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Characterization and Identification of Certain Halobacteria Isolated from Saline Soil in Jeddah.

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ABSTRACT

Extreme environments like marine salterns and sea shores are inhabiting by a variety of bacteria that are well-adapted to such environments. Four different bacterial genera were isolated from hypersaline soil of Jeddah shores. Our isolates are cultured and purified in nutrient agar medium supplemented with 10 % NaCl. The isolates tolerated high concentration of NaCl up to 25 %. These genera are identified as *Bacillus* sp. YHSA15, *Virgibacillus* sp. YHSA41, *Halomonas alikaliphila* YHSA35 and *Nocardiopsis dassonvillei* YHSA42 based on their morphological, biochemical characteristics and phylogenetic analyses inferred from 16S rRNA gene sequence. Biochemical and nutritional tests showed that strains; Bacillus sp. YHSA15, *H. alikaliphila* YHSA35 and *N. dassonvillei* YHSA42 were halophilic bacteria. They cannot grow in the absence of NaCl. While, *Virgibacillus* sp. YHSA41 was considered as halotolerant bacteria.

Keywords: Halobacteria; Saline Soil; *Bacillus*; *Virgibacillus*; *Halomonas*; *Nocardiopsis*

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INTRODUCTION

Marine salterns and sea shores are habitats for a large variety of halophilic or halotolerant microorganisms that differ throughout the entire gradient of salt concentration [1]. Halophiles are salt-loving organisms that flourish in saline environments and can be classified as slightly, moderately or extremely halophilic, depending on their requirement for sodium chloride [2-4]. Halotolerant microorganisms are capable of growing in the absence as well as in the presence of salts [5-8]. Halophilic and halotolerant microorganisms are important for numerous industrial processes [9]. They have a great role in current science and technology by using in breed salt tolerant crops, many industrial important enzymes, proteins and antibiotics [10]. They also represent a potential source for commercially important bioactive compounds and their bioremediation capabilities are also noticeable [11, 12]. Several bacterial genera belonging to these microorganisms have been observed such as *Virgibacillus, Bacillus, Pseudomonas, Staphylococcus, Flavobacterium, Acinetobacter, Oceanobacillus, Vibrio, Micrococcus, Alteromonas, Escherichia coli* [8,10,13-15], *Nocardiopsis* (Li) and *Halomonas* [16, 17]. These genera play an important role in the organic matter decomposition and cycling of nutrients. For classifying and identifying bacterial species, some biochemical assays are utilized to determine chemical components within a microorganism. These tests differentiate and identify bacteria by identifying specific bacterial metabolites as a function of available nutrients. Genomic methods have been routinely applied in microbiology [18-22]. 16S rRNA sequences constitute the largest gene-specific data set, and the number of entries in accessible databases is increasing continually [23]. Sequence databases currently contain over a million full-length 16S rRNA gene sequences representing a broad phylogenetic spectra that are a useful benchmark for identifying bacteria [24]. These making 16S rRNA-based identification of unknown bacterial isolates more and more likely. Generally, the analysis of 16S rRNA sequences is a simple and common method for the identification of microorganisms. The present study point out biochemical and molecular characterization of four halobacteria isolated from hyper-saline environment of Jeddah shore.

MATERIALS AND METHODS

Sampling

Soil samples were collected from Red Sea shore at Jeddah province. The samples were collected in sterile polythene bags, transported to the laboratory aseptically and stored at 4 ºC.

Isolation and identification

Soil samples were serially diluted from 10^{-1} to 10^{-6} using sterile distilled water. 0.1ml from each dilution was placed and spread onto nutrient agar (NA) plates supplemented with 10% NaCl as a selective medium. Plates were incubated at 35 ºC for 30 days. Mannitol Salt Agar (MSA) containing 7.5 % NaCl [25] was used to confirm the salt tolerance ability of the isolates. All isolates were subjected to higher salt concentrations ranging from 10 to 30 % NaCl in their culture medium. Four highly salt tolerate isolates were purified for identification. Pure cultures were stored at 4 ^{o}C.

Morphological characterization

Gram staining, motility and colony color were determined in 24-hour cultures in liquid NA medium supplemented with 5 % NaCl. Tyrosine agar [26] was used to determine the production of melanoid pigment. Soluble pigment production was tested on solid Mueller Hinton (MH) medium containing 5 g/L tyrosine [27]. Isolates were grown separately in Schaeffer's sporulation medium [28] for 24 h at 37 ºC. Spores were visualized by staining with malachite green and light microscopy.

