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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. condimenti, 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; phynotypic; 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 un-inoculated 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 hallo 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 size709 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 $MgCl_2$ (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. [a]Average 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 $5x10^4$/gram fresh tissue, while the lowest number of bacteria was $1x10^2$ 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
Mophological 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. condimenti,* 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	***Cronobacter condiment* LMG26250** ***Cronobactermalonaticus*BR-1** ***Cronobactersakazakii*BQ16**
2	709	92.53 88.34 81.46	***Cronobacter condiment* LMG26250** ***Cronobactermalonaticus*BR-1** ***Cronobactersakazakii*BQ16**
3	709	94.23 94.09 94.08	***Bacillus firmu*PX28** ***Bacillus oceanisediminis*A3a** ***Bacillus subtilis* HAU5**
4	709	96.37	***Bacillus subtilis* D221**
5	709	91.49 91.35 91.35	***Alcaligenesfaecalis* EGU38** ***Pusillimonas*sp.** ***Rhodobactersphaeroides* L.**
6	709	--	**--**
7	709	76.82 76.77	***Bacillus pumilus*p51H01** ***Bacillus safensis*SDS101**
8	709	92.53 88.34 81.46	***Cronobacter condiment* LMG26250** ***Cronobactermalonaticus*BR-1** ***Cronobactersakazakii*BQ16**

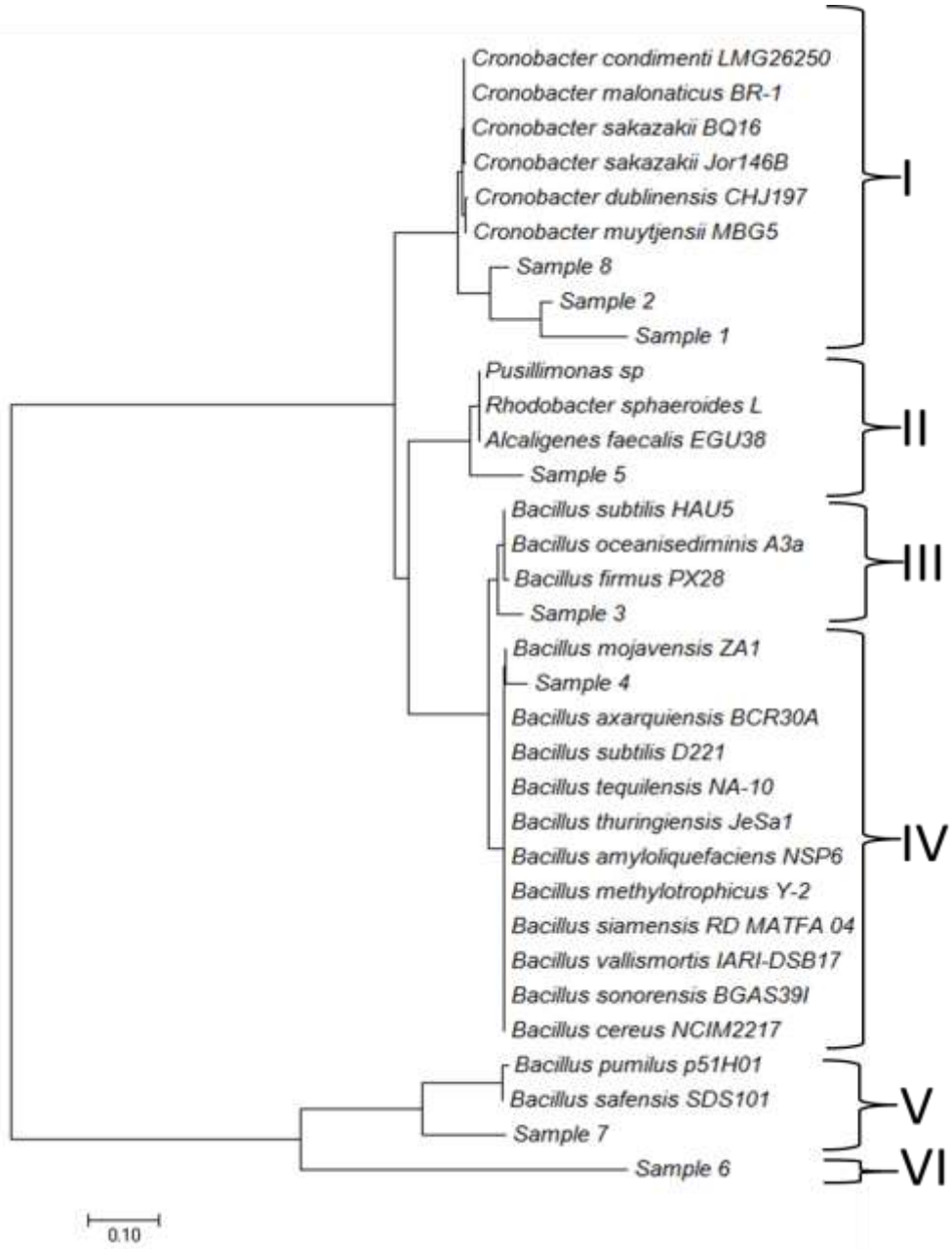


Figure 1: The maximum likelihood phylogenetic tree constructed using our eight isolated sequences and similar sequences collected from the NCBI database site.

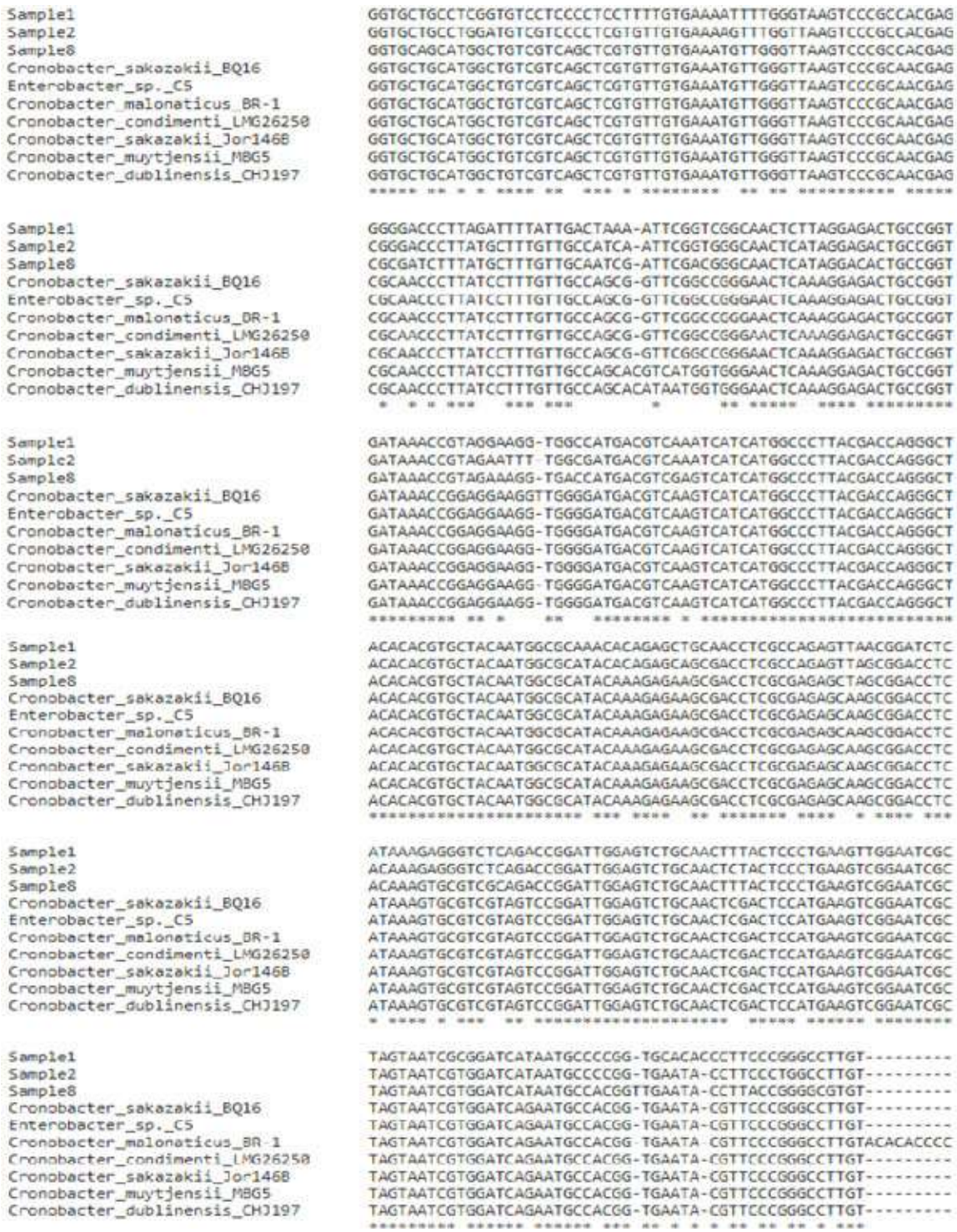
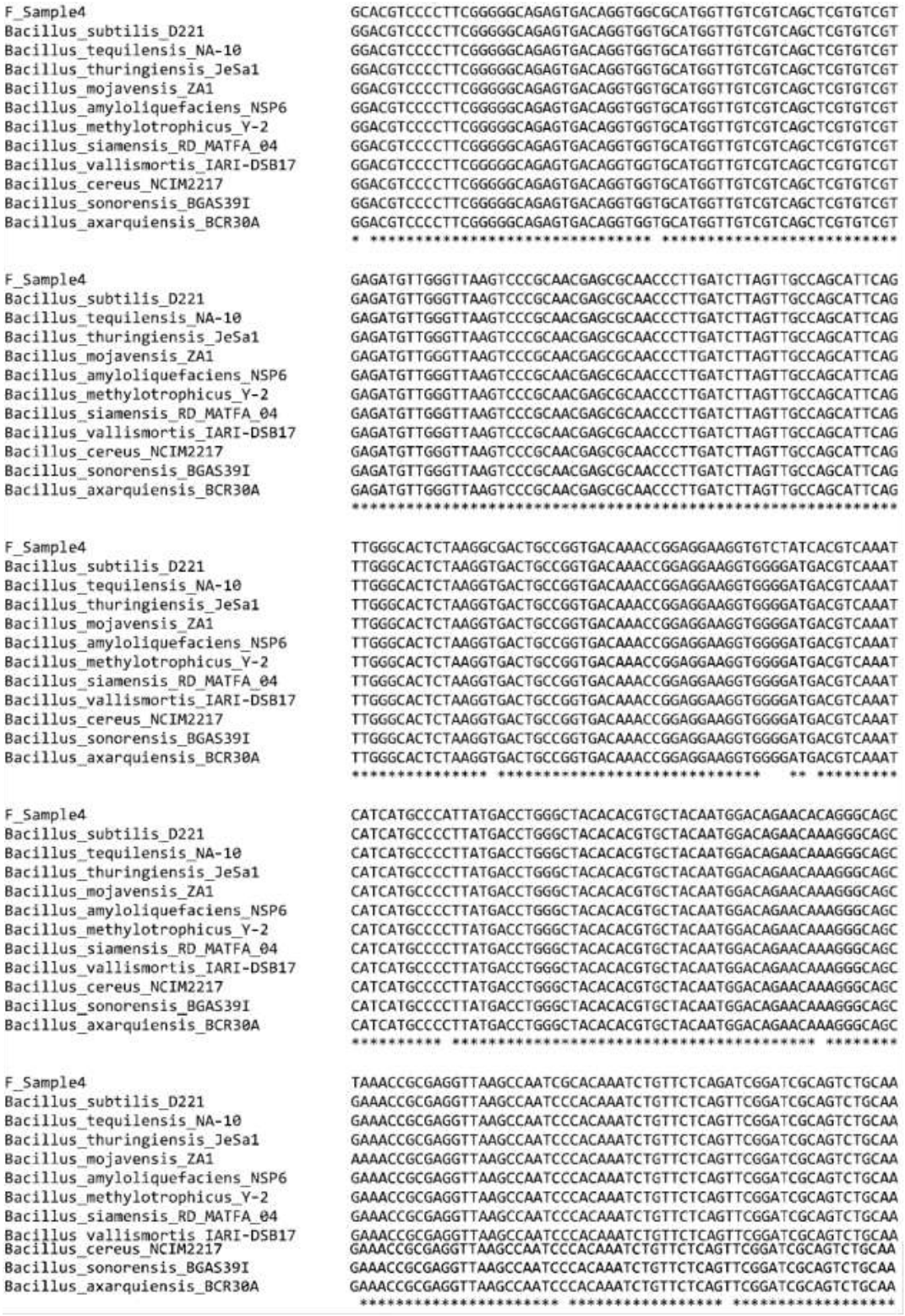


Figure 2: Partial sequence alignment for the PCR products of the 16S rRNA genes of three bacterial samples isolated from salted fish (isolate 1, isolate 2 and isolate 8) and other high-similarity sequences published in the NCBI-GenBank database.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Bacillus_subtilis_D221 GGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGT
Bacillus_tequilensis_NA-10 GGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGT
Bacillus_thuringiensis_JeSa1 GGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGT
Bacillus_mojavensis_ZA1 GGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGT
Bacillus_amyloliquefaciens_NSP6 GGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGT
Bacillus_methylotrophicus_Y-2 GGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGT
Bacillus_siamensis_RD_MATFA_04 GGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGT
Bacillus_vallismortis_IARI-DSB17 GGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGT
Bacillus_cereus_NCIM2217 GGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGT
Bacillus_sonorensis_BGAS39I GGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGT
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F_Sample4 GAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAG
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Bacillus_tequilensis_NA-10 GAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAG
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Bacillus_mojavensis_ZA1 GAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAG
Bacillus_amyloliquefaciens_NSP6 GAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAG
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Bacillus_vallismortis_IARI-DSB17 TTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAAT
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Bacillus_mojavensis_ZA1 CATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGC
Bacillus_amyloliquefaciens_NSP6 CATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGC
Bacillus_methylotrophicus_Y-2 CATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGC
Bacillus_siamensis_RD_MATFA_04 CATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGC
Bacillus_vallismortis_IARI-DSB17 CATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGC
Bacillus_cereus_NCIM2217 CATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGC
Bacillus_sonorensis_BGAS39I CATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGC
Bacillus_axarquiensis_BCR30A CATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGC
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Bacillus_thuringiensis_JeSa1 GAAACCGCGAGGTTAAGCCAATCCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAA
Bacillus_mojavensis_ZA1 AAAACCGCGAGGTTAAGCCAATCCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAA
Bacillus_amyloliquefaciens_NSP6 GAAACCGCGAGGTTAAGCCAATCCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAA
Bacillus_methylotrophicus_Y-2 GAAACCGCGAGGTTAAGCCAATCCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAA
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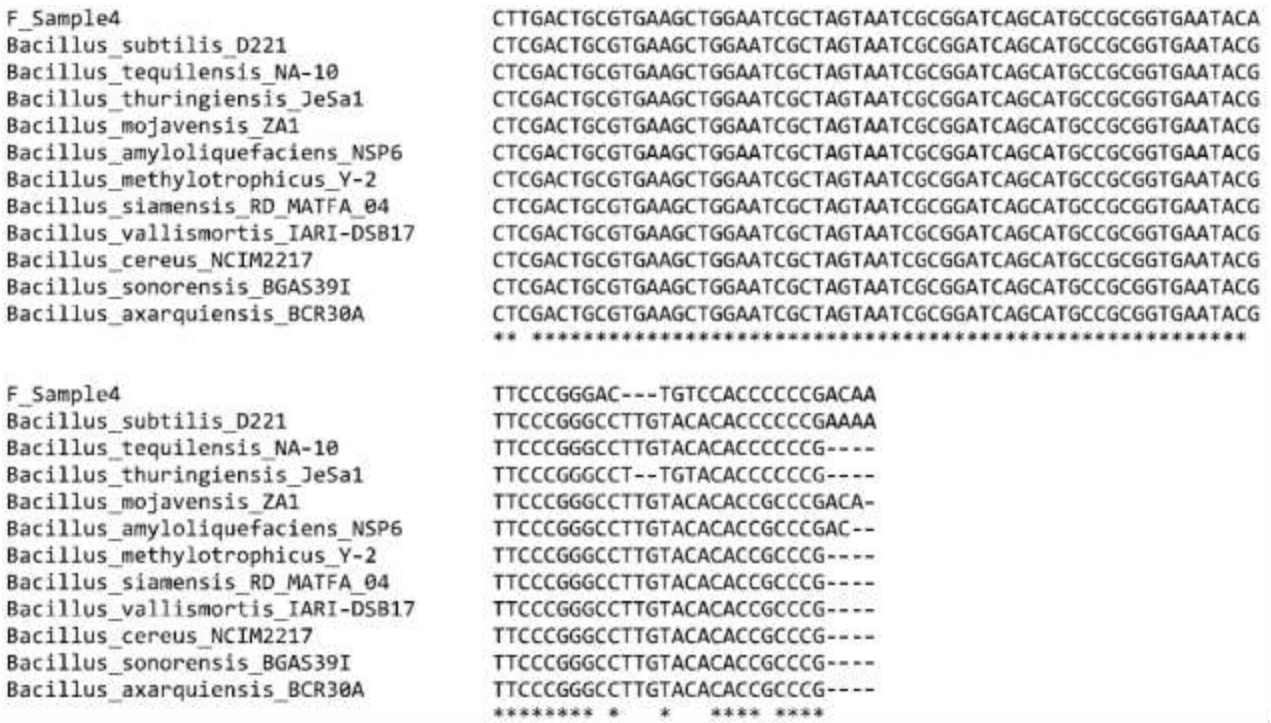

Figure 3: Partial sequence alignment for the PCR products of the 16S rRNA genes of a bacterial isolates isolated from salted fish (isolate 4) and eleven other high-similarity sequences published in the NCBI-GenBank database.

