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Characterization of Some Extremely Halophilic Bacteria Isolated from Salt Marshes of Gamasa Egypt

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ABSTRACT

Halophiles can be classified as extreme, moderate and slight halophiles. Many extremophiles produce unique biotechnological and commercial materials. Salinity stress is one of the major factors negatively affecting bacterial growth. Cellular biochemical changes could be monitored via protein pattern in response to different NaCl concentrations. So our work aimed to do a comparison among salt-tolerant bacteria in their response to different NaCl concentrations via halotolerance test, antimicrobial susceptibility, amino acid profile and protein banding pattern. From haloterance test of eleven bacterial isolates, STB5, STB8 and *Shigella dysenteriae* were more tolerance to NaCl. From 16S rRNA gene sequencing, the two isolates STB5 and STB8 were identified as *Halomonas caseinilytica* and *Paraliobacillus quinghaiensis*. From 32used antibiotics only 5 antibiotics, cefotaxime, ampicillin, penicillin G, ceftazidime and aztreonam, showed resistance. From SDS PAGE, the appearance or absence of high or low molecular weight bands may be directly associated with the bacterial response to salt. Protein profile of tested halophilic bacteria under different concentrations of NaCl% (0, 5% and 20%) showed variable polymorphism in *Halomonas caseinilytica* MG199079*, Paraliobacillus quinghaiensis* MG250199 and *Shigella dysenteriae*. Environment-specific patterns of amino acids profile were observed. Based on the obtained results, it could be concluded that the three organisms *Halomonas caseinilytica* MG199079*, Paraliobacillus quinghaiensis* MG250199 and *Shigella dysenteriae,* are extreme halophilic bacteria, they were different in of amino acids accumulation protein profile and in different concentration of NaCl.

Keywords: *Halomonas caseinilytica*, *Paraliobacillus quinghaiensis* and *Shigella dysenteriae*, Salinity, tolerance indices

INTRODUCTION

Halophiles or salt loving are microorganisms from different species that live, grow, and multiply at different levels of NaCl concentrations or over a wide range of salinity (Kanekar, 2012). Extreme and moderate halophilic microorganisms have been isolated not only from hypersaline environments but also from alkaline environments such as the alkaline brines of Wade Natrun in Egypt (Hedi *et al.*, 2009).

Halophiles can be classified as extreme halophiles that can survive in over NaCl concentration, moderate halophiles that can survive in 5.0 – 15.0 % NaCl concentration and slight halophiles that can survive in 2.0-5.0% NaCl concentrations. Extreme halophiles, not only tolerate but also require NaCl concentrations above10 % to survive, and their optimal growth is often obtained above 20% NaCl concentration (Anwar, 2012). Many extremophilic microorganisms have evolved unique properties of considerable biotechnological and commercial significance (Margesin and Schinner, 2001). So, they are already used for some biotechnological processes, such as the bioremediation of wastes and pollutant oil recovery, searching for new drugs (Del Moral, 1994).

Halophilic microorganisms have variable mechanisms to adapt with their environmental conditions. These mechanisms are either to produce low molecular weight molecules that are synthesized and transported in response to the salinity condition (Mimura *et al.*, 1994), or inorganic ions that are used not only to balance the osmotic pressure but also to protect the enzyme activity (lyehara Ogawa *et al.*, 1984; Gilbert and Summers, 1988).

Salinity stress is one of the major factors negatively affecting growth and productivity in living organisms including bacteria (Adam *et al.*, 2015). In hypersaline ecosystem, the additive effect of high concentrations heavy metals strongly influences microorganisms living there. So, microorganisms thrive in such environments must be adapted to both of high salinity and heavy metals. The external osmolarity is a limiting physical factor that determines the ability of organisms to survive in a given habitat. Bacterial and plant cellular responses to high osmolarity are remarkably similar or closely parallels in the mechanisms because both organisms accumulate the same set of cytoplasmic solutes under high salinity (Csonka, 1989).

In response to extracellular changes in salinity concentrations, halophilic bacteria can synthesize and accumulate several low molecular weight compounds or compatible solutes such as sugars, amino acids and/or amino acid derivatives (Wood, 2001). Compatible solutes do not interrupt the main metabolism but also they are able to stabilize proteins even if they accumulate at high concentrations (Brown, 1976). They also protect cells and subcellular structures from hazards of low or high temperature, drying and oxygen free radicals (Saum and Müller, 2007).

Cellular biochemical changes could be monitored via protein pattern or SDS-PAGE analysis which define changes in protein profiles in response to different NaCl concentrations (Booyens, 2014). So our work aimed to do comparison among STB5, STB8and *Shigella dysenteriae* bacteria in their response to different NaCl concentrations via antimicrobial susceptibility test, amino acid profile and protein banding

MATERIALS AND METHODS

Bacterial isolates

Eight isolates were obtained from solid samples; from different off shore salt marshes located after 10 kilometres from west of Gamasa on Damietta road, and cultured on L.B media containing 10% NaCl. Another 3 isolates; *Staph aureus, E.coli* and *Shigella dysenteriae* were obtained from Mansoura University Hospitals (MUHs), Mansoura, Egypt. The bacterial isolate were maintained by storing on LB media at 4°c and sub culturing every month.