Scanning electron microscopy (SEM)

Bacterial cells were harvested, fixed, dehydrated and embedded essentially as described by McDougall *et al*. [29]. Specimens were sputter coated with gold-palladium alloy and observed with a JEOL JSM-6390LA Scanning Electron Microscope (SEM) at 15KV. (JEOL Electron Microscopy Ltd. Japan).

Biochemical characterization

Growth at different salt concentrations was determined by streaking each inoculum onto the surface of solid NA media with different concentrations of NaCl 0, 5, 10, 15, 20, 25 and 30 % (w/v). Inoculated dishes were incubated at 37 °C for 5 to 20 days. The temperature range was determined as above by incubating the bacteria at temperatures of 5, 15, 20, 25, 30, 40, 45, 50 and 55 °C. The pH growth range was determined in a similar way on NA media havening different pH values had ranged from 5 to 11 adjusted by HCl or NaOH. The ability to grow anaerobically was evaluated on solid medium incubated in jars with the GasPak envelops (BBL). H_2S production was tested in tubes containing liquid MH medium supplemented with 0.01 % (w/v) of L-cysteine. Lead-acetate paper used as indicator for the gas [30]. Haemolysis was studied in solid MH medium supplemented with 5 % (v/v) defibrinated sheep blood. Oxidase reaction was performed according to Kovács [31]. Catalase was determined by adding 10 volumes of 3% H_2O_2 to each strain culture on their solid medium. Indol production was tested in liquid MH medium using Kovács' reagent [32]. Methyl red and Voges-Proskauer were tested using methyl red and Barrit's reagent [33], respectively. Starch agar is a differential medium that tests the ability of an organism to produce certain exoenzymes, including a-amylase and oligo-1,6-glucosidase. It contains the following components (g/l): peptone 5, KCl 1, NH_4SO_4 1, $MgSO_4.7H_2O$ 0.5, NaH_2PO_4 1, soluble starch 30 and agar 20. pH was adjusted at 7.2 [34, 35]. Iodine has been added to starch agar plate. The zone of clearing surrounding the isolates indicate that they able to hydrolyze starch. Cultures were inoculated into tubes of nutrient gelatin (nutrient broth, 100ml; Difco gelatin, 12 g; pH 7.0) and incubated at 37 °C for 14 days. After they had been cooled to 4 ºC, they were observed for liquefaction of the gelatin. Casein hydrolysis was indicated by a clear zone around bacterial growth on solid MH medium plus an equal quantity of skimmed milk [36]. Cellulose-degrading ability of bacterial isolates was performed by streaking on the cellulose Congo-Red agar media with the following composition: KH_2PO_4 0.5 g, $MgSO_4$ 0.25 g, cellulose 2 g, agar 15 g, Congo-Red 0.2 g, gelatin 2 g; distilled water 1L pH 6.8–7.2 [37]. Chitin hydrolysis was measured by the halo diameter of enzyme diffusion on the chitinase production medium (0.2 % colloidal chitin, 0.4% NH_4NO_3, 0.05 % K_2HPO_4, 0.05 % $MgSO_4$-$7H_2O$, 0.05 % KCl, 0.001 % $FeSO_4$-$7H_2O$, and 2% agar). Production of acid from carbohydrates was determined by the methods of Gordon *et al*. [38]. Finally, Urea hydrolysis was detected with Christensen's medium [39].

Antimicrobial susceptibility tests

Antimicrobial susceptibility was assayed using the diffusion agar method [40]. The used antimicrobial compounds (MASTRING-S^{TM}) were: Ampicillin (25 µg) Tetracycline (30 µg), Chloramphenicol (30 µg), Levofloxacin (5 µg), Flucloxacillin (5 mcg), Tobramycin (10mcg), Ofloxacin (5 mcg), Norfloxacin (10mcg), Cefotaxime (30 µg), Imipenem (10 µg), Bacitracin (10 µg), Penicillin G(10 µg), Polymyxin B (300 U), Gentamicin (10 µg) and Neomycin (30 µg).

Molecular characterization

DNA extraction

Genomic DNA was extracted from the overnight cultures of the selected bacterial strains which grown separately in 1.0 ml of nutrient broth. The bacterial cells were washed thrice with 0.9 % NaCl and used to extract total genomic DNA using DNA extraction kit (Macrogen) and the extracted DNA was purified by Macrogen extraction Kit following the manufacture protocol (Macrogen).

