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Full Length Research Paper

Diversity of culturable moderately halophilic and halotolerant bacteria in a marsh and two salterns a protected ecosystem of Lower Loukkos (Morocco)

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To study the biodiversity of halophilic bacteria in a protected wetland located in Loukkos (Northwest, Morocco), a total of 124 strains were recovered from sediment samples from a marsh and salterns. 120 isolates (98%) were found to be moderately halophilic bacteria; growing in salt ranges of 0.5 to 20%. Of 124 isolates, 102 were Gram-positive while 22 were Gram negative. All isolates were identified based on 16S rRNA gene phylogenetic analysis and characterized phenotypically and by screening for extracellular hydrolytic enzymes. The Gram-positive isolates were dominated by the genus *Bacillus* (89%) and the others were assigned to *Jeotgalibacillus, Planococcus*, *Staphylococcus* and *Thalassobacillus*. The Gram negative isolates were dominated by the genus *Vibrio* (41%) and the others were assigned to *Halomonas*, *Psychrobacter, Marinobacterium*, *Pseudoalteromonas*, *Salinivibrio* and *Photobacterium.* The growth of strains obtained under different physico-chemical conditions and the screening for hydrolytic enzymes showed a high diversity even within the same species.

Key words: Halophilic bacteria, Lower Loukkos, sediment, biodiversity, 16S rRNA gene sequencing, hydrolytic enzymes.

INTRODUCTION

The "Complex of lower Loukkos" is a protected ecosystem of 3600 ha. It is a unique wetland type on Morocco's Atlantic coastline, including estuarine waters, shallow marine waters, salt marsh, freshwater swamps and floodplains, in addition to rice paddies in drained areas and a number of abandoned salterns. The main activities around the site are agriculture, livestock raising, salt production, and tourism. In 1996, it was identified as a Moroccan Site of Biological and Ecological Interest (SBEI) and since 2005; it belongs to Wetlands of

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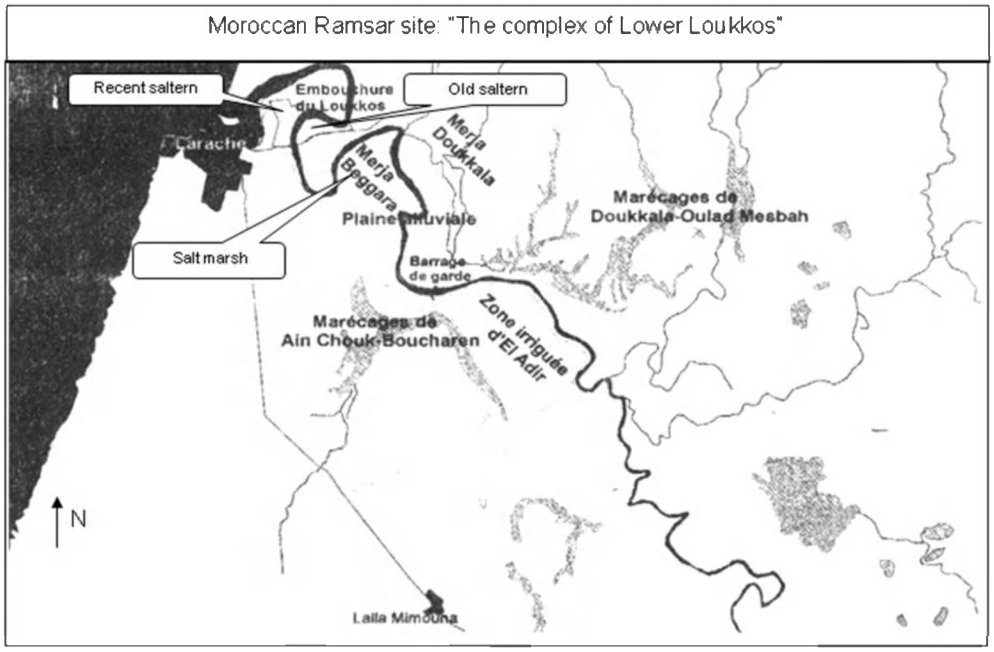

Figure 1. Location of the sampling sites (http://www.wetlands.org/Reports/Countrymaps/Morocco/1MA012/1MA012map.jpg).

international importance as part of the intergovernmental Ramsar Convention on Wetlands: (http://www.ramsar.org/cda/ramsar/display/main/main.jsp?zn=ramsar&cp=1-26-76%5E16152_4000_0). This is the first study reporting on culturable halophilic isolates in a salt marsh and two salterns located in the protected Lower Loukkos site.

