Merritt, D.E. Kadouri, G.A. O'Toole, Growing and analyzing static biofilms, *Curr. Protoc. Microbiol.*, Chapter 1(2005) Unit 1B.1.
- D. Mack, W. Fischer, A. Krokotsch, K. Leopold, R. Hartmann, H. Egge, R. Laufs, The intercellular adhesin involved in biofilm accumulation of *Staphylococcus epidermidis* is a linear beta-1,6-linked glucosaminoglycan: purification and structural analysis. *J. Bacteriol.*, 178 (1996) 175-183.
- X. Wang, J.F. Preston, T. Romeo, The pgaABCD locus of *Escherichia coli* promotes the synthesis of a polysaccharide adhesin required for biofilm formation, *J. Bacteriol.*, 186 (2004) 2724-2734.
- S.B. McGee, C.A. Orenge, M.E. Kunik, V.A. Molinari, R.H. Workman, Delirium in geropsychiatric patients: patient characteristics and treatment outcomes, *J. Geriatr. Psychiatry Neurol.*, 10 (1997) 7-10.
- R. Rohban, M.A. Amoozegar, A. Ventosa, Screening and isolation of halophilic bacteria producing extracellular hydrolyses from Howz Soltan Lake, Iran, *J. Ind. Microbiol. Biotechnol.*, 36 (2009) 333-340.
- C. Sánchez Porro, S. Martin, E. Mellado, A. Ventosa, Diversity of moderately halophilic bacteria producing extra-cellular hydrolytic enzymes, *J. Appl. Microbiol.*, 94 (2003) 295-300.
- M. Le Chevanton, M. Garnier, G. Bougaran, N. Schreiber, E. Lukomska, J.B. Bérard, E. Fouilland, O. Bernard, J.P. Cadoret, Screening and selection of growth-promoting bacteria for *Dunaliella* cultures, *Algal Res.*, 2 (2013) 212-222.
- J. Liu, A.J. Lewitus, P. Brown, S.B. Wilde, Growth-promoting effects of a bacterium on raphidophytes and other phytoplankton, *Harmful Algae*, 7 (2008) 1-10.
- A. Tahrioui, E. Quesada, I. Llamas, The hanR/hanI quorum-sensing system of *Halomonas anticariensis*, a moderately halophilic bacterium, *Microbiology*, 157 (2011) 3378-87.

22. A. Tahrioui, M. Schwab, E. Quesada, I. Llamas, Quorum sensing in some representative species of halomonadaceae, *Life (Basel)*, 3 (2013) 260-75.
23. B.A. Dođaner, L.K. Yan, H. Youk, Autocrine signaling and quorum sensing: Extreme ends of a common spectrum, *Trends Cell Biol.*, 26 (2016) 262-271.
24. K. Montgomery, J.C. Charlesworth, R. Lebard, P.T. Visscher, B.P. Burns, Quorum Sensing in Extreme Environments, *Life*, 3 (2013) 131-148.
25. H.P. Steenackers, I. Parijs, K.R. Foster, J. Vanderleyden, Experimental evolution in biofilm populations, *FEMS Microbiol. Rev.*, 40 (2016) 373-397.
26. A. Kılıç, E. Uyanık, Tuz Gölü'nde oluşan kirlenmenin göl üzerindeki etkilerinin araştırılması, 4. Endüstriyel Hammaddeler Sempozyumu 18-19 Ekim, (2001) 135-145.
27. M.P. Schultz, J.A. Bendick, E.R. Holm, W.M. Hertel, Economic impact of biofouling on a naval surface ship, *Biofouling.*, 27 (2011) 87-98.