PCR and sequencing experiments

Purified DNAs were used by Macrogen Company of Korea to conduct PCR experiments and sequencing. The volume of the PCR reaction, the PCR reaction conditions and the forward and reverse primers all were adjusted and conducted by the Macrogen Company. The forward primer 5`-27F 5' (AGA GTT TGA TCM TGG CTC AG)3' and the reverse primer 5`-1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3' were used for the amplification of nearly the complete 16S rDNA gene. Sequencing reaction was performed using a PRISM BigDye Terminator v3.1 Cycle sequencing Kit. The reaction conditions and reading of the products were conducted by Macrogen.

The collected data were used for phylogenetic analyses after unalignable and gap-containing sites were deleted (1350 bp in total). The aligned nucleotide sequences can be obtained from the corresponding author upon request. The analyses were done by maximum-parsimony (MP) and neighbor-joining (NJ) by heuristic searches with the TBR branch swapping and 10 random taxon additions. Bootstrap replications were adjusted for both methods to be 10000 replications. The maximum-likelihood method in PAUP* 4.0b10 [41] was also used with NNI branch swapping and axis taxon additions and 200 bootstrap replications. The general reversible model (GTR+I+G) and parameters optimized by Modeltest 3.0 [42] were used.

RESULTS

Four selected bacterial strains showing a highly salt tolerant were submitted to extensive morphological, physiological, biochemical, nutritional and antimicrobial susceptibility testes (Table 1).

Description of strain YHSA15

Strain YHSA15 has a creamy yellow color colony, rod shaped cells (1.5-1.7 X 0.8-1.0 µm) (Fig 1a), forming endospores. The cells were motile. The isolate required aerobic/anaerobic conditions for growth and grow in nutrient medium containing 5-25 % NaCl (Table 1). It cannot grow in the absence of NaCl. Optimal temperature was 37-40 ºC. the temperature range was 15-50 ºC. pH range was between 6 up to 10.5. Gram reaction, methyl red, hemolytic activity, catalase, nitrate reductase and oxidase are positive. *Bacillus* sp. YHSA15 tested negative for the following: diffusible pigment, melanin production, Voges-Proskauer, indole production, H_2S production and acid production from L-xylose, lactose, raffinose and D-fructose. It was able to utilize casein (Fig 2a), starch, cellulose, chitin and gelatin as a sole carbon source. It was unable to utilized urea. The isolate tested against fifteen antibiotics listed in Table (2). The results obtained showed that our strain varied in its susceptibility to all antimicrobials used. It was resistant to levofloxacin, pencillin G and polymyxin B (Fig 3a). The following antibiotics inhibit the growth of *Bacillus* sp. YHSA15: ampicillin, tetracycline, chloramphenicol, flucloxacillin, tobramycin, ofloxacin, norfloxacin, cefotaxime, imipenem, bacitracin, gentamicin and neomycin.

Table 1 Morphological, biochemical and nutritional characteristics of the selected strains: YHSA15, YHSA41, YHSA35 and YHSA42.

Characteristic	Bacterial strain			
	YHSA15	YHSA41	YHSA35	YHSA42
Pigmentation	Creamy yellow	Creamy faint yellow	Creamy white	White to off white
Diffusible pigment	-	-	-	+
Melanin production	-	-	-	+
Cell shape	Rods	Rods	Rods	Aerial hyphae
Length	1.5-1.7µm	1.9-2.2µm	1.5-2.9µm	1.5-2 µm (spore)
Diameter	0.8-1.0µm	0.5-0.6µm	0.8-0.9µm	0.8-0.9 µm (spore)
Motility	+	+	+	ND
Soprulation	+	+	+	+
Growth at:				
0.0% NaCl	-	+	-	-
5% NaCl	+	+	+	+
10% NaCl	+	+	+	+
15% NaCl	+	+	+	+
20% NaCl	+	+	+	+
25% NaCl	+	+	+	Weak
30% NaCl	-	-	-	-
Anaerobic growth	±	-	-	-
Temperature range	15- 50ºC	10- 50ºC	5-50ºC	15-45ºC
pH range	6-10.5	6.5-10	7-10	6.5-8.5
Gram reaction	+	+	-	+
Methyl red	+	-	+	+