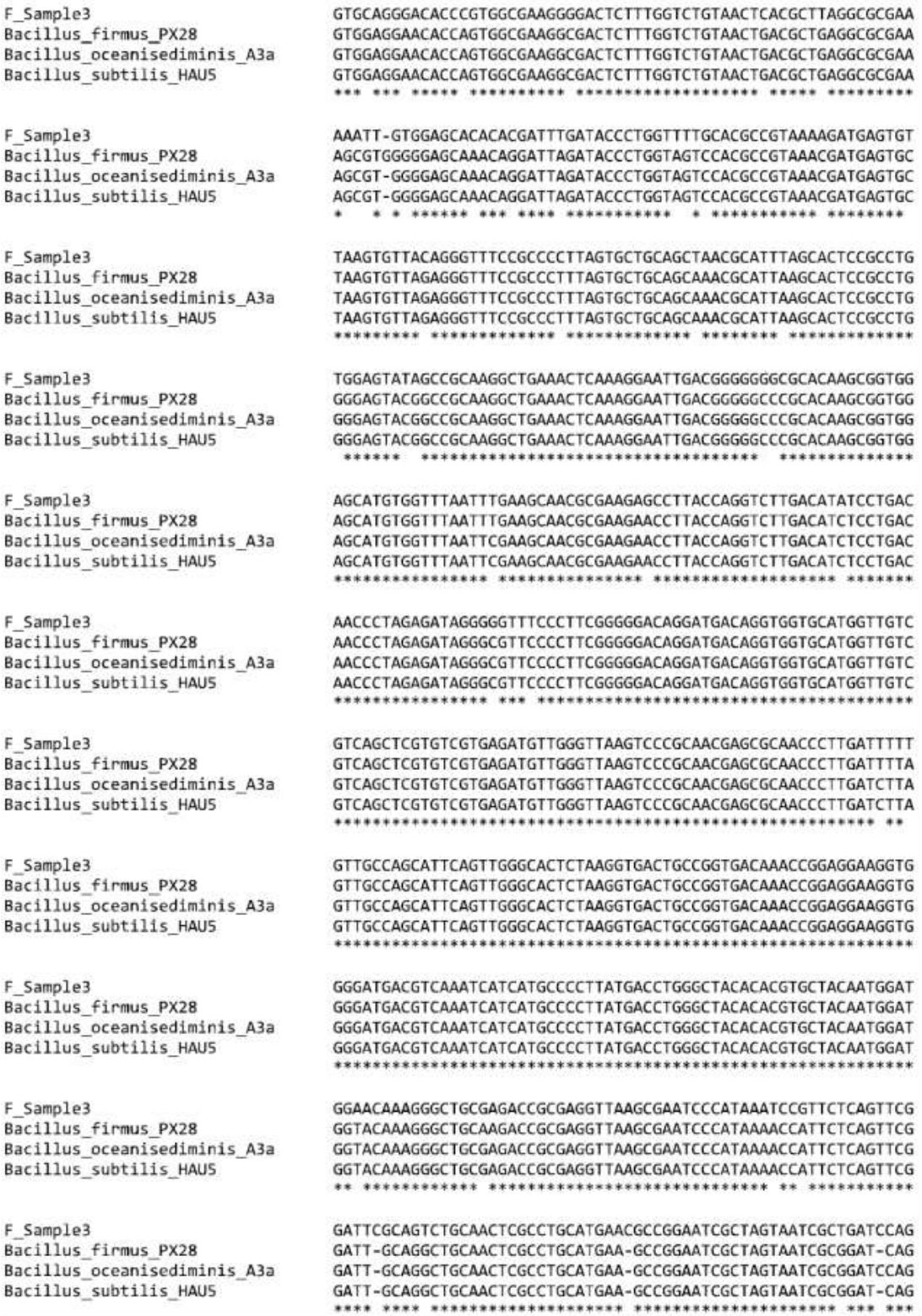

Figure 4 : Partial sequence alignment for the PCR products of the 16S rRNA genes of a bacterial sample isolated from salted fish (isolate 3) and high-similarity *B. oceanisediminis*, *B. firmus* and *B. subtilis* sequences published in the NCBI-GenBank database.

