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Characterization and 16S rDNA identification of halophilic bacteria

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Salinity-tolerant bacteria from the surface of salted fish samples were isolated on Ashby's medium. The isolates were purified and identified based on morphology, biochemical tests, phenotypic and 16S rDNA sequences. The results showed a total of eight isolates; isolates No. 1, 2 and 8 presented in a phylogenetic cluster with species belonging to the genus *Cronobacter* and were closely related to *C. condimentii*, *C. malonaticus*, *C. sakazakii* BQ16, *C. sakazakii* Jor1468, *C. dublinensis* and *C. muytjensii*. Isolate No. 3 was most closely related to *Bacillus oceanisediminis*A3a, *Bacillus firmus* PX28 and *Bacillus subtilis* HAU5. Isolate No. 4 was closely related to *Bacillus mojavensis*ZA1 and then to several other species of *Bacillus*. Isolate No. 7 was closely related to *Bacillus pumilus* p51H01 and *Bacillus safensis* SDS101. Isolate No. 5 was clustered with three different genera: *Alcaligenes faecalis*, *Rhodobacter sphaeroides* and *Pusillimonas*_sp. Isolate No. 6 was separated alone in the last cluster. Further studies are required to identify these isolates, which are potential type strains for novel species.

The bacteria grew over a wide range of salinity, i.e., 10-30‰ w/v NaCl, while none of the bacteria isolated at the highest salinity range (30 to 33%) grew at 15% salt concentration. Salinity-tolerant bacterial isolates have attracted the attention of agriculturists as soil inoculums to improve plant growth and yield in saline soils. The widespread use of halotolerant bacteria in these saline water bodies is of great interest for future research and biotechnological development.

Keywords: salt tolerance; salinity; halophilic bacteria; phenotypic; 16S rDNA

INTRODUCTION

Halophiles use different physiological, morphological and genetic mechanisms to deal with environmental conditions (Oren, 2008). A high-saline environment could cause proteins, including enzymes, to denature, leading to reduced enzyme activity and DNA damage (DasSarma and DasSarma, 2006), which could result in changes in bacterial genetic material. Halophilic microorganisms can be isolated from various sources, such as salted fish (Hezayen et al., 2010), brine wells (Xiang et al., 2008), salt lakes (Swan et al., 2010), salterns (Bardavid et al., 2007) and salt mines (Chen et al., 2007). Insertions and/or repeat elements as well as

specific protein-coding or structural genes, act as phylogenetic markers. Among these markers, rRNA molecules such as 5S, 16S, 23S and spacers can be used for phylogenetic analyses, but the 5S rRNA and 23S rRNA genes have restricted use. However, the 16S rRNA gene is the most commonly used marker (Mora and Amann, 2001). The first use 16S rRNA gene sequences for phylogenetic tree construction occurred in 1985 (Lane et al., 1985). 16S rRNA has become the most widely used reliable marker for the taxonomic classification and phylogenetic analysis of microorganisms (Tringe and Hugenholtz, 2008 and Yang et al., 2016)

The goal of this study was to identify the

bacterial unknowns isolated from salted fish by morphological and 16S rRNA genes.

MATERIALS AND METHODS

Sample collection

Eight samples of salted fish were obtained from Aswan Governorate, Upper Egypt. Approximately 10 grams of fish tissue was collected from each fish and placed in clean sterile glass bottles. The bottles were kept in an ice-chest box, transported to the laboratory and processed within 24 hrs. The salinity of the fish tissue was determined immediately in the laboratory using electrical conductivity (EC), and pH was measured by a EC and pH meter. All experiments were carried out in duplicate, with uninoculated tubes serving as control experiments.

Bacterial strains and growth conditions

Sixteen bacterial strains were isolated and grown on enriched nutrient agar (NA) medium, which contained the following components (g/l): beef extract, 3 sources of growth factors, peptone extract, 5.0 g of energy source, and 15 g/l agar. The medium solidified, and pH was adjusted to 7.2. The bacterial strains cultured on basal medium contained the following components (g/l): NaCl 225, MgSO₄ 7 H₂O 5 S, Mg⁺⁺ source, KCl 2.0 K⁺ source and yeast extract 1.0, which was used for bacterial growth on different substrates, as well as for testing enzymatic and biochemical activities and measuring the ability of the strains to grow on the saline media. The eight isolates grown on basal media were assigned the symbol "a" (1a-8a), while the other bacteria grown on NA were used as a control.

Morphological and physiological tests:

The colonies of the isolate halo bacteria were described on the NA and NA with NaCl media. Bacteria were studied after isolation by performing preliminary microscopic examinations of the following characteristics: form (the shape of the colony), margins, pigmentation, elevation and gram staining results. Pure cultures of randomly selected bacterial isolates were identified on the basis of their colonial morphology and cellular morphology characteristics according to the method of Cowan and Steel (1960). A series dilution was prepared with 1 g fish tissue in a glass tube and then cultivated in Petri dishes and incubated at 28 °C for 4 days. The bacterial colonies were diagnosed, purified in isolation medium several times and then transferred to a

refrigerator until their use in further experiments. Then, plates were incubated at 28°C for 48 h. Different distinct colonies were repeatedly sub-cultured every 48 h onto newly prepared NA plates until pure cultures were obtained. The bacterial cell growth response to sodium chloride was examined in solid nutrient and basal nutrient salt media. Then, deep glucose agar tests were conducted to determine the oxygen requirements of the microorganisms incubated at 40° C for 3 days.

Biochemical tests:

Biochemical tests were performed according to the method of Balleroni (1984) to determine the microorganisms' requirements for oxygen. Deep glucose agar tests were performed based on catalase and oxidase activities, nitrate reduction and starch hydrolysis. The oxidase activity tests were conducted according to Kovace (1956). Catalase activity was shown by adding drops of 3% hydrogen peroxidase to agar colonies; oxygen bubbles indicated a positive test, according to Facklam (1995). The hydrolyse starch (amylose and amylopectin) was determined following Bailey (1986) using basal salt agar medium supplemented with 1 soluble starch (% w/v).

DNA isolation and PCR conditions

The bacterial genome was purified according to Wilson (1987) from a 5 ml liquid culture of eight isolated bacteria. DNA was re-suspended in 100 µl of Tris-EDTA (TE) buffer (pH 8.0) and stored at -20°C.

A partial fragment of 16S rDNA (expected size 709 bp) was amplified and sequenced using universal primers published by Sauer et al., 2005: 16S rDNA forward 5'-GTGTAGCGGTGAAATGCG-3' and 16S rDNA reverse 5'-ACGGGCGGTGTGTACAA-3'. A 25 µl PCR mix was prepared as follows: 5 µl of 5X green buffer, 4 µL of MgCl₂ (25 mM), 0.25 µl of GoTaq DNA polymerase (5 U/µl) (GoTaq® Hot Start Polymerase, Promega), 1 µl of primers (100 µM), 0.5 µl of dNTPs (10 mM) and 50 ng DNA of tested bacterial isolate, and nuclease-free water to the final volume of 25 µl. The PCR steps were as follows: 94°C for 5 min, followed by 30 cycles of 94°C for 45 sec, 55°C for 45 sec for annealing and 72°C for 1 min for elongation. The amplification fragment with a length of 709 bp was visualized with GelRed Nucleic Acid Gel Stain (Biotium) after gel electrophoresis in a 1.5% agarose gel (Genetics).