Voges-proskauer	-	-	+	+
Hemolytic activity	+	+	+	-
Indole production	-	+	-	-
H_2S production	-	-	+	-
Catalase	+	+	-	-
Nitrate reductase	+	+	+	+
Oxidase	+	+	+	+
Acid production from				
Sucrose	+	+	+	+
Galactose	+	-	+	+
D-xylose	+	+	+	-
L-xylose	-	+	+	-
Lactose	-	-	+	-
D- glucose	+	+	+	+
Raffinose	-	+	ND	+
D-Fructose	-	+	+	+
Maltose	+	+	+	-
Mannitol	+	+	+	-
Decomposition of:				
Starch	+	+	-	-
Gelatin	+	+	-	+
Casein	+	+	+	+
Cellulose	+	+	-	+
Chitin	+	+	+	-
Urea	-	+	-	-

(+) : Positive reaction; (-):Negative reaction; ND: Not detected

Table 2: Antibiotic susceptibility of the selected strains: YHSA15, YHSA41, YHSA35 and YHSA42.

	Bacterial strain			
Characteristic	YHSA15	YHSA41	YHSA35	YHSA42
Ampicillin	+	+	+	+
Tetracycline	+	+	+	-
Chloramphenicol	+	-	+	+
Levofloxacin	-	-	+	+
Flucloxacillin	+	+	+	+
Tobramycin	+	+	+	+
Ofloxacin	+	+	+	+
Norfloxacin	+	+	+	-
Cefotaxime	+	+	+	+
Imipenem	+	+	+	+
Bacitracin	+	-	-	+
Penicillin G	-	-	-	+
Polymyxin B	-	-	-	+
Gentamicin	+	+	+	+
Neomycin	+	+	+	+

(+) : Positive reaction (-) : Negative reaction

Description of strain YHSA41

Strain YHSA41 has a creamy faint yellow color colony, rod shaped cells (1.9-2.2 X 0.5-0.6 µm) (Fig 1b), forming endospores and motile cells. The isolate grow aerobically in nutrient medium containing 0.0-25 % NaCl. Optimal temperature was 37-40 ºC. The temperature range was 10-50 ºC. pH range was between 6.5 up to 10. Gram reaction, hemolytic activity, indole production, catalase, nitrate reductase and oxidase are positive. While, it showed negative for the following reactions: diffusible pigment, melanin production, methyl red, Voges-proskauer, H_2S production, acid production from galactose and lactose. It utilized various sugars

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including D-xylose, L-xylose, D-glucose, raffinose, D-fructose, maltose and mannitol to produce acid. Both decomposition of starch, casein cellulose, chitin and gelatin liquefaction are positive. Among the antibiotics tested, the *Virgibacillus* sp. YHSA41 was resistant to chloramphenicol, levofloxacin bacitracin, pencillin G and polymyxin B (Fig 3b).

Description of strain YHSA35

Strain YHSA35 has a creamy white color colony, rod shaped cells (1.5-2.9 X 0.8-0.9 µm) (Fig 1c), forming endospores. The cells were motile. It tested negative for the diffusible pigment and melanin production. The isolate required aerobic conditions for growth and grow in nutrient medium containing 5-25% NaCl. Like strain YHSA15, it cannot grow in the absence of NaCl. Optimal temperature was 35-40 ºC. The temperature range of growth was 5-50 ºC. pH range was between 7 up to 10.5. Gram reaction, indole production and catalase activity are negative. It showed positive to methyl red, Voges-Proskauer, hemolytic activity, H_2S production, nitrate reductase and oxidase activities. It has the ability to produce acids from all tested sugars except Raffinose (not detected) (Table 1). It produced hydrolytic enzymes such proteases and chitinase capable of hydrolyzing casein and chitin, respectively. It was unable to utilized starch, gelatin, cellulose nor chitin. It unutilized urea. Antimicrobial activity tests obtained showed that *H. alikaliphila* YHSA35 varied in its susceptibility to all antimicrobials used. It was resistant to bacitracin, pencillin G and polymyxin B (Fig 3c).