Halotolerance tests

To select the halotolerant isolates as well as to order the isolates according to their degree in tolerance to NaCl concentrations, the eleven isolates were cultured on LB

media containing 0, 5, 10, 15, 20, 25, and 30% NaCl at 37°C and shaking at 150 rpm for 6 days.

16s RNA sequences

For molecular identification of halotolerant isolates, the 16SrRNA gene of the isolates was sequenced. The sequencing reaction mixtures were performed in the 9700 Thermal Cycler at a total volume of 20µL (7µL of the purified PCR product and 13µL of the sequencing Module) by adjusting the thermal cycling condition to 96° C for 10 sec, 50°C for 5sec and 60°Cfor 4 sec (25 cycles).Then the excess dye termination and primers were removed from the cycle sequencing reaction using DyeExTM 2.0 Spin Lit (Qiagen PN 63204). The generated sequences were analyzed by Finch TV software and the phylogenetic tree was generated via Sea View software using the closest published type strain sequences. Sequences of the isolates were submitted to the Gen Bank on NCBI.

Characterization and identification of the isolates

- **Morphology and Gram staining**

Fresh samples were examined by phase contrast microscope without staining as well as fixed bacterial samples were stained by Gram stain and examined by light microscope using oil immersion lens. Bacteria films were prepared for Gram staining and examined under light microscope according to Collee *et al.*, (1996); Benson *et al.*, (2000)

- **Motility test**

Single stab by halophilic isolates in semi- solid agar medium and the condition favouring motility were performed according to Baron and Finegold,(1990).Non motile bacteria give confirmed growth to the stab-line while motile bacteria give diffused growth.

- **Oxidase test**

A colony of halotolerant bacteria was transferred to 6 cm square piece of whatman filter paper saturated with 1-2 drops of 1 % oxidase reagent (Tetra methyl para phenylene diamino dehydroxide) according to (Hemraj *et al.*, 2013). A positive result was indicated by the development of purple colour in few seconds while no change in colour with negative result**.**

- ***Catalase test***

At room temperature, a drop of diluted 3% hydrogen peroxide was added to 6 days old culture according to the effervescence could be observed instantly as a positive result (Facklam and Elliott, 1995).

Antimicrobial susceptibility test

The antimicrobial susceptibility to antibiotic has been performed by using Instrument BD phoenix automated microbiology.

SDS – PAGE

The standard method for separating and differentiating proteins according of bacteria isolates (Laemmli, 1970). The denatured gel consists of two different layers, a stacking gel (4%) on top of separating gel (12%). Main components of the gel are monomer solution, ammonium per sulphate (Claps *et al.*, 2002) and Tetramethylethylenediamine (TEMED). APS and TEMED are responsible for polymerization of monomer through free radical reaction**.**

Amino acid profile

Amino acid analysis is a technique based on ion exchange liquid chromatography, used in a wide range of application areas to provide a qualitative composition.To investigate the compositions of free amino acids in *Halomonas caseinilytica, Paraliobacillus quinghaiensis and Shigella dysenteriae* in response to the external NaCl concentrations amino acid profile was performed according to Mimura *et al.*, (1994).

Halomonas caseinilytica, Paraliobacillus quinghaiensis and *Shigella dysenteriae* was grown aerobically at 37°C in a L.B medium containing various NaCl concentrations (0, 5 and 20%), incubated in shaker incubator at 150 rpm for 10 days. Ten day old cultures were processed on amino acid analyzer Sykam5433. A standard solution of amino acids was obtained from Wako Chemicals, Japan. Protein was measured by the method of Lowry *et al.*, (1951). With bovine serum albumin as a standard. Determinations of Asp/Asn and Glu/Gln were considered together for the analysis, because measurements did not distinguish between the two amino acids in the pairs

RESULTS

1. Isolation of halotolerant bacteria

Eleven halotolerant bacterial isolates were obtained by screening on LB medium containing 10% NaCl. (Table. 1).

Table 1. Halotolerance test of halotolerant bacterial isolates using different concentrations of sodium chloride (0, 5, 15, 20, 25 and 30%).MUBL: Mansoura University Bacteriology lab, MUH: Mansoura University hospital.

Isolate No	Source	Concentrations of NaCl					
		٠%	5%	15%	20%	25%	30%
STB1		+	+	+	+	-	-
STB 2		+	+	+	+	-	-
STB 3		+	+	+	+	+	+
STB 4		+	+	+	-	-	-
STB 5	MUBL	+	+	+	+	+	+
STB 6		+	+	+	-	-	-
STB 7		+	+	+	-	-	-
STB 8		+	+	+	+	+	+
9 *Staph aureus*		+	+	+	+	+	-
10 *E.coli*	MUH	+	+	+	-	-	-
11 *Shigella dysenteriae*		+	+	+	+	+	-

(+): growth , (-): No growth

2. Haloterance test

From haloterance test of eleven halotolerant bacterial isolates (Table 1), all eleven isolates afforded the stress of NaCl till 15%. While at 20, 25 and 30% NaCl STB3, STB5 and STB8, could grow. Since all isolates are able to survive in the presence of 15% NaCl concentration, they considered as extreme halotolerant bacterial isolates. STB5 and STB8

besides *Shigella dysenteriae* were subjected for further identification and characterization.