Sequencing

The 16S rDNA PCR products were used for sequencing by the 3100 Genetic Analyser sequencer (Applied Biosystems). To find similar sequences, the 16S rDNA sequences of the isolates were compared with sequences available in the NCBI GenBank database by a BLAST search(<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Three partial sequences of 16S rDNA were obtained from <http://blast.ncbi.nlm.nih.gov/Blast.cgi>, and two of our sequences were used to construct the UPGMA phylogenetic tree. Phylogenetic analyses were conducted in MEGA4 (Tamura et al., 2007). Sequence alignments were carried out using the site <http://www.ebi.ac.uk/Tools/msa/clustalw2/>.

RESULTS

Table (1) shows that the salted fish samples contained 34.1% fresh weight and 71.22% dry weight organic matter contents. Total soluble salts (TSS) and total salts (TS) were relatively high (100.95 mg/g and 133.2, respectively). The pH value was approximately 7.0-8.5, and the samples were slightly alkaline. The contents of elements related to salinity were 32.5, 0.038, 1.5 and 0.25 mg/g for and Na, Ca, K, and Mg, respectively.

Table (1) Chemical characteristics of salted fish samples *

Parameter ^a	Average SD ^b
OM mg/g	339.1±8.2
MC%	53.1±1.2
pH	7-8.5
TS mg/g fresh weight	133.2±7.5
TSS mg/g fresh weight	100.95±5.5
Na	32.5±8.08
Ca	0.038±0.01
K	1.5±0.4
Mg	0.25±0.1

*OM: Organic matter, MC%: Moisture content percentage,

TS: Total salts. TSS: Total soluble salts. ^b Standard deviation. ^aAverage of 30 samples

The results showed that a number of bacterial colonies isolated from the salted fish were different (Table 2). The highest number of bacteria was 5×10^4 /gram fresh tissue, while the lowest number of bacteria was 1×10^2 gram fresh tissue. Morphological, physiological and biochemical tests diagnosed eight bacterial isolates. Morphologically and biologically, isolates 1, 2 and 8 were gram-negative; regarding shape, five isolates appeared rod-long and rod-shaped. The numbers 1, 2, 3, 4, 5, 6, 7 and 8 indicate bacterial

growth on NA. and 1a, 2a, 3a, 4a, 5a, 6a, 7a and 8a indicate bacterial growth on NB media with NaCl. The results of the biochemical tests for all bacteria strains were positive for catalase and starch. Some of the strains were positive for oxides and nitrate reduction, while these tests were negative for other strains. The physiological data show that five bacterial isolates were mandatory aerobic bacteria, and 3 bacterial isolates were facultative aerobic bacteria. The pH for all microbes was approximately 7.5-8.5.

To identify eight bacterial strains isolated from salted fish, we used morphological and molecular approaches. A partial sequence of 16S rDNA was utilized to characterize the strains at the molecular level. After isolation and 16S rDNA sequencing, the search programs Entrez and BLAST were used to find similar sequences published in the NCBI database. A maximum likelihood phylogenetic tree was constructed using our sequences and similar sequences from the NCBI database. The phylogenetic tree presents six clusters, as shown in Fig. (1). The first cluster contains isolates No. 1, 2 and 8, which present with some species belonging to the genus *Cronobacter* and are closely related to the species *C. condimenti*, *C. malonaticus*, *C. sakazakii* BQ16, *C. sakazakii* Jor1468, *C. dublinensis* and *C. muytjensii*. In the second cluster, isolate 5 is found with *Alcaligenes faecalis*, *Rhodobacter sphaeroides* and *Pusillimonas*_sp. Isolate 3 is in a third cluster that includes *Bacillus oceanisediminis*, *Bacillus firmus* and *Bacillus subtilis* HAU5. Moreover, isolate 4 is in a fourth cluster and is closely related to *Bacillus mojavensis*ZA1 and then to several species of *Bacillus*, such as *B. subtilis* D221, *B. tequilensis*, *B. mojavensis*, *B. thuringiensis*, *B. amyloliquefaciens*, *B. methylotrophicus*, *B. siamensis*, *B. vallismortis*, *B. cereus*, *B. sonorensis* and *B. axarquiensis*. The fifth cluster consists of isolate No. 7, which is closely related to *Bacillus pumilus* p51H01 and *B. safensis* SDS101. Isolate 6 is found alone in a unique cluster.

To prove the pedigree of our unknown isolates, alignments of partial 16Sr DNA sequences of these isolates were created using <http://www.ebi.ac.uk/Tools/msa/clustalo/>. The results are shown in Fig. (2).

Table (2): The numbers 1 to 8 indicate different bacterial samples, and the numbers 1a to 8a indicate bacteria grown on NB media with NaCl

Tests Bacteria	Characteristics	1 1a	2 2a	3 3a	4 4a	5 5a	6 6a	7 7a	8 8a
Morphological tests	G. Stain	G-	G-	G+	G+	G-	G+	G+	G-
	Margins	convex	wavy	wavy	wavy	m.entire	m.entire	convex	convex
	color	-	-	white	white	purple	-	white	-
	Elevation	Flat	Flat	umbonate	umbonate	Flat	Flat	umbonate	Flat
	shape	Rod-long	Rod-long	rods	Rods	Rod-long	Rod-long	Rods	Rod-long
Biological Tests	Catales	+	+	++	++	++	+	++	+
	Oxidase	-	-	--	--	+	+	-	-
	Nitrat redicase	+	+	-	-	-	-	-	+
	Starch hydrolysis	+	+	+	++	+	+	+	+
Physiological tests	O ₂ . depending	f. anaer	f. anaer	aerobic	aerobic	aerobic	aerobic	aerobic	f. anaer
	pH	7.5	8	7	7.5	8	8.5	7.5	8

Our isolates exhibited high similarities among each other (Table 3). Isolate 1 is 88.63% and 82.97% similar to isolate 2 and isolate 8, respectively. Furthermore, isolate 2 is 89.7% similar to isolate 8. Additionally, Fig. (2) shows the similarities between isolates 1, 2 and 8 and other species belonging to the genus *Cronobacter* from the NCBI database. The highest similarities are found between isolates 1, 2 and 8 and the species *C. sakazakii*, *C. malonaticus* and *C. condiment*, with percentages of 81.46%, 88.34% and 92.53, respectively.

Moreover, based on the similarity matrix Fig. (3), isolate 4 belongs to the genus *Bacillus* and is more than 96% similar to *B. subtilis*, *B. mojavensis*, *B. amyloliquefaciens*, *B. axarquiensis*, *B. sonorensis*, *B. cereus*, *B. vallismortis*, *B. siamensis*, *B. methylotrophicus*, *B.*

tequilensis and *B. thuringiensis*. The highest similarity is between isolate 4 and *B. subtilis*, with a percentage of 96.37%. The lowest similarity is 96.06% between isolate 4 and *B. amyloliquefaciens*. Isolate 3 shows 94.09%, 94.23% and 94.08% similarity to *B. oceanisediminis*, *B. firmus* and *B. subtilis*, respectively (Fig. (4)). In addition, as shown in Fig. (5), isolate 7 displays 76.82% and 76.77% similarity to *B. pumilus* and *B. safensis*, respectively. Furthermore, Isolate 5 shows similarity to *Pusillimonas*, *Rhodobacter sphaeroides* and *Alcaligenes faecalis*, with percentages of 91.35%, 91.35% and 91.49%, respectively (Fig. (6)). However, the isolate 6 sequences do not match any sequences in the NCBI database.