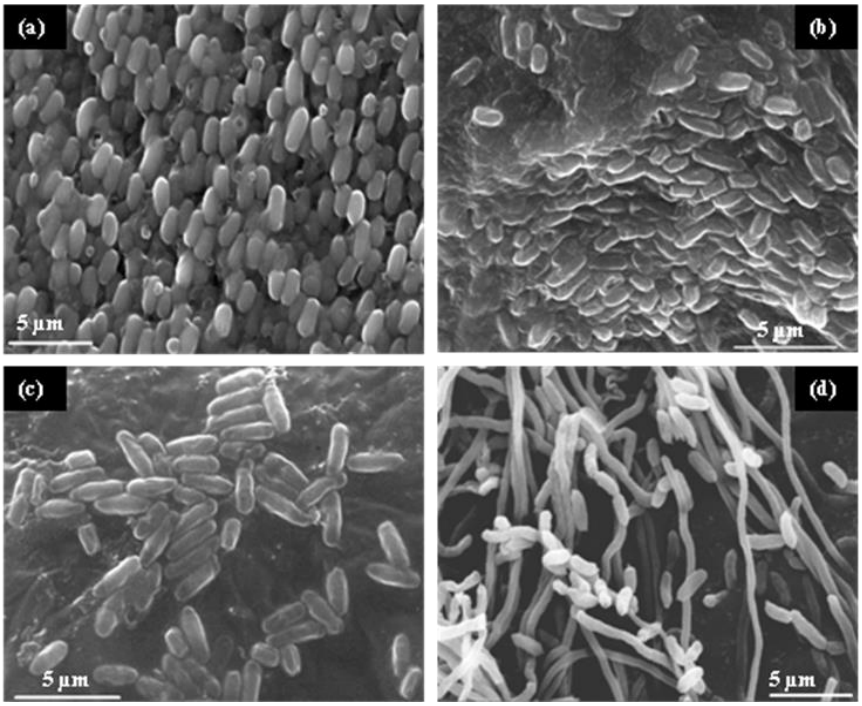

Fig 1: Scanning electron microscopy for isolated strains: (a) YHSA15, (b) YHSA41, (c) YHSA35 and (d) YHSA42.

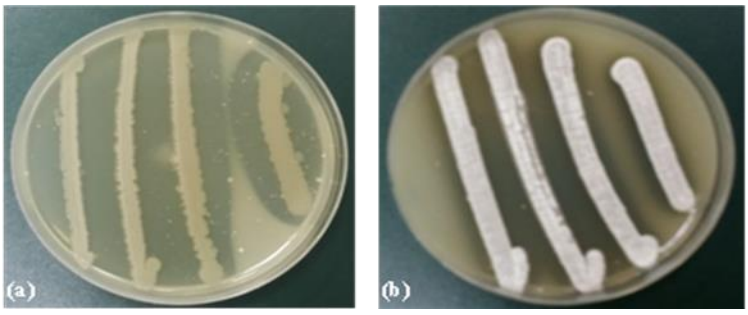

Fig 2: Casein decomposition by (a) strain YHSA15 and (b) YHSA42.

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Description of strain YHSA42

The biochemical and physiological properties of strain YHSA42 are summarized in Tables (1) and (2). The organism produced chemically diverse compounds with a sample range of biological activity. It tested positive for the diffusible pigment and melanin production. It produced aerial smooth spores (1.5-2 X 0.8-0.9 µm). They were born solitary, clustered and in chain carried on straight hypha (Fig 3d). No growth occurred in the absence of NaCl. The optimum growth was at 10-15 %. A weak growth was obtained at 25 %. However, the temperature range was 15-45 °C. The optimum temperature was observed at 35 °C. pH range was between 6.5 up to 8.5. It showed positive to Gram reaction, methyl red, Voges-Proskauer, nitrate reductase and oxidase activities. Hemolytic activity, indole production, H_2S production and catalase activity are negative. Strain YHSA42 utilized sucrose, galactose, D-glucose and raffinose as sole carbon sources for growth and produced acids from these carbohydrates (Table 1). While, negative results were obtained using D-xylose, L-xylose, lactose, maltose and mannitol. This actinomycete was able to decompose or hydrolyze gelatin, casein and cellulose, but neither starch and chitin nor urea. Among the antibiotics tested, strain YHSA42 was susceptible to all tested antibiotics except tetracycline and norfloxacin (Table 2).