3. Morphological and biochemical characters of halotolerant bacteria

The 3 selected strains STB5, STB8 and *Shigella dysenteriae* were characterized morphologically and biochemically (Table 2). STB5 is a Gram negative bacterium, motile, short rods or oval in shape, occur singly or in pairs showing white colony. Its growth in NaCl concentrations was up to 20% NaCl concentration. Light yellow after 2 days. Catalase positive, oxidase negative. STB8 is a Gram positive bacterium, motile, oxidase positive, and catalase positive. Its growth in NaCl concentrations was up to 20%. *Shigella dysenteriae* is a Gram-negative bacterium, non-motile, produce catalase oxidase negative besides its growth in NaCl concentrations up to 20%.

Table 2. Morphological and Biochemical characters of *Halomonas caseinilytica, Paraliobacillus quinghaiensis and Shigella dysenteriae* grown on LB medium.

Characteristics	*H. caseinilytica*	*P. quinghaiensis*	*S. dysenteriae*
Morphology	Short rods	Rods	Rods
Pigmentation	White	White	White
Oxidase	+	+	+
catalse	+	+	-
Motility	+	+	-
Salt range (%, w/v)			
Control no NaCl	+	+	+
5% NaCL	+	+	+
20% NaCL	+	+	+
Gram stain	-ve	+ve	-ve

4. Molecular identification of halotolerant bacterial isolates

From 16S rRNA gene sequencing, the two selected isolates STB5 and STB8 were identified as *Halomonas caseinilytica* (Fig. 1) and *Paraliobacillus quinghaiensis* (Fig.2). *Halomonas caseinilytica* was submitted to gene bank with accession numbers MG199079 while *Paraliobacillus quinghaiensis* were submitted to gene bank with accession numbers MG250199. From phylogenic analysis, *Halomonas caseinilytica* showed high levels of sequence similarity with members of the genus *H.elongata* 97%, H. *halmophila* 97%, *H. sabkhae* 96% and *H. eurihalina* 95%. The isolate *Paraliobacillus quinghaiensis* showed high similarity with members of genus *Bacillus* and given the name *Paraliobacillus quinghaiensis* EUI35728 91%, *Virgibacillus picturae* 90%, *Bacillus marismortui* 90% *and Bacillus circulans* 84%.

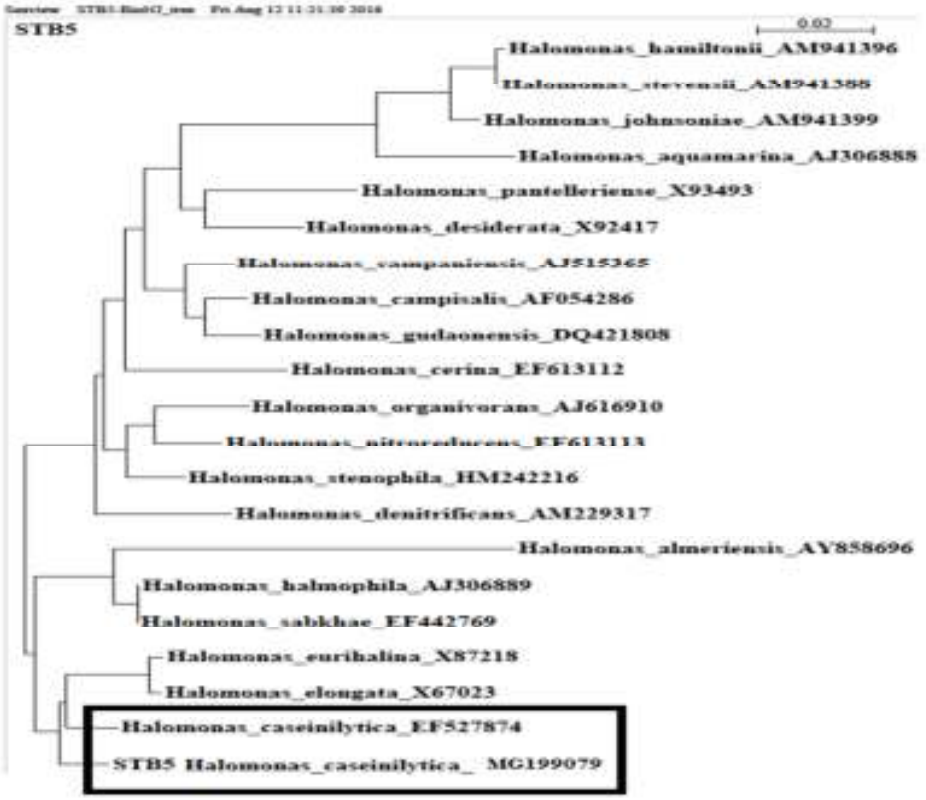

Fig.1. The phylogenic tree of the strains *Halomonas caseinilytica* (STB5) based on the full 16S rRNA gene sequences using the distance methods.