Table 3. 16S rRNA sequencing analysis results for isolates from salted fish.

Isolate number	No. of bp sequenced	Similarity with nearest type strain (%)	Tentative identification based on nearest neighbour
1	709	92.53 88.34 81.46	<i>Cronobacter condiment</i> LMG26250 <i>Cronobacter malonaticus</i> BR-1 <i>Cronobacter sakazakii</i> BQ16
2	709	92.53 88.34 81.46	<i>Cronobacter condiment</i> LMG26250 <i>Cronobacter malonaticus</i> BR-1 <i>Cronobacter sakazakii</i> BQ16
3	709	94.23 94.09 94.08	<i>Bacillus firmus</i> PX28 <i>Bacillus oceanisediminis</i> A3a <i>Bacillus subtilis</i> HAU5
4	709	96.37	<i>Bacillus subtilis</i> D221
5	709	91.49 91.35 91.35	<i>Alcaligenes faecalis</i> EGU38 <i>Pusillimonas</i> sp. <i>Rhodobacter sphaeroides</i> L.
6	709	--	--
7	709	76.82 76.77	<i>Bacillus pumilus</i> sp51H01 <i>Bacillus safensis</i> SDS101
8	709	92.53 88.34 81.46	<i>Cronobacter condiment</i> LMG26250 <i>Cronobacter malonaticus</i> BR-1 <i>Cronobacter sakazakii</i> BQ16