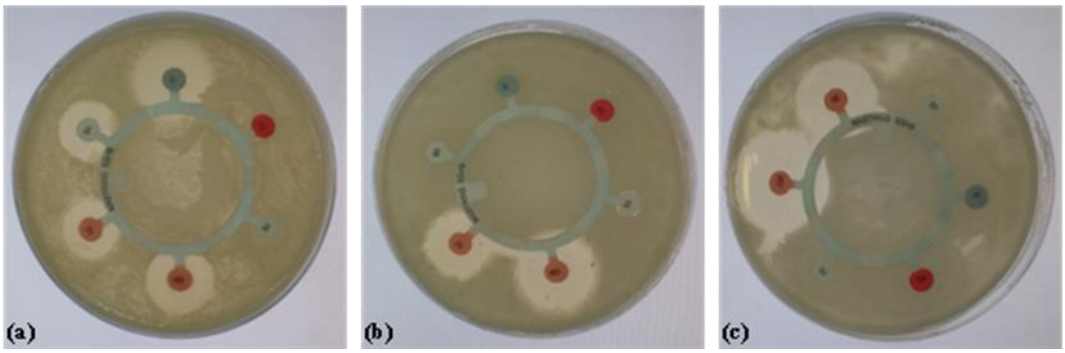

Fig 3: Comparison of the results of the antibiotic sensitivity assay for strains: (a) YHSA15, (b) YHSA41 and (c) YHSA35 against bacitracin (BA), chloramphenicol (C), penicillin (G), polymyxin (B), gentamicin (GM) and neomycin (NE).

Molecular study

The aligned 1350 bp of the 16S rDNA gene exhibited 900 constant nucleotides and 450 variables sites. Among the variable sites, 40 were parsimony uninformative and 410 were informative under parsimony criterion. The parsimony tree constructed showed consistency index (CI = 0.85), homology index (HI = 0.149), retention index (RI = 0.95) and rescaled consistency index (RCI = 0.81). The base composition for the studied strains were A= 23.06%, C= 23.91%, G= 33.47% and T= 19.56%.

The sequenced fragment of 16S rDNA gene has been sequenced for 4 different bacterial strains. These data have been treated with Blastn program (http://www.ncbi.nlm.nih.gov) for searching for their closely related strain sequences already found in the Genbank database. Sequences with identity of 99 to 95 % to these four strains have been collected from the database. The collected taxa were *Bacillus firmus* (NR_025842), *Bacillus nealsonii* (NR_044546), *Halomonas venusta* (NR_042069), *Halomonas alkaliphila* (NR_042256), *Halomonas stevensii* (NR_115088), *Virgibacillus marismortui* (NR_028873), *Virgibacillus salarius* (NR_041270), *Virgibacillus olivae* (NR_043572), *Virgibacillus sediminis* (NR_042743), *Nocardiopsis dassonvillei* (NR_074635), *Nocardiopsis halotolerans* (NR_025422), *Nocardiopsis nikkonensis* (NR_112904), *Nocardiopsis xinjiangensis* (NR_025095). The closely related *Pseudonocardia saturnea* (NR_042006) was used as an out group for tree rooting. A single tree was shown by the three used analytical methods with typical tree topology and the neighbor-joining tree was selected to be presented herein (Fig 4). The four studied strains were distinguished into four different species belonging to four different genera which were *Bacillus, Virgibacillus, Halomonas* and *Nocardiopsis*. Each of which was clustered among its congeneric species with 100 % bootstrap support. Strain YHSA15 was identified as *Bacillus* sp. (bootstrap = 78 % for MP and NJ). Strain YHSA35 was identified as *H. alkaliphila* (bootstrap=63, 100 % for MP and NJ). Strain YHSA41 was identified either as *V. salaries* or *V. marismortui* (bootstrap = 63, 92, 62 % for MP, NJ and ML, respectively) while, strain YHSA42 was identified as *N. dassonvillei* (bootstrap = 98, 99, 100 % for MP, NJ and ML, respectively). In parallel to the tree

topology, the calculated genetic distance showed the smallest values between *Bacillus* sp. YHSA15 and *B. firmus* (D= 0.006) and between YHSA41 and either *V. marismortui* and *V. salaruis* (D= 0.0007). With respect to YHSA35, the smallest genetic distances were between the strain and both *Halomonas alkaliphila* and *Halomonas stevensii* (D=0.0007). YHSA42 exhibited the closest distance with *N. dassonvillei* (D=0.0007).

The sequences of present isolates (*Bacillus* sp. YHSA15, *Virgibacillus* sp. YHSA41, *H. alikaliphila* YHSA35 and *N. dassonvillei* YHSA42) were submitted to NCBI Genbank database with their accession numbers (KU744851, KU744851, KU744851, and KU744851, respectively).

DISCUSSION

Halotolerant bacteria are a group of microorganisms able to grow in culture media containing a wide range of NaCl (1-33 %) or in the absence of NaCl [43]. Several studies have been conducted on their ecology, taxonomy and phylogeny as well as their biotechnological applications [3, 44, 45]. Our work aimed to characterize high salt tolerant bacteria. The results of phenotypic characteristics and biochemical testes were reasonable to differentiate the isolated genera. Nevertheless, the differentiation within the species on the basis of their phenotypic characteristics and biochemical testes has so far proved quite difficult. Furthermore, the characteristics selected by some authors to describe a species might be different from those selected by other authors. Identification of the selected isolates was carried out according to their morphological, biochemical and nutritional tests based on Bergey's Manual of Systematic Bacteriology 2nd edition [46, 47].