Halophilic bacteria and archaea have a worldwide distribution and have been isolated from a wide variety of habitats, including areas of high salt concentrations, e.g. saline lakes, saline desert soils, salterns, salt marshes, salt mines, salted hides or foods (Oren, 2002; Ventosa et al., 2008). Halophilic bacteria are either Gram negative or Gram positive and exhibit aerobic, facultatively anaerobic or obligatory anaerobic metabolism (Johnson et al., 2007). Moderately halophilic bacteria, growing in media containing 3 to 15% (w/v) NaCl are widely distributed in various marine environments and have been frequently isolated from crystallizer ponds, saline soil, salt marshes and solar salterns (Oren, 2002; Müller and Oren, 2003; Baati et al., 2010).

Among these saline environments, salterns and salt marshes are interesting model systems for studying halophilic bacteria. It has been reported that such environments are a valuable source of novel micro-organisms, many of which have biotechnological potential for the production of compatible solutes, hydrolytic enzymes or exopolysaccharides (Quesada et al., 2004; Yeon et al., 2005; Ventosa et al., 2008).

Several problems associated with the accuracy of conventional method for halophilic bacterial systematic have been reported (Kushner, 1993). The study of bacterial diversity in the recent years has involved a combination of chemotaxonomy, phenotypic and genotypic characterization, in order to lead to a stable bacterial taxonomy (Prakash et al., 2007). Identification of halophilic bacteria has been based on both classical and molecular techniques (Ventosa et al., 2008), including microscopy (Johnson et al., 2007), polar lipid and pigment profiles (Litchfield et al., 2000), molecular and genetic fingerprinting (Yeon et al., 2005), FAME (Yoon and Oh, 2005; Johnson et al., 2007), analysis of bacteriorhodopsin gene fragments as a molecular marker (Papke et al., 2003), fluorescence *in situ* hybridization (FISH) and 16S rRNA gene sequencing (Yeon et al., 2005; Maturrano et al., 2006).

In this report on culturable moderately halophilic and halotolerant isolates from a marsh and two salterns in Lower Loukkos, bacteria were characterized using a combination of phenotypic properties, genomic fingerprinting and phylogenetic analysis based on 16S rRNA genes.

MATERIALS AND METHODS

Sites studied

The location of the two salterns and the salt marsh studied are shown in the map (Figure 1). They are situated near Larache (north-west coast of Morocco) at 35°11'N, 006°07'W and 35°11'N006°08'W and at 35°10'N, 006°06'W respectively.

Sampling of the sediment

Seven samples were obtained from the top layer (10 cm) of sedi-#ments. Samples were immediately transported in thermo boxes and stored at 4°C for subsequent analysis. The temperature was measured using an Ama-Digit ad 20th digital thermometer and the pH using a calibrated pH meter (8521 Hanna Instruments, Amorim, Portugal). The total salt concentration was determined by titration according to the method of Mohr (Sheen and Kahler, 1938).

Enumeration and Isolation of halophilic bacteria

An amount of 15 g of sediment of each sample was homogenized in 15 ml of autoclaved saline water (at 5% NaCl). The number of viable bacteria was estimated as follows: 10 fold serial dilutions were prepared using autoclaved saline water (at 5% NaCl) and 0.1 ml of each serially diluted sample was plated on Columbia agar (Biokar Diagnostics, Beauvais, France) supplemented with 2, 3, 5 or 10% (w/vol.) of NaCl and incubated at 30°C for 48 to 72 h.

Based on the colony characteristics, bacteria were picked up and plated on Columbia agar medium containing 5% of salt until axenic cultures were obtained