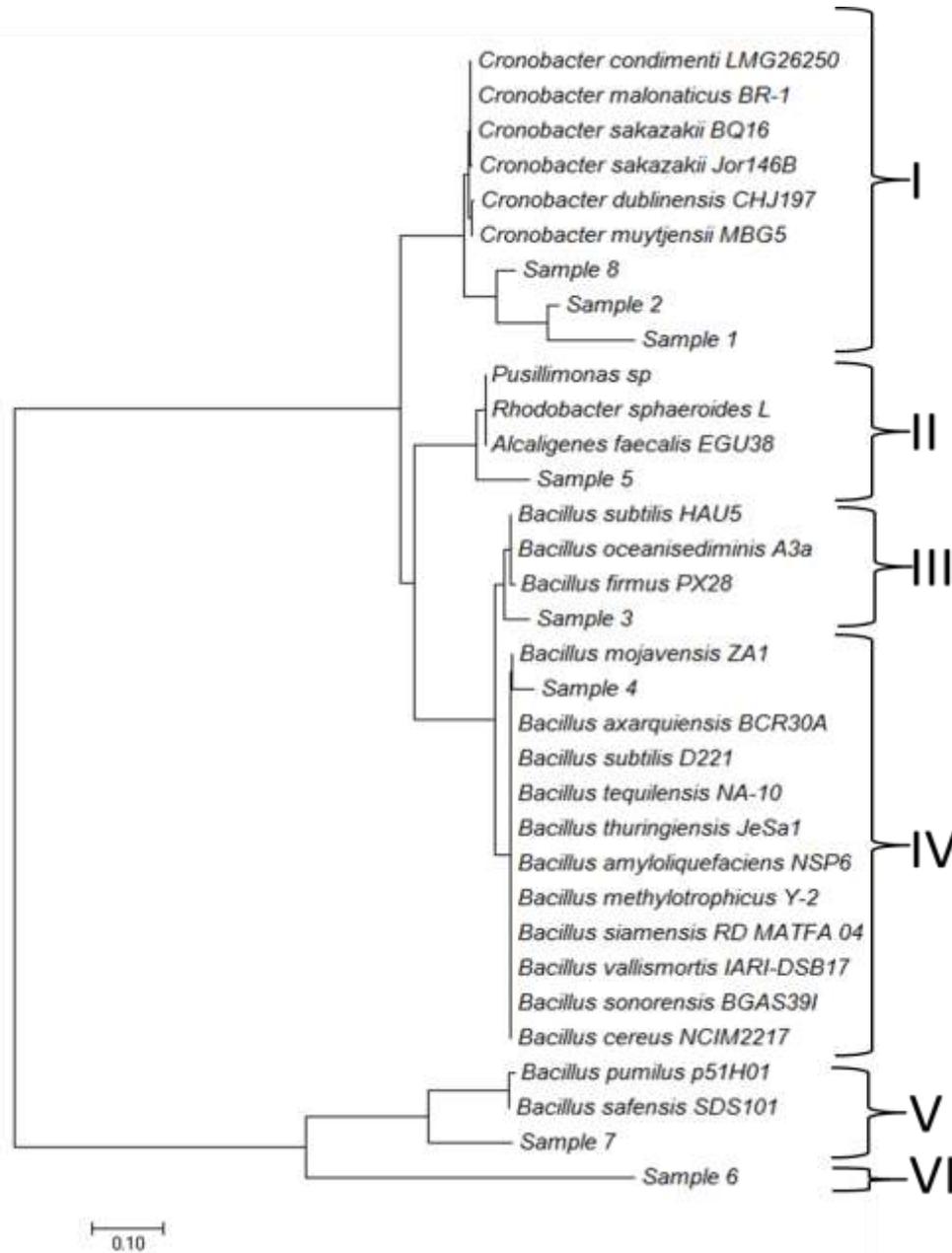


Figure 1: The maximum likelihood phylogenetic tree constructed using our eight isolated sequences and similar sequences collected from the NCBI database site.

```

Sample1      GGTGCTGCCTCGGTGTCCTCCCTCCTTTGTGAAAATTTTGGGTAAGTCCC GCCCACGAG
Sample2      GGTGCTGCCTCGGTGTCCTCCCTCCTTTGTGAAAATTTTGGGTAAGTCCC GCCCACGAG
Sample8      GGTGCTGCCTCGGTGTCCTCCCTCCTTTGTGAAAATTTTGGGTAAGTCCC GCCCACGAG
Cronobacter_sakazakii_BQ16  GGTGCTGCATGGCTGTCGTGTCAGCTCGTGTGTTGTGAAAATGTTGGGTTAAGTCCC GCCCACGAG
Enterobacter_sp._C5      GGTGCTGCATGGCTGTCGTGTCAGCTCGTGTGTTGTGAAAATGTTGGGTTAAGTCCC GCCCACGAG
Cronobacter_malonaticus_BR-1  GGTGCTGCATGGCTGTCGTGTCAGCTCGTGTGTTGTGAAAATGTTGGGTTAAGTCCC GCCCACGAG
Cronobacter_condimentii_LMG26250  GGTGCTGCATGGCTGTCGTGTCAGCTCGTGTGTTGTGAAAATGTTGGGTTAAGTCCC GCCCACGAG
Cronobacter_sakazakii_Jor146B  GGTGCTGCATGGCTGTCGTGTCAGCTCGTGTGTTGTGAAAATGTTGGGTTAAGTCCC GCCCACGAG
Cronobacter_muytjensii_MBG5  GGTGCTGCATGGCTGTCGTGTCAGCTCGTGTGTTGTGAAAATGTTGGGTTAAGTCCC GCCCACGAG
Cronobacter_dublinensis_CHJ197  GGTGCTGCATGGCTGTCGTGTCAGCTCGTGTGTTGTGAAAATGTTGGGTTAAGTCCC GCCCACGAG
*****

Sample1      GGGGACCCCTTAGATTTTATTGACTAAA-ATTCGGTCCGCAACTCTTAGGAGACTGCCGGT
Sample2      GGGGACCCCTTAGATTTTATTGACTAAA-ATTCGGTCCGCAACTCTTAGGAGACTGCCGGT
Sample8      GGGGACCCCTTAGATTTTATTGACTAAA-ATTCGGTCCGCAACTCTTAGGAGACTGCCGGT
Cronobacter_sakazakii_BQ16  CGCGATCTTTATGCTTTGTTGCCAATCG-ATTCGACGGGCAACTCATAGGACTGCCGGT
Enterobacter_sp._C5      CGCAACCCCTTATCCTTTGTTGCCAAGCG-GTTCGGCCGGGAACTCAAAGGAGACTGCCGGT
Cronobacter_malonaticus_BR-1  CGCAACCCCTTATCCTTTGTTGCCAAGCG-GTTCGGCCGGGAACTCAAAGGAGACTGCCGGT
Cronobacter_condimentii_LMG26250  CGCAACCCCTTATCCTTTGTTGCCAAGCG-GTTCGGCCGGGAACTCAAAGGAGACTGCCGGT
Cronobacter_sakazakii_Jor146B  CGCAACCCCTTATCCTTTGTTGCCAAGCG-GTTCGGCCGGGAACTCAAAGGAGACTGCCGGT
Cronobacter_muytjensii_MBG5  CGCAACCCCTTATCCTTTGTTGCCAAGCG-GTTCGGCCGGGAACTCAAAGGAGACTGCCGGT
Cronobacter_dublinensis_CHJ197  CGCAACCCCTTATCCTTTGTTGCCAAGCG-GTTCGGCCGGGAACTCAAAGGAGACTGCCGGT
*****

Sample1      GATAAACCGTAGGAAAGG-TGGCCATGACGTCAAATCATCATGGCCCTTACGACCAGGGCT
Sample2      GATAAACCGTAGGAAAGG-TGGCCATGACGTCAAATCATCATGGCCCTTACGACCAGGGCT
Sample8      GATAAACCGTAGGAAAGG-TGGCCATGACGTCAAATCATCATGGCCCTTACGACCAGGGCT
Cronobacter_sakazakii_BQ16  GATAAACCGTAGGAAAGG-TGGCCATGACGTCAAATCATCATGGCCCTTACGACCAGGGCT
Enterobacter_sp._C5      GATAAACCGTAGGAAAGG-TGGCCATGACGTCAAATCATCATGGCCCTTACGACCAGGGCT
Cronobacter_malonaticus_BR-1  GATAAACCGTAGGAAAGG-TGGCCATGACGTCAAATCATCATGGCCCTTACGACCAGGGCT
Cronobacter_condimentii_LMG26250  GATAAACCGTAGGAAAGG-TGGCCATGACGTCAAATCATCATGGCCCTTACGACCAGGGCT
Cronobacter_sakazakii_Jor146B  GATAAACCGTAGGAAAGG-TGGCCATGACGTCAAATCATCATGGCCCTTACGACCAGGGCT
Cronobacter_muytjensii_MBG5  GATAAACCGTAGGAAAGG-TGGCCATGACGTCAAATCATCATGGCCCTTACGACCAGGGCT
Cronobacter_dublinensis_CHJ197  GATAAACCGTAGGAAAGG-TGGCCATGACGTCAAATCATCATGGCCCTTACGACCAGGGCT
*****

Sample1      ACACACGTGCTACAATGGCGCATAACAAGAGAAAGCGACCTCGCGAGAGTAAACGGATCTC
Sample2      ACACACGTGCTACAATGGCGCATAACAAGAGAAAGCGACCTCGCGAGAGTAAACGGATCTC
Sample8      ACACACGTGCTACAATGGCGCATAACAAGAGAAAGCGACCTCGCGAGAGTAAACGGATCTC
Cronobacter_sakazakii_BQ16  ACACACGTGCTACAATGGCGCATAACAAGAGAAAGCGACCTCGCGAGAGTAAACGGATCTC
Enterobacter_sp._C5      ACACACGTGCTACAATGGCGCATAACAAGAGAAAGCGACCTCGCGAGAGTAAACGGATCTC
Cronobacter_malonaticus_BR-1  ACACACGTGCTACAATGGCGCATAACAAGAGAAAGCGACCTCGCGAGAGTAAACGGATCTC
Cronobacter_condimentii_LMG26250  ACACACGTGCTACAATGGCGCATAACAAGAGAAAGCGACCTCGCGAGAGTAAACGGATCTC
Cronobacter_sakazakii_Jor146B  ACACACGTGCTACAATGGCGCATAACAAGAGAAAGCGACCTCGCGAGAGTAAACGGATCTC
Cronobacter_muytjensii_MBG5  ACACACGTGCTACAATGGCGCATAACAAGAGAAAGCGACCTCGCGAGAGTAAACGGATCTC
Cronobacter_dublinensis_CHJ197  ACACACGTGCTACAATGGCGCATAACAAGAGAAAGCGACCTCGCGAGAGTAAACGGATCTC
*****

Sample1      ATAAAGAGGGTCTCAGACCGGATTGGAGTCTGCAACTTTACTCCCTGAAGTGGAAATCGC
Sample2      ATAAAGAGGGTCTCAGACCGGATTGGAGTCTGCAACTTTACTCCCTGAAGTGGAAATCGC
Sample8      ATAAAGAGGGTCTCAGACCGGATTGGAGTCTGCAACTTTACTCCCTGAAGTGGAAATCGC
Cronobacter_sakazakii_BQ16  ATAAAGTGCCTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTGGAAATCGC
Enterobacter_sp._C5      ATAAAGTGCCTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTGGAAATCGC
Cronobacter_malonaticus_BR-1  ATAAAGTGCCTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTGGAAATCGC
Cronobacter_condimentii_LMG26250  ATAAAGTGCCTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTGGAAATCGC
Cronobacter_sakazakii_Jor146B  ATAAAGTGCCTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTGGAAATCGC
Cronobacter_muytjensii_MBG5  ATAAAGTGCCTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTGGAAATCGC
Cronobacter_dublinensis_CHJ197  ATAAAGTGCCTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTGGAAATCGC
*****

Sample1      TAGTAATCGCGGATCATAATGCCCGG-TGCACACCCTTCCCGGGCCTTGT-----
Sample2      TAGTAATCGTGGATCATAATGCCCGG-TGAATA-CCTTCCCTGGCCCTTGT-----
Sample8      TAGTAATCGTGGATCATAATGCCCGG-TGAATA-CCTTACC6GGGCGTGT-----
Cronobacter_sakazakii_BQ16  TAGTAATCGTGGATCAGAATGCCACGG-TGAATA-CGTTCCCGGGCCTTGT-----
Enterobacter_sp._C5      TAGTAATCGTGGATCAGAATGCCACGG-TGAATA-CGTTCCCGGGCCTTGT-----
Cronobacter_malonaticus_BR-1  TAGTAATCGTGGATCAGAATGCCACGG-TGAATA-CGTTCCCGGGCCTTGTACACACCCC
Cronobacter_condimentii_LMG26250  TAGTAATCGTGGATCAGAATGCCACGG-TGAATA-CGTTCCCGGGCCTTGT-----
Cronobacter_sakazakii_Jor146B  TAGTAATCGTGGATCAGAATGCCACGG-TGAATA-CGTTCCCGGGCCTTGT-----
Cronobacter_muytjensii_MBG5  TAGTAATCGTGGATCAGAATGCCACGG-TGAATA-CGTTCCCGGGCCTTGT-----
Cronobacter_dublinensis_CHJ197  TAGTAATCGTGGATCAGAATGCCACGG-TGAATA-CGTTCCCGGGCCTTGT-----
*****
    
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Figure 2: Partial sequence alignment for the PCR products of the 16S rRNA genes of three bacterial samples isolated from salted fish (isolate 1, isolate 2 and isolate 8) and other high-similarity sequences published in the NCBI-GenBank database.

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F_Sample4
Bacillus_subtilis_D221
Bacillus_tequilensis_NA-10
Bacillus_thuringiensis_JeSa1
Bacillus_mojavensis_ZA1
Bacillus_amyloliquefaciens_NSP6
Bacillus_methylotrophicus_Y-2
Bacillus_siamensis_RD_MATFA_04
Bacillus_vallismortis_IARI-DSB17
Bacillus_cereus_NCIM2217
Bacillus_sonorensis_BGAS39I
Bacillus_axarquiensis_BCR30A

GCACGTCCTCCGCGGGCAGAGTGACAGGTGGCGCATGGTTGTCGTGACGTCGTGTCGT
GGACGTCCTCCGCGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTGACGTCGTGTCGT
GGACGTCCTCCGCGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTGACGTCGTGTCGT
GGACGTCCTCCGCGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTGACGTCGTGTCGT
GGACGTCCTCCGCGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTGACGTCGTGTCGT
GGACGTCCTCCGCGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTGACGTCGTGTCGT
GGACGTCCTCCGCGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTGACGTCGTGTCGT
GGACGTCCTCCGCGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTGACGTCGTGTCGT
GGACGTCCTCCGCGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTGACGTCGTGTCGT
GGACGTCCTCCGCGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTGACGTCGTGTCGT
*****

F_Sample4
Bacillus_subtilis_D221
Bacillus_tequilensis_NA-10
Bacillus_thuringiensis_JeSa1
Bacillus_mojavensis_ZA1
Bacillus_amyloliquefaciens_NSP6
Bacillus_methylotrophicus_Y-2
Bacillus_siamensis_RD_MATFA_04
Bacillus_vallismortis_IARI-DSB17