It was then confirmed with 16S rDNA sequencing. Like all haloarchaea, our isolates affected the environmental conditions. Many of which grow best above room temperature. We observed that our isolates grow best at temperature ranging from 35 to 40 ºC. This result was in agreement with those obtained by many others who use 37 ºC in their laboratories [48, 49]. The salt response of halophilic and halotolerant microbes can be affected by growth temperature and vice versa [50]. Based on the pH, the four isolates are considered as neutrophiles bacteria. This agree with Vreeland *et al*. [51] and Caton *et al*. [14] who isolated many halophilic and halotolerant microbes growing best in media with pHs from 6.8 to 7.5.

The description of strain YHSA15 using phenotypic characteristics and biochemical testes is relatively identical to *Bacillus firmus* that identified by Rao and Narasu [52]. The genus *Virgibacillus* was proposed by Heyndrickx *et al*. [53]. Compared with *V. salarius* strain YIM kkn3^T was identified by Chen *et al*. [54] to which our strain YHSA41 shared several features, but both differed in their ability to hydrolysis both casein and starch. Strain YHSA41 can grow in the absence of NaCl but strain YIM kkn3^T didn't. The description of strain YHSA41 is relatively identical to *V. marismortui* that was identified by Heyrman *et al*. [55]. Strain YHSA35 shared several features with *H. alikaliphila*, *H. venusta* and *H. stevensi*, but it was alkaliphilic, required higher NaCl concentration in the medium for optimal growth than both related *Halomonas* species. Moreover, it can grow at 25 % NaCl. Strain YHSA35 also differed from these three species in some physiological testes. It can hydrolysis casein and cannot grow in the absence of NaCl. These results aren't in agreement with those recorded by Romano *et al*. [56] who found that the growth of *H. alkaliphila* strain 18bAG^T occurs in the absence of NaCl and gives negative results with casein hydrolysis. On the other side, strain YHSA35 shared several features with *H. alikaliphila* strain 18bAG^T. Concerning *Halomonas*, our results are in agreement with those recorded by Ventosa *et al*. [44]. They reported that this genus can tolerate or require a high salt concentration for growth. Most of the biochemical tests gave similar results to those published by Mata *et al*., [16], Romano *et al*. [56] and Yang *et al*. [57], who described many species of *Halomonas*. The genus *Nocardiopsis* was first described by Meyer [58] for bacteria that were previously classified as either *Streptothrix dassonvillei* [59], *Nocardia dassonvillei* [60], or *Actinomadura dassonvillei* [61] on the basis of their morphological characteristics and cell wall type [58]. Members of *N. dassonvillei* have been isolated by many researchers from a large variety of natural habitats such as soil and marine sediments, different plant and animal materials as well as human patients [62]. It belongs to the family *Nocardiopsaceae.* In this study, most of the biochemical tests agreed with those published by Beau *et al*. [63], Evtushenko *et al*. [64] and Sun *et al*. [62]. Nevertheless, the present results did not coincide with the other previously published data.