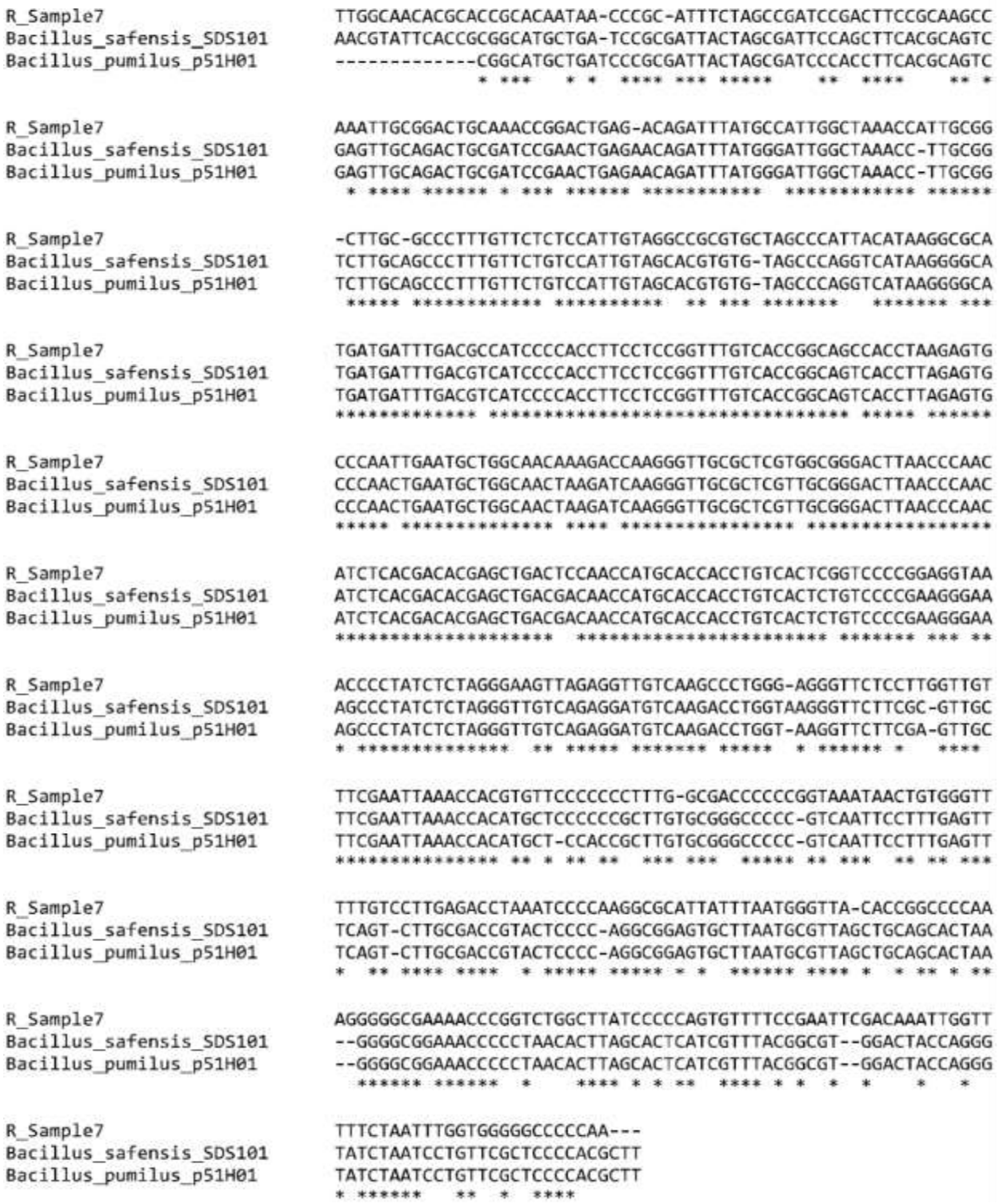

Figure 5: Partial sequence alignment for the PCR products of the 16S rRNA genes of a bacterial sample isolated from salted fish (isolate 7) and high-similarity *B. pumilus* and *B. safensis* sequences published in the NCBI-GenBank database

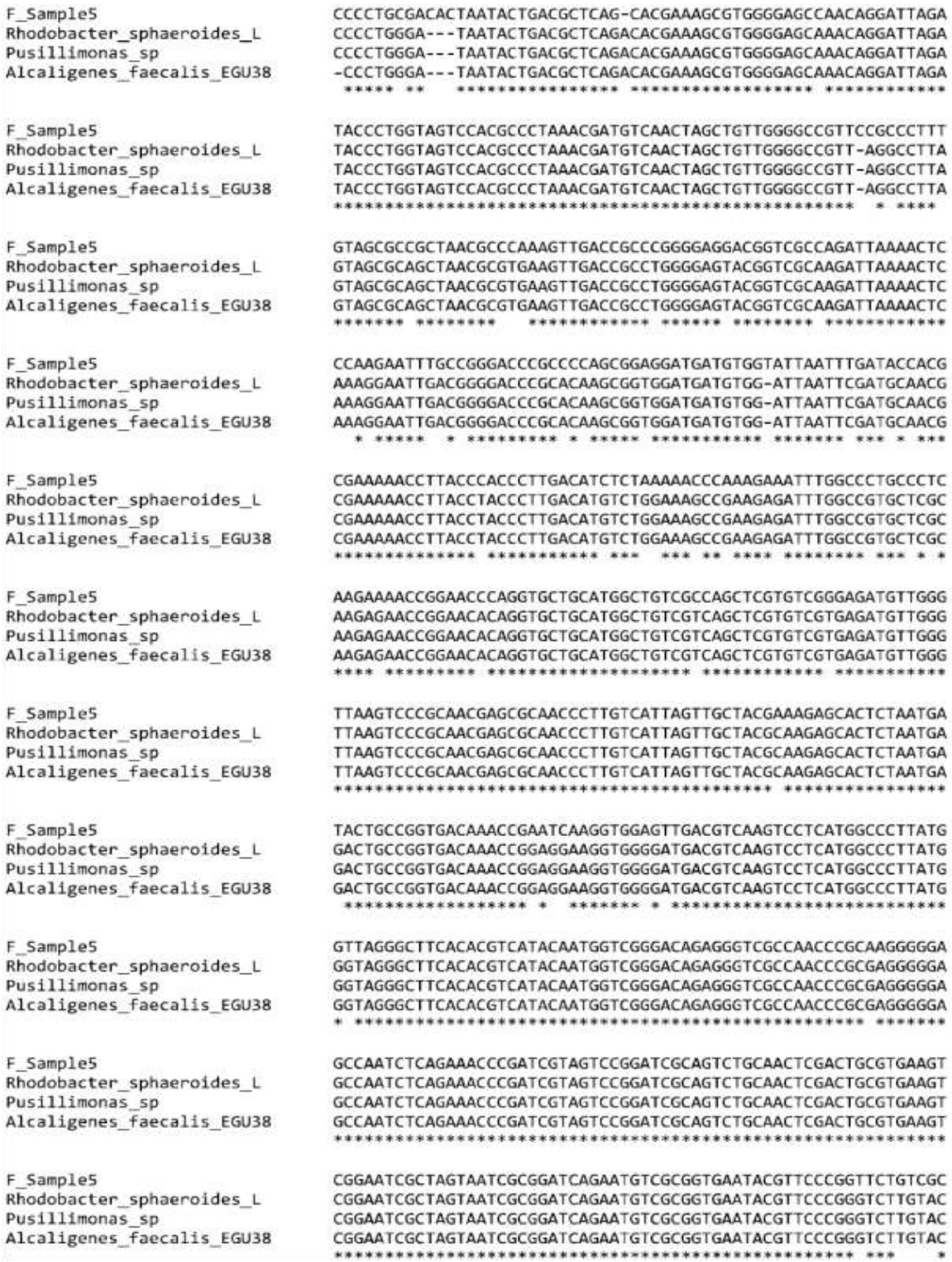

Figure 6: Partial sequence alignment for the PCR products of the 16S rRNA genes of a bacterial sample isolated from salted fish (isolate 5) and high-similarity *Pusillimonas, Rhodobacter sphaeroides* and *Alcaligenes faecalis* sequences published in the NCBI-GenBank database.

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 (Bardavid*et al*. 2007, Chen *et al.* 2007, Xiang *et al*. 2008, Swan *et al*. 2010 and Hezayen*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. condimenti, 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*p51H01 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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