Bacillus_cereus_NCIM2217
Bacillus_sonorensis_BGAS39I
Bacillus_axarquiensis_BCR30A

GAGATGTTGGGTTAAGTCCCAGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTGAG
*****

F_Sample4
Bacillus_subtilis_D221
Bacillus_tequilensis_NA-10
Bacillus_thuringiensis_JeSa1
Bacillus_mojavensis_ZA1
Bacillus_amyloliquefaciens_NSP6
Bacillus_methylotrophicus_Y-2
Bacillus_siamensis_RD_MATFA_04
Bacillus_vallismortis_IARI-DSB17
Bacillus_cereus_NCIM2217
Bacillus_sonorensis_BGAS39I
Bacillus_axarquiensis_BCR30A

TTGGGCACCTAAGGCGACTGCCGGTGACAAACCGAGGAAGGTGTCTATCAGCTCAAA
TTGGGCACCTAAGGCGACTGCCGGTGACAAACCGAGGAAGGTGGGGATGACGTCAAAT
TTGGGCACCTAAGGCGACTGCCGGTGACAAACCGAGGAAGGTGGGGATGACGTCAAAT
TTGGGCACCTAAGGCGACTGCCGGTGACAAACCGAGGAAGGTGGGGATGACGTCAAAT
TTGGGCACCTAAGGCGACTGCCGGTGACAAACCGAGGAAGGTGGGGATGACGTCAAAT
TTGGGCACCTAAGGCGACTGCCGGTGACAAACCGAGGAAGGTGGGGATGACGTCAAAT
TTGGGCACCTAAGGCGACTGCCGGTGACAAACCGAGGAAGGTGGGGATGACGTCAAAT
TTGGGCACCTAAGGCGACTGCCGGTGACAAACCGAGGAAGGTGGGGATGACGTCAAAT
TTGGGCACCTAAGGCGACTGCCGGTGACAAACCGAGGAAGGTGGGGATGACGTCAAAT
TTGGGCACCTAAGGCGACTGCCGGTGACAAACCGAGGAAGGTGGGGATGACGTCAAAT
*****

F_Sample4
Bacillus_subtilis_D221
Bacillus_tequilensis_NA-10
Bacillus_thuringiensis_JeSa1
Bacillus_mojavensis_ZA1
Bacillus_amyloliquefaciens_NSP6
Bacillus_methylotrophicus_Y-2
Bacillus_siamensis_RD_MATFA_04
Bacillus_vallismortis_IARI-DSB17
Bacillus_cereus_NCIM2217
Bacillus_sonorensis_BGAS39I
Bacillus_axarquiensis_BCR30A

CATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGC
CATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGC
CATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGC
CATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGC
CATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGC
CATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGC
CATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGC
CATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGC
CATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGC
*****

F_Sample4
Bacillus_subtilis_D221
Bacillus_tequilensis_NA-10
Bacillus_thuringiensis_JeSa1
Bacillus_mojavensis_ZA1
Bacillus_amyloliquefaciens_NSP6
Bacillus_methylotrophicus_Y-2
Bacillus_siamensis_RD_MATFA_04
Bacillus_vallismortis_IARI-DSB17
Bacillus_cereus_NCIM2217
Bacillus_sonorensis_BGAS39I
Bacillus_axarquiensis_BCR30A

TAAACCGGAGGTTAAGCCAATCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAA
GAAACCGGAGGTTAAGCCAATCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAA
GAAACCGGAGGTTAAGCCAATCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAA
GAAACCGGAGGTTAAGCCAATCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAA
GAAACCGGAGGTTAAGCCAATCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAA
GAAACCGGAGGTTAAGCCAATCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAA
GAAACCGGAGGTTAAGCCAATCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAA
GAAACCGGAGGTTAAGCCAATCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAA
GAAACCGGAGGTTAAGCCAATCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAA
GAAACCGGAGGTTAAGCCAATCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAA
*****
    
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F_Sample3          GTGCAGGGACACCCGTGGCGAAGGGGACTCTTTGGTCTGTAACCTACGCTTAGGCGCGAA
Bacillus_firmus_PX28  GTGGAGGAACACAGTGGCGAAGGCGACTCTTTGGTCTGTAACCTGACGCTGAGGCGCGAA
Bacillus_oceanisediminis_A3a  GTGGAGGAACACAGTGGCGAAGGCGACTCTTTGGTCTGTAACCTGACGCTGAGGCGCGAA
Bacillus_subtilis_HAU5  GTGGAGGAACACAGTGGCGAAGGCGACTCTTTGGTCTGTAACCTGACGCTGAGGCGCGAA
*** **

F_Sample3          AAATT-GTGGAGCACACAGATTGATACCCTGGTTTTGCACGCCGTAAGATGAGTGT
Bacillus_firmus_PX28  AGCGTGGGGAGCAAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGAGTGC
Bacillus_oceanisediminis_A3a  AGCGT-GGGGAGCAAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGAGTGC
Bacillus_subtilis_HAU5  AGCGT-GGGGAGCAAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGAGTGC
*  * *

F_Sample3          TAAGTGTACAGGGTTTCCGCCCTTAGTGCTGCAGCTAACGCATTTAGCACTCCGCTG
Bacillus_firmus_PX28  TAAGTGTTAGAGGGTTTCCGCCCTTAGTGCTGCAGCAAAACGCATTAAGCACTCCGCTG
Bacillus_oceanisediminis_A3a  TAAGTGTTAGAGGGTTTCCGCCCTTAGTGCTGCAGCAAAACGCATTAAGCACTCCGCTG
Bacillus_subtilis_HAU5  TAAGTGTTAGAGGGTTTCCGCCCTTAGTGCTGCAGCAAAACGCATTAAGCACTCCGCTG
*****

F_Sample3          TGGAGTATAGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGGCGCACAAAGCGTGG
Bacillus_firmus_PX28  GGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGGCCGACAAGCGTGG
Bacillus_oceanisediminis_A3a  GGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGGCCGACAAGCGTGG
Bacillus_subtilis_HAU5  GGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGGCCGACAAGCGTGG
*****

F_Sample3          AGCATGTGGTTAATTTGAAGCAACGCGAAGAGCCTTACCAGGTCTTGACATATCCTGAC
Bacillus_firmus_PX28  AGCATGTGGTTAATTTGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATATCCTGAC
Bacillus_oceanisediminis_A3a  AGCATGTGGTTAATTTGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATATCCTGAC
Bacillus_subtilis_HAU5  AGCATGTGGTTAATTTGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATATCCTGAC
*****

F_Sample3          AACCTAGAGATAGGGGTTTCCCTTCGGGGACAGGATGACAGGTGGTGCATGGTTGTC
Bacillus_firmus_PX28  AACCTAGAGATAGGGGTTTCCCTTCGGGGACAGGATGACAGGTGGTGCATGGTTGTC
Bacillus_oceanisediminis_A3a  AACCTAGAGATAGGGGTTTCCCTTCGGGGACAGGATGACAGGTGGTGCATGGTTGTC
Bacillus_subtilis_HAU5  AACCTAGAGATAGGGGTTTCCCTTCGGGGACAGGATGACAGGTGGTGCATGGTTGTC
*****

F_Sample3          GTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCAGCAACGAGCGCAACCTTGATTTT
Bacillus_firmus_PX28  GTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCAGCAACGAGCGCAACCTTGATTTT
Bacillus_oceanisediminis_A3a  GTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCAGCAACGAGCGCAACCTTGATTTT
Bacillus_subtilis_HAU5  GTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCAGCAACGAGCGCAACCTTGATTTT
*****

F_Sample3          GTTGCCAGCATTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGAGGAAGGTG
Bacillus_firmus_PX28  GTTGCCAGCATTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGAGGAAGGTG
Bacillus_oceanisediminis_A3a  GTTGCCAGCATTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGAGGAAGGTG
Bacillus_subtilis_HAU5  GTTGCCAGCATTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGAGGAAGGTG
*****

F_Sample3          GGGATGACGTCAAATCATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGAT
Bacillus_firmus_PX28  GGGATGACGTCAAATCATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGAT
Bacillus_oceanisediminis_A3a  GGGATGACGTCAAATCATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGAT
Bacillus_subtilis_HAU5  GGGATGACGTCAAATCATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGAT
*****

F_Sample3          GGAACAAAGGGCTGCGAGACCGGAGGTTAAGCGAATCCCATAAAACCTTCTCAGTTCCG
Bacillus_firmus_PX28  GGTACAAGGGCTGCGAGACCGGAGGTTAAGCGAATCCCATAAAACCTTCTCAGTTCCG
Bacillus_oceanisediminis_A3a  GGTACAAGGGCTGCGAGACCGGAGGTTAAGCGAATCCCATAAAACCTTCTCAGTTCCG
Bacillus_subtilis_HAU5  GGTACAAGGGCTGCGAGACCGGAGGTTAAGCGAATCCCATAAAACCTTCTCAGTTCCG
**

F_Sample3          GATTCGCAGTCTGCAACTCGCCTGCATGAACGCCGGAATCGTAGTAATCGCTGATCCAG
Bacillus_firmus_PX28  GATT-GCAGGCTGCAACTCGCCTGCATGAA-GCCGGAATCGTAGTAATCGCGGAT-CAG
Bacillus_oceanisediminis_A3a  GATT-GCAGGCTGCAACTCGCCTGCATGAA-GCCGGAATCGTAGTAATCGCGGATCCAG
Bacillus_subtilis_HAU5  GATT-GCAGGCTGCAACTCGCCTGCATGAA-GCCGGAATCGTAGTAATCGCGGAT-CAG
****
    
```