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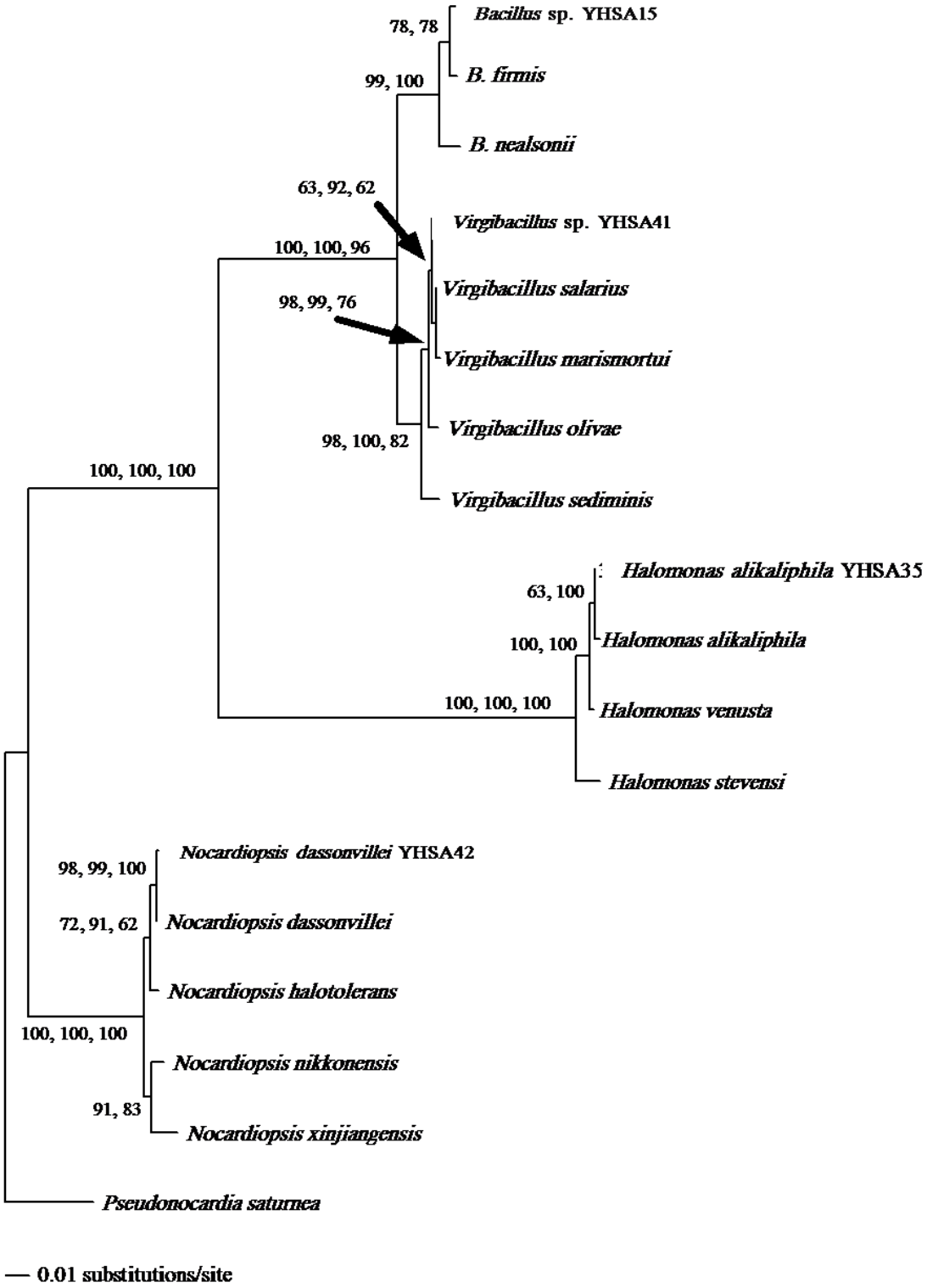

Fig 4: Neighbor-joining tree constructed from 1350 bp of 16S rDNA gene for 4 bacterial isolates belonging to different genera. Values at nodes represent the bootstrap support for MP, NJ and ML methods, respectively.

In disagreement with the present results, Meyer [58] reported that *N. dassonvillei* type strain IMRU 509^T was not able to liquefy gelatin. However, and in accordance with our results, Lejbkowicz *et al*. [65] reported a positive reaction for gelatin by *N. dassonvillei* . Strain YHSA42 can grow at 20 % NaCl at 35 ºC as an optimum temperature. These results are not in agreement with those obtained by Lechevalier and Lechevalier [61] and Evtushenko *et al*. [64]. To the contrary, they confirmed that *N. dassonvillei* type strain IMRU 509^T cannot grow at 20 % NaCl. Moreover, the catalase test was positive as reported by Meyer [58] and Beau *et al*.

[63], but in this study the catalase gave negative result. In conclusion, based on the outcome of the morphological, biochemical and physiologycal characteristics, antibiotic sensitivity and molecular characterization test isolated halobacteria were identified as *Bacillus* sp. YHSA15, *Virgibacillus* sp. YHSA41, *Halomonas alikaliphila* YHSA35 and *Nocardiopsis dassonvillei* YHSA42. The results from our study revealed that, these strains are moderately halotolerant bacteria. And according to their biochemical activation, it could be exploited directly for many potential applications in environmental safety, like industrial waste treatment, leather, food, enzyme and polymer industries; production of different stress compatible solutes, saline agricultural fields and transgenic plants for stress tolerance.

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