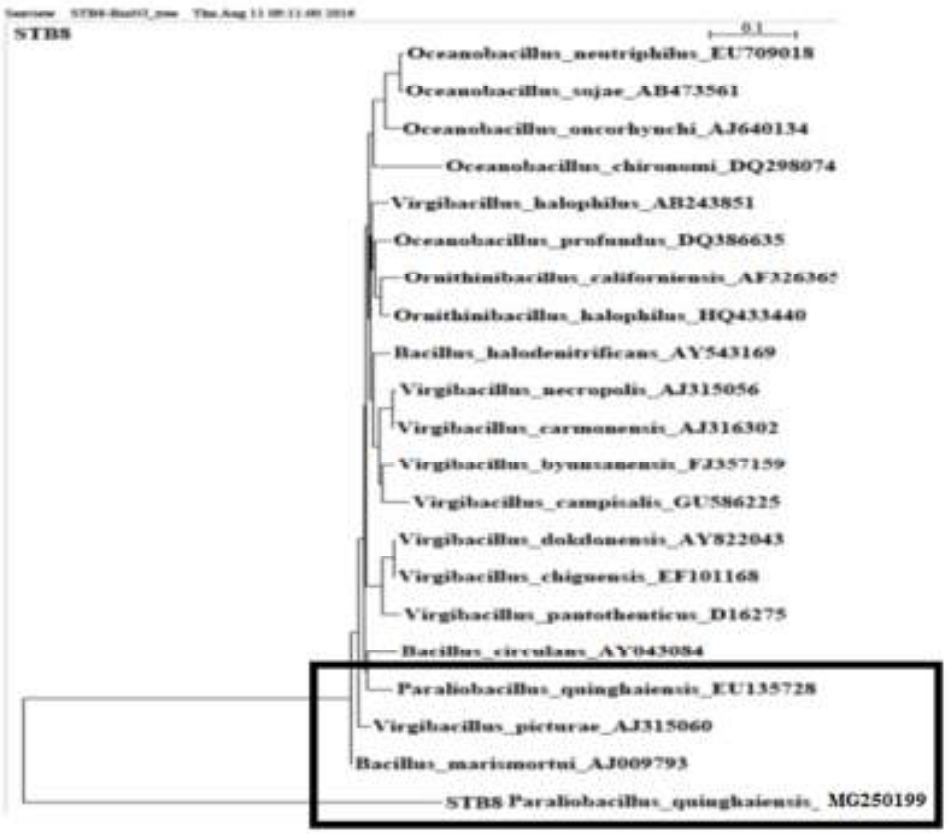

Fig.2. The phylogenic tree of the strains *Paraliobacillus quinghaiensis* (STB8) based on the full 16S rRNA gene sequences using the distance methods

5. Antimicrobial susceptibility test

The antibacterial tests of the *Halomonas caseinilytica* MG199079*, Paraliobacillus quinghaiensis* MG250199 and *Shigella dysenteriae* showed that, bacterial species vary in their sensitivity to different antibiotic agents. From 32 antibiotics only 5 antibiotics (cefotaxime , ampicillin , penicillin G , ceftazidime , aztreonam) showed resistance in 6 cases as a total resistance (Table 3).

Table 3. The antibiotic susceptibility of *Halomonas caseinilytica, Paraliobacillus quinghaiensis, and Shigella dysenteriae* bacteria using 32 antibiotics. (BD phoenix Automated microbiology Instrument)

No	Antimicrobial	*H. caseinilytica*	*P. quinghaiensis*	*S. dysenteriae*
1	Gentamicin – Syn	ND	ND	ND
2	Gentamicin	S	S	S
3	Imipenem	S	S	S
4	Cefoxitin	ND	ND	I
5	Cefotaxime	S	S	R
6	Ampicillin	R	R	S
7	Penicillin G	S	R	ND
8	Oxacillin	S	S	ND
9	Amoxicillin – Clavulante	S	S	S
10	Daptomycin	S	S	ND
11	Trimethoprim – Sulfamethoxazole	S	S	S
12	Teicoplanin	S	S	ND
13	Vancomycin	S	S	ND
14	Clindamycin	S	I	ND
15	Erythromycin	S	S	ND
16	Fusidic Acid	ND	ND	ND
17	Linezolid	S	S	ND
18	Mupirocin High level	S	S	ND
19	Nitrofurantion	S	S	ND
20	Ciprofloxacin	S	S	ND
21	Moxifloxacin	S	S	ND
22	Rifampin	S	S	ND
23	Tetracycline	S	I	ND
24	Amikacin	ND	ND	S
25	Ampicillin	ND	ND	S
26	Ertapenem	ND	ND	I
27	Meropenem	ND	ND	S
28	Ceftazidime	ND	ND	R
29	Ceftriaxone	ND	ND	S
30	Cefepime	ND	ND	S
31	Aztreonam	ND	ND	R
32	Piperacillin – Tazobactam	ND	ND	S

S: sensitive, R: resistant, I: intermediate, ND: not detected

Halomonas caseinilytica MG199079 was resistant to ampicillin while *Paraliobacillus quinghaiensis* MG250199 was resistant to two antibiotics ampicillin and penicillin G while *Shigella dysenteriae* was multidrug resistant to three antibiotics; cefotaxime, ceftazidime, aztreonam

5. SDS- PAGE analysis

Results of protein banding analysis of *Halomonas caseinilytica* MG199079, Paraliobacillus *quinghaiensis* MG250199 and *Shigella dysenteriae* (Tables 4, 5 and 6 respectively) under NaCl% concentrations (0%, 5% and 20%) were documented in Fig.3. For *H. caseinilytica* MG199079, the total 15 protein bands ranging from 9.172 KDa to 93.557 KDa were distributed as: 8 monomorphic bands, 3 unique bands, 4 polymorphic bands. Thirteen bands per lane were recorded in both 0% and 5% NaCl concentrations while the number of bands were reduced to 9 bands per lane in 20% NaCl concentration.

For *Paraliobacillus quinghaiensis* MG250199, the total 18 protein bands ranging from11.210 KDa to141.964 KDa were distributed as: 7 monomorphic bands and 11 polymorphic bands. Nine bands per lane were recorded in 0% NaCl concentration while the number of bands per lane in 5% and 20% NaCl concentrations were increased to 17 bands.

For *Shigella dysenteriae* the total 17 protein bands ranging from 9.471KDa to 143.742KDa were distributed as: 8 monomorphic bands, 9 polymorphic bands. Fourteen bands per lane were recorded in 0% NaCl concentration and reduced to 12 bands per lane in 5% NaCl concentration as well as the reduction in number of bands per lane recorded 10 bands in 20% NaCl concentration.

Table 4. Analysis of the protein pattern of *H. caseinilytica* grown on LB broth media conting different concentration of NaCl (0%, 5% and 20% NaCl)

Band no	RF	Polymorphism	Mol .Wt of bands	0%	5%	20%
1	0.203	Monomorphic	93.557	+	+	+
2	0.232	Monomorphic	85.486	+	+	+
3	0.269	Polymorphic	76.187	+	+	-
4	0.300	Monomorphic	69.125	+	+	+
5	0.351	Monomorphic	58.906	+	+	+
6	0.410	Monomorphic	49.029	+	+	+
7	0.447	Unique	43.784	+	-	-
8	0.486	Monomorphic	38.746	+	+	+
9	0.530	Polymorphic	33.817	+	+	-
10	0.563	Monomorphic	30.471	+	+	+
11	0.627	Monomorphic	25.047	+	+	+
12	0.675	Unique	21.536	-	+	-
13	0.837	Polymorphic	12.989	+	+	-
14	0.874	Polymorphic	11.576	+	+	-
15	0.949	Unique	9.172	-	-	+
No. of bands /lane				13	13	9
No. Total bands					15	
No. monomorphic bands					8	
No. unique					3	
No. polymorphic bands					4	

Table 5. Analysis of the protein pattern of *Paraliobacillus quinghaiensis* grow on LB broth media conting different concentrations of NaCl 0%, 5% and 20% NaCl)

Band	RF	Polymorphism	Mol .Wt of bands	0%	5%	20%
1	0.069	Polymorphic	141.964	-	+	+
2	0.136	Polymorphic	115.071	-	+	+
3	0.108	Monomorphic	107.623	+	+	+
4	0.183	Monomorphic	99.573	+	+	+
5	0.202	Monomorphic	93.849	+	+	+
6	0.242	Polymorphic	82.865	-	+	+
7	0.275	Polymorphic	74.723	-	+	+
8	0.315	Polymorphic	68.540	+	+	-
9	0.336	Monomorphic	61.876	+	+	+
10	0.364	Monomorphic	56.602	+	+	+
11	0.397	Polymorphic	51.161	+	-	+
12	0.392	Polymorphic	42.323	-	+	+
13	0.443	polymorphic	32.617	-	+	+
14	0.592	Polymorphic	27.88	-	+	+
15	0.636	polymorphic	24.290	-	+	+
16	0.413	Polymorphic	17.055	-	+	+
17	0.799	Monomorphic	14.642	+	+	+
18	0.796	Monomorphic	11.210	+	+	+
No .of ands /lane				9	17	17
No. Total bands					18	
No. monomorphic bands					7	
No. unique					0	
No. polymorphic bands					11	

Table 6. Analysis of the protein pattern of *Shigella dysenteries* on LB broth media conting different concentrations of NaCl (0%, 5% and 20% NaCl)

Band	RF	Polymorphism	Mol .Wt	0%	5%	20%
1	0.065	Polymorphic	143.742	+	-	-
2	0.134	Polymorphic	115.966	+	+	-
3	0.156	Polymorphic	108.293	+	+	-
4	.175	Polymorphic	102.075	+	-	-
5	0.216	Polymorphic	89.721	+	-	-
6	0.251	Monomorphic	80.503	+	-	-
7	0.290	Polymorphic	71.312	+	-	-
8	0.327	Polymorphic	63.616	-	+	+
9	0.369	Monomorphic	53.2827	+	+	+
10	0.421	Monomorphic	47.474	+	+	+
11	0.495	Polymorphic	37.651	-	+	+
12	0.545	Monomorphic	32.275	+	+	+
13	0.571	Monomorphic	29.70	+	+	+
14	0.641	Monomorphic	23.931	+	+	+
15	0.744	Polymorphic	17.415	-	+	+
16	0.575	Monomorphic	13.553	+	+	+
17	0.939	Monomorphic	9.471	+	+	+
No .of bands /lane				14	12	10
No. Total bands					17	
No. monomorphic bands					8	
No. unique					0	
No. polymorphic bands					9	

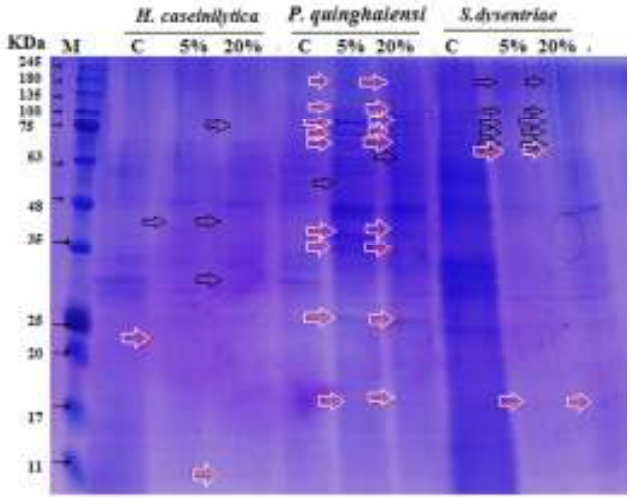

Fig. 3. SDS-PAGE of *Halomonas caseinilytica, Paraliobacillus quinghaiensis* and *S. dysenteriae* bacteria cultured in different NaCL concentration (0%, 5and 20%) stained with colloidal coomassie blue stained Gel. M is the prestained protein marker.

6. Amino acid profile

The concentration and types of amino acids of halotolerant salt-tolerant bacteria *Halomonas caseinilytica* MG199079*, Paraliobacillus quinghaiensis* MG250199 and *Shigella dysenteriae* were variable (Table. 7, 8, 9). For *Halomonas caseinilytica* MG199079*,* all types of amino acids were recorded in 0% and 5% NaCl concentrations but cysteine could not be detected in 20% NaCl concentration. Methionine recorded the lowest amount of amino acids. The most-accumulated free amino acids in *Halomonas caseinilytica* MG199079 was glutamic acid/glutamine (11.7% and 13.1%) in 0% and 20% NaCl concentrations respectively. Glycine was recorded as 14.4% in 5% NaCl concentration. Histidine amino acid recorded 10.4%value in 0% NaCl concentration, then decreased to 4.2% value in 5% NaCl concentration and again increased to 11.2% value in 20% value NaCl.

For *Paraliobacillus quinghaiensis* MG250199, at 0% and 20% NaCl concentration treatments recorded the 20 types of amino acids were recorded but cysteine could not be detected in 5% NaCl concentration. The most-accumulated free amino acids in *Paraliobacillus quinghaiensis* MG250199 was histidine showing 13.8%, 9.7% and 12.6% at o%,5%and 20% NaCl concentrations respectively while isoleucine showed the lowest amount of amino acids.

For *Shigella dysenteriae*, the 0% and 5% NaCl treatments recorded all types of amino acids were recorded at0% and 5%NaCl concentration, however cysteine could not be detected. The most-accumulated free amino acids was glutamic acid/glutamine representing 15.7% at 0% NaCl concentration whereas glycine recorded 17.0% and 17.8% in 5% and 20% NaCl concentrations respectively. The minimum amounts recorded were isoleucine (2.1%) in 0% NaCl concentration, and tyrosine (0.9%) in 5% NaCl concentration besides methionine (1.0%) in 20%. glutamic acid/glutamine recorded 15.7 in 0% NaCl concentrations than decreasing to 10.3% with 5% NaCl concentration and again increased to 11.4% value in 20% NaCl concentration.

Table 7. Intracellular free amino acid profile concentration and abundance in *Halomonas caseinilytica* grown in LB medium with 0%, 5% and 20% NaCl concentration.

NO	Amino acid	NaCl Control 0%	NaCl 5%	NaCl 20%
		Amount (%)	Amount (%)	Amount (%)
1	ASP+Asn	9.5	9.1	8.4
2	THR	3.7	3.4	3.7
3	SER	4.0	3.6	3.6
4	GLU+Gln	11.7	11.6	13.1
5	PRO	6.2	9.9	7.3
6	GLY	5.6	14.4	6.3
7	ALA	6.1	9.8	8.9
8	CYS	3.1	4.4	ND
9	VAL	4.5	3.4	3.3
10	MET	2.2	1.6	2.2
11	ILE	3.5	2.2	2.4
12	LEU	7.4	4.7	4.6
13	TYR	4.4	1.6	5.0
14	PHE	3.9	1.9	4.2
15	HIS	10.4	4.2	11.2
16	LYS	6.5	4.4	6.7
17	AMMONIA	2.8	3.1	6.8
18	ARG	4.5	6.8	2.4

.ND, not detected.

ASP: Aspartic , ASN: Asparagine, THR: Threonine ,SER: Serine , GLU: Glutamic , GLN: Glutamine , PRO : Proline , GLY : Glycine , ALA : Alanine , CYS : cysteine ,VAL : Valine , MET : Methionine , ILE : isoleucine , LEU : Leucine, TYR : Tyrosine , PHE: Phenylalanine, HIS : Histidine, LYS : -Lysine , AMMONIA , ARG : Arginine.

Table 8. Intracellular free amino acid profile concentration and abundance in *Paraliobacillus quinghaiensis* grown in LB medium with 0%, 5% and 20% NaCl concentrations

NNO	Amino acid	NaCl control	NaCl 5%	NaCl 20%
		Amount (%)	Amount (%)	Amount (%)
11	ASP+Asn	8.5	8.7	2.4
22	THR	3.1	3.4	3.9
33	SER	3.8	4.1	4.8
34	GLU+Gln	11.3	13.7	11.0
35	PRO	6.2	7.8	8.8
36	GLY	6.1	8.2	6.9
37	ALA	7.3	10.0	6.7
38	CYS	3.6	ND	5.8
39	VAL	2.8	2.8	3.4
310	MET	2.6	2.2	2.4
311	ILE	2.1	1.9	2.2
312	LEU	4.4	3.7	4.5
313	TYR	4.6	4.6	4.2
314	PHE	2.5	2.6	2.7
315	HIS	13.8	9.7	12.6
316	LYS	6.8	6.8	6.8
317	AMMONIA	8.0	7.1	4.2
118	ARG	2.5	2.5	6.8

.ND, not detected

ASP: Aspartic , ASN: ,THR: Threonine ,SER: Serine , GLU: Glutamate , GLN: Glutamine ,PRO : Proline , GLY : Glycine , ALA : Alanine , CYS : cysteine ,VAL : Valine , MET : Methionine , ILE : isoleucine , LEU : Leucine, TYR : Tyrosine , PHE: Phenylalanine, HIS : Histidine, LYS : -Lysine , AMMONIA , ARG : Arginine.

Table 9. Intracellular free amino acid profile concentration and abundance in *Shigella dysenteriae* grown in LB medium 0%, 5% and 20% NaCl concentration.

NO	Amino acid	NaCl control	NaCl 5%	NaCl 20%
		Amount (%)	Amount (%)	Amount (%)
11	ASP+Asn	9.1	7.5	7.5
12	THR	3.4	2.7	2.7
13	SER	3.7	3.4	3.5
14	GLU+Gln	15.7	10.3	11.4
15	PRO	9.4	12.9	13.4
16	GLY	6.6	17.0	17.8
17	ALA	9.7	9.2	13.9
18	CYS	ND	8.9	ND
19	VAL	2.9	2.6	2.6
110	MET	2.2	1.2	1.0
111	ILE	2.1	1.5	1.6
112	LEU	4.4	3.5	3.4
113	TYR	3.6	0.9	0.9
114	PHE	2.5	1.5	1.4
115	HIS	9.4	3.5	4.5
116	LYS	6.2	4.1	5.2
117	AMMONIA	6.2	2.3	2.1
118	ARG	2.7	6.7	7.1

.ND, not detected

ASP: Aspartic , ASN: ,THR: Threonine ,SER: Serine , GLU: Glutamate , GLN: Glutamine ,PRO : Proline , GLY : Glycine , ALA : Alanine , CYS : cysteine ,VAL : Valine , MET : Methionine , ILE : isoleucine , LEU : Leucine, TYR : Tyrosine , PHE: Phenylalanine, HIS : Histidine, LYS : -Lysine , AMMONIA , ARG : Arginine.

DISCUSSION

Hypersaline environments, are active environments inhabited by a variety of novel microbial strains with novel properties. Halophiles are divided into three categories: slight halophiles, which grow optimally at low salinities of 2-5 % NaCl, moderate halophiles, which grow optimally at salinities of 5-20 NaCl; and extreme halophiles that grow optimally at salinities greater than 20 % NaCl (Kushner, 1993). In this study, the three phenotypically and molecularly identified bacteria *Halomonas caseinilytica* MG199079, *Paraliobacillus quinghaiensis* MG250199 and *Shigella dysenteriae* are considered extreme halophiles.

In bacteria, the resistance to antibiotics could be used as a selectable marker and is important for persistence and competition with other bacteria. The extensive use of antibiotics has developed resistant populations of staphylococci (Periti *et al.*, 1998). This development may be due to mutations or transfer of plasmids, that carry a number of different antibiotic resistance genes, between different bacteria (Hassan, 2010). Halophilic bacteria have plasmids in their cells (Ghosh *et al.*, 2010).

In this study, from 32 antibiotics, *Halomonas caseinilytica* MG199079 and *Paraliobacillus quinghaiensis* MG250199 were resistant to 5 antibiotics; cefotaxime ampicillin, penicillin G, ceftazidime and aztreonam. Whereas, *Shigella dysenteriae* was resistant to three antibiotics; cefotaxime, ceftazidime and aztreonam.

The mechanism of stress tolerance exists in all living systems with minor modifications from bacteria to higher plants. As abiotic stress ultimately affects the cellular gene-expression machinery, it is evident that a large number of genes are up or down regulated (Shiozaki *et al.*, 2005). Molecular adaptation of halophiles, to high salinity is might be render to the presence of proteins and amino acids composition exhibiting halotolerance in different microorganisms with varying levels of salt tolerance (Anwar, 2012).

From SDS PAGE, the appearance or absence of high or low molecular weight bands may be directly associated with the bacterial response to salt stress (Hassan and Mahgoub, 2016). Protein profile of tested halophilic bacteria under different concentrations of NaCl% (0%, 5% and 20%) showed variable polymorphism in *Halomonas caseinilytica* MG199079, *Paraliobacillus quinghaiensis* MG250199 and *Shigella dysenteriae*.

Transmembrane proteins or porins are encoded genes such as *ompA* and *OmpK36* (Nikaido, 2001). That are cation or anion selective channels (Samartzidou and Delcour, 1999). Investigations of the transmembrane topology of proteins give the impression that transmembrane segments are not located randomly in the sequences (Tusnady, 1998).

A common adaptation is the decrease in hydrophobic amino acids visible in the most recent works distinguished amino acids critical in poring structure and function in bacteria (Simonet *et al.*, 2000).

Nevertheless, specific sets of amino acids were enriched in specific habitats, creating molecular signatures. In this work, the environment-specific patterns of amino acids profile were observed. For *Halomonas caseinilytica* MG199079 in 0%NaCl ASP+Asn, .GLU+Gln and HIS recorded high percentage. While in 5% NaCl: GLY, PRO and ALA recorded high percentage and in 20% NaCl GLU+GLn, ALA and ASP +Asn recorded high percentage for *Paraliobacillus quinghaiensis* MG250199 in 0%NaCl HIS, GLU +Gln and ASP + Asn recorded high percentage, while in 5% NaCl, GLU + Gln, ALA and HIS recorded high percentage. and in 20% NaCl: HIS, GLU and PRO recorded high.*Shigella dysenteriae* in NaCl control% GLU + Gln, ALA and PRO recorded high percentage. While in 5% NaCl: GLY, PRO and GLU +Gln recorded high percentage. and in 20% NaCl: GLY, ALA and PRO recorded high percentage.

CONCLUSION

In this work three bacterial strains, *Halomonas caseinilytica* MG199079, *Paraliobacillus quinghaiensis* MG250199 and *Shigella dysenteriae* were isolated, identified and their salinity tolerance was evaluated. Under NaCl different concentrations the isolates were classified as extreme halophilic bacteria that vary in accumulation of amino acids in the protein profile.

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توصيف لبعض البكتريا المحبة للملوحه العالية ا لمعزولة من المستنقعات الملحيه جمصه -مصر

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يمكن تصنيف البكتريا المحبة للملوحة الي شديدة ومتوسطة وقليلة التحمل. العديد من البكتريا المتحملة للملوحة العالية تنتج مواد ذات اهمية بيوتكنولوجية و تجارية فريدة. الإجهاد الملحي هو أحد العوامل الرئيسية التي تؤثر سلبا على نمو البكتيريا. و يمكن ملاحظة التغيرات البيوكيميائية الخلوية عبرالتغيرات في نمط البروتينات استجابة لتراكيزات كلوريد الصوديوم المختلفة. هدف البحث هو عزل وتصنيف بعض البكتيريا التي تتحمل الملوحة واستجابتها لتركيزات كلوريد الصوديوم المختلفة من خلال اختبار(halotolerance test)،زكذلك مقارنة حساسية البكتريا للمضادات الحيوية ، والتغير في نمط الأحماض الأمينية والبروتينات . نتيجة لاختبار تحمل الملوحة لاحدي عشر عزلة بكتيرية (halotolerance test)، كانت العزلات STB5 و STB8 بالاضافة الي الشيجلا دايسنتاريا أكثر تحملاللتركيزات المختلفة من كلوريد الصوديوم. من خلال تسلسل تتابع الجين rRNA 16S ، تم تعريف العزلتين STB5 و STB8 علي انهما caseinilytica *Halomonas* و*quinghaiensis* Paraliobacillus. ومن خلال اختبار الحساسية للمضادات الحيوية أظهرت البكتريا المقاومة لخمس فقط من المضادات الحيوية وهي، سيفوتاكسيم ، أمبيسيلين ، البنسلين G ، سيفتازيديم والأزترونام. من خلال تجربة التفريد الكهربائي للبروتين (SDS PAGE) ، قد يرجع ظهور أو عدم ظهور أوزيادة أو نقص كمية البروتين الي ارتباطً مباشر بالاستجابة البكتيرية للملح. أظهر التفريد الكهربائي للبروتينات البكترية المحبة للملوحة تحت تراكيز مختلفة من NaCl٪ (٠ ، ٥٪ و ٢٠٪) تعدد انماط البروتينات في السلالات المختلفة Halomonas caseinilytica MG199079، Paraliobacillus quinghaiensis MG250199 و Shigella dysenteriae.وايظا لوحظت انماط معينة من بالأحماض الأمينية مع التركيزات المختلفة للملوحة. وأخيرا ظهر ان البكتريا الثلاثة Halomonas caseinilytica MG199079، Paraliobacillus quinghaiensis MG250199 و Shigella dysenteriae ، هي البكتيريا المتطرفة halophilic extreme، بكتريا متحملة للملوحة العالية ومتنوعة في النمط البروتيني وكذلك الأحماض الأمينية استجابة للتركيزات المختلفة من كلوريد الصوديوم.