Figure 4 : Partial sequence alignment for the PCR products of the 16S rRNA genes of a bacterial sample isolated from salted fish (isolate 3) and high-similarity *B. oceanisediminis*, *B. firmus* and *B. subtilis* sequences published in the NCBI-GenBank database.

```

R_Sample7          TTGGCAACACGCACCGCACAAATAA-CCC GC-ATTTCTAGCCGATCCGACTTCCGCAAGCC
Bacillus_safensis_SDS101 AACGTATTCACCGCGGCATGCTGA-TCCGCGATTACTAGCGATTCCAGTTCACGCAGTC
Bacillus_pumilus_p51H01 -----CGGCATGCTGATCCCGCATTACTAGCGATCCACCTTCACGCAGTC
                    *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

R_Sample7          AAATTGCGGACTGCAAACCGGACTGAG-ACAGATTTATGCCATTGGCTAAACCATTGCGG
Bacillus_safensis_SDS101 GAGTTGCAGACTGCGATCCGAACGAGAACAGATTTATGGGATTGGCTAAACC-TTGCGG
Bacillus_pumilus_p51H01 GAGTTGCAGACTGCGATCCGAACGAGAACAGATTTATGGGATTGGCTAAACC-TTGCGG
                    *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

R_Sample7          -CTTGC-GCCCTTTGTTCTCTCCATTGTAGGCCGCGTGTAGCCCATACATAAGGGCGCA
Bacillus_safensis_SDS101 TCTTGCAGCCCTTTGTTCTGTCCATTGTAGCACGTGTG-TAGCCAGTTCATAAGGGGCA
Bacillus_pumilus_p51H01 TCTTGCAGCCCTTTGTTCTGTCCATTGTAGCACGTGTG-TAGCCAGTTCATAAGGGGCA
                    *****

R_Sample7          TGATGATTTGACGCCATCCCCACCTTCTCCGGTTTGTACCAGGACACCTAAGAGTG
Bacillus_safensis_SDS101 TGATGATTTGACGTCATCCCCACCTTCTCCGGTTTGTACCAGGACACCTAAGAGTG
Bacillus_pumilus_p51H01 TGATGATTTGACGTCATCCCCACCTTCTCCGGTTTGTACCAGGACACCTAAGAGTG
                    *****

R_Sample7          CCCAATTGAATGCTGGCAACAAGACCAAGGGTTGCGCTCGTGGCGGGACTTAACCCAAC
Bacillus_safensis_SDS101 CCCAATGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTGGCGGGACTTAACCCAAC
Bacillus_pumilus_p51H01 CCCAATGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTGGCGGGACTTAACCCAAC
                    *****

R_Sample7          ATCTCAGCACGAGCTGACTCCAACCATGCACCACCTGTCACTCGTCCCCGAGGTAA
Bacillus_safensis_SDS101 ATCTCAGCACGAGCTGACGACAACCATGCACCACCTGTCACTCTGTCCCCGAGGGAA
Bacillus_pumilus_p51H01 ATCTCAGCACGAGCTGACGACAACCATGCACCACCTGTCACTCTGTCCCCGAGGGAA
                    *****

R_Sample7          ACCCCTATCTCTAGGGAAGTTAGAGTTGTCAAGCCCTGGG-AGGGTTCCTTGGTTGT
Bacillus_safensis_SDS101 AGCCCTATCTCTAGGGTTGTCAAGGATGTCAAGACCTGGTAAGGGTTCCTCGC-GTTGC
Bacillus_pumilus_p51H01 AGCCCTATCTCTAGGGTTGTCAAGGATGTCAAGACCTGGT-AAGGTTCTTCGA-GTTGC
                    *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

R_Sample7          TTCGAATTAACCACGTGTTCCCCCCTTTG-GCGACCCCCGGTAAATAACTGTGGGTT
Bacillus_safensis_SDS101 TTCGAATTAACCACATGCTCCCCCGCTTGTGCGGGCCCC-GTCAATTCCTTTGAGTT
Bacillus_pumilus_p51H01 TTCGAATTAACCACATGCT-CCACCGCTTGTGCGGGCCCC-GTCAATTCCTTTGAGTT
                    *****

R_Sample7          TTTGTCTTGAGACCTAAATCCCCAAGGCGCATTATTTAATGGGTTA-CACCGGCCCAA
Bacillus_safensis_SDS101 TCAGT-CTTGCAGCGTACTCCCC-AGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTAA
Bacillus_pumilus_p51H01 TCAGT-CTTGCAGCGTACTCCCC-AGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTAA
                    *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

R_Sample7          AGGGGCGGAAAACCCGGTCTGGCTTATCCCCAGTGTTTTCCGAATTCGACAAATTGGTT
Bacillus_safensis_SDS101 --GGGGCGGAAAACCCCTAACACTTAGCACTCATCGTTTACGGCGT--GGACTACAGGG
Bacillus_pumilus_p51H01 --GGGGCGGAAAACCCCTAACACTTAGCACTCATCGTTTACGGCGT--GGACTACAGGG
                    *****

R_Sample7          TTTCTAATTTGGTGGGGCCCCCAA---
Bacillus_safensis_SDS101 TATCTAATCCTGTTGCTCCCCACGCTT
Bacillus_pumilus_p51H01 TATCTAATCCTGTTGCTCCCCACGCTT
                    *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
    
```

Figure 5: Partial sequence alignment for the PCR products of the 16S rRNA genes of a bacterial sample isolated from salted fish (isolate 7) and high-similarity *B. pumilus* and *B. safensis* sequences published in the NCBI-GenBank database

```

F_Sample5          CCCCTGCGACACTAATACTGACGCTCAG-CACGAAAGCGTGGGGAGCCAACAGGATTAGA
Rhodobacter_sphaeroides_L CCCCTGGGA---TAATACTGACGCTCAGACACGAAAGCGTGGGGAGCAAACAGGATTAGA
Pusillimonas_sp    CCCCTGGGA---TAATACTGACGCTCAGACACGAAAGCGTGGGGAGCAAACAGGATTAGA
Alcaligenes_faecalis_EGU38 -CCCTGGGA---TAATACTGACGCTCAGACACGAAAGCGTGGGGAGCAAACAGGATTAGA
*****

F_Sample5          TACCCTGGTAGTCCACGCCCTAAACGATGTCAACTAGCTGTTGGGGCCGTTCCGCCCTTT
Rhodobacter_sphaeroides_L TACCCTGGTAGTCCACGCCCTAAACGATGTCAACTAGCTGTTGGGGCCGTT-AGGCCTTA
Pusillimonas_sp    TACCCTGGTAGTCCACGCCCTAAACGATGTCAACTAGCTGTTGGGGCCGTT-AGGCCTTA
Alcaligenes_faecalis_EGU38 TACCCTGGTAGTCCACGCCCTAAACGATGTCAACTAGCTGTTGGGGCCGTT-AGGCCTTA
*****

F_Sample5          GTAGCGCCGCTAACGCCCAAAGTTGACCGCCCGGGGAGGACGGTCGCCAGATTA AAAACTC
Rhodobacter_sphaeroides_L GTAGCGCAGCTAACCGGTGAAGTTGACCGCCTGGGGAGTACGGTCGCAAGATTA AAAACTC
Pusillimonas_sp    GTAGCGCAGCTAACCGGTGAAGTTGACCGCCTGGGGAGTACGGTCGCAAGATTA AAAACTC
Alcaligenes_faecalis_EGU38 GTAGCGCAGCTAACCGGTGAAGTTGACCGCCTGGGGAGTACGGTCGCAAGATTA AAAACTC
*****

F_Sample5          CCAAGAATTTGCCGGGACCCGCCCCAGCGGAGGATGATGTGGTATTAATTTGATACCAG
Rhodobacter_sphaeroides_L AAAGGAATTGACGGGGACCCGCACAAGCGGTGGATGATGTGG-ATTAATTCGATGCAACG
Pusillimonas_sp    AAAGGAATTGACGGGGACCCGCACAAGCGGTGGATGATGTGG-ATTAATTCGATGCAACG
Alcaligenes_faecalis_EGU38 AAAGGAATTGACGGGGACCCGCACAAGCGGTGGATGATGTGG-ATTAATTCGATGCAACG
* *****

F_Sample5          CGAAAAACCTTACCCACCCTTGACATCTCTAAAAACCCAAAGAAATTTGGCCCTGCCCTC
Rhodobacter_sphaeroides_L CGAAAAACCTTACCTACCCTTGACATGCTCGAAAGCCGAAGAGATTTGGCCGTGCTCGC
Pusillimonas_sp    CGAAAAACCTTACCTACCCTTGACATGCTCGAAAGCCGAAGAGATTTGGCCGTGCTCGC
Alcaligenes_faecalis_EGU38 CGAAAAACCTTACCTACCCTTGACATGCTCGAAAGCCGAAGAGATTTGGCCGTGCTCGC
*****

F_Sample5          AAGAAAACCGGAACCCAGGTGCTGCATGGCTGTCGCCAGCTCGTGTCTGGGAGATGTTGGG
Rhodobacter_sphaeroides_L AAGAGAACCGGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCTGGGAGATGTTGGG
Pusillimonas_sp    AAGAGAACCGGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCTGGGAGATGTTGGG
Alcaligenes_faecalis_EGU38 AAGAGAACCGGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCTGGGAGATGTTGGG
****

F_Sample5          TTAAGTCCCGAACGAGCGCAACCCTTGTATTAGTTGCTACGAAAGAGCACTCTAATGA
Rhodobacter_sphaeroides_L TTAAGTCCCGAACGAGCGCAACCCTTGTATTAGTTGCTACGAAAGAGCACTCTAATGA
Pusillimonas_sp    TTAAGTCCCGAACGAGCGCAACCCTTGTATTAGTTGCTACGAAAGAGCACTCTAATGA
Alcaligenes_faecalis_EGU38 TTAAGTCCCGAACGAGCGCAACCCTTGTATTAGTTGCTACGAAAGAGCACTCTAATGA
*****

F_Sample5          TACTGCCGGTGACAAACCGAATCAAGGTGGAGTTGACGTCAAGTCCCTCATGGCCCTTATG
Rhodobacter_sphaeroides_L GACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCCTCATGGCCCTTATG
Pusillimonas_sp    GACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCCTCATGGCCCTTATG
Alcaligenes_faecalis_EGU38 GACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCCTCATGGCCCTTATG
*****

F_Sample5          GTTAGGGCTTACACGTATACAATGGTCGGGACAGAGGGTCGCCAACCCGCAAGGGGGA
Rhodobacter_sphaeroides_L GGTAGGGCTTACACGTATACAATGGTCGGGACAGAGGGTCGCCAACCCGCGAGGGGGA
Pusillimonas_sp    GGTAGGGCTTACACGTATACAATGGTCGGGACAGAGGGTCGCCAACCCGCGAGGGGGA
Alcaligenes_faecalis_EGU38 GGTAGGGCTTACACGTATACAATGGTCGGGACAGAGGGTCGCCAACCCGCGAGGGGGA
*

F_Sample5          GCCAATCTCAGAAACCCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGT
Rhodobacter_sphaeroides_L GCCAATCTCAGAAACCCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGT
Pusillimonas_sp    GCCAATCTCAGAAACCCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGT
Alcaligenes_faecalis_EGU38 GCCAATCTCAGAAACCCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGT
*****

F_Sample5          CGGAATCGCTAGTAATCGCGGATCAGAATGTCGCGGTGAATACGTTCCCGGTTCTGTGAC
Rhodobacter_sphaeroides_L CGGAATCGCTAGTAATCGCGGATCAGAATGTCGCGGTGAATACGTTCCCGGTTCTGTGAC
Pusillimonas_sp    CGGAATCGCTAGTAATCGCGGATCAGAATGTCGCGGTGAATACGTTCCCGGTTCTGTGAC
Alcaligenes_faecalis_EGU38 CGGAATCGCTAGTAATCGCGGATCAGAATGTCGCGGTGAATACGTTCCCGGTTCTGTGAC
*****
    
```

Figure 6: Partial sequence alignment for the PCR products of the 16S rRNA genes of a bacterial sample isolated from salted fish (isolate 5) and high-similarity *Pusillimonas*, *Rhodobacter sphaeroides* and *Alcaligenes faecalis* sequences published in the NCBI-GenBank database.

DISCUSSION

Much attention has been given to halophilic bacteria, especially moderately halophilic bacteria. These bacteria have been isolated from salted fish, brine wells, salt lakes, salterns and salt mines (Bardavidet *et al.* 2007, Chen *et al.* 2007, Xiang *et al.* 2008, Swan *et al.* 2010 and Hezayenet *et al.*, 2010). Several studies have been carried out on the biotechnological applications of halophilic bacteria, including the production of bioactive compounds (antibiotics), and studies have also investigated their phylogenetic characteristics (Vreeland 1992, Ventosa *et al.*, 1998 and Vahed *et al.*, 2011). Based on morphological and biochemical tests and 16S rRNA gene sequencing, we identified eight bacterial strains isolated from salted fish. The chemical analysis of the salted fish samples showed that the fresh weight, dry weight organic matter content, TSS, TS, elements related to salinity (Na, Ca and Mg) and pH results are in agreement with Youssef *et al.* (2003). The growth curve data showed that the bacterial strains isolated from saline media had a similar acceleration action when grown on NA media and nutrient broth media with NaCl, and NaCl had no effect on the velocity of phase, which is consistent with Omotoyinbo (2016).

Halophiles grow optimally at different salinity concentrations, which can be divided into three classes: low salinities of 20-50 ppt, moderate salinities of 50-200 ppt and high salinities > 200 pptNaCl (DasSarma and DasSarma, 2006). Salinity-tolerant bacterial isolates have attracted the attention of agriculturists as soil inoculums to improve plant growth and yield in saline soils. The widespread use of halotolerant bacteria is of great interest for future research and biotechnological development (DasSarma and DasSarma, 2006).

Phylogenetic analysis based on 16S rDNA sequencing indicated that three isolates (No. 1, 2 and 8) were members of the genera *Cronobacter* and closely related to the species *C. condimentii*, *C. malonaticus*, *C. sakazakii* BQ16, *C. sakazakii* Jor1468, *C. dublinensis* and *C. muytjensii*. Isolate No. 3 was most closely related to *Bacillus oceanisediminis*A3a, *Bacillus firmus* PX28 and *Bacillus subtilis* HAU5. Furthermore, isolate No. 4 was closely related to *Bacillus mojavensis* ZA1 and then to several species of *Bacillus*. Isolate No. 7 was closely related to *Bacillus pumilus*sp51H01 and *Bacillus safensis* SDS101. A previous study reported that gram-positive bacteria assigned to *Bacillus* were extensively represented in saline soils (Ventosa *et al.*, 2008).

Most of these bacteria were classified as halotolerant microorganisms, which are able to grow, in most cases, in NaCl concentrations up to 25% (Kushner, 1985)

Isolate No. 5 was clustered with three different genera: *Alcaligenes faecalis*, *Rhodobacter sphaeroides* and *Pusillimonas*_sp. Isolate No. 6 was separated alone in the last cluster. Further studies are required to identify these isolates, which are potential type strains for novel species.

CONCLUSION

The results of this work showed that some Salinity-tolerant bacterial isolates have been isolated and identified by morphological, biochemical and molecular properties. Also, this study suggested that Salinity-tolerant bacterial isolates will be used to have attracted the attention of agriculturists as bacterial fertilizers can improve plant growth and yield in saline soils.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

This work was carried out in collaboration between all authors. Authors MA-E, RK and KAA performed the conception of the manuscript, designed the study. MA-E and RK designed and performed the experiments and also wrote the manuscript .Authors MA-E, RK and KAA performed the data analysis and interpretation of the manuscript. Authors MA-E and RK complete the drafting of the manuscript. Authors MA-E, KAA and RK completed critical revision of the manuscript for important intellectual content. All authors read and approved the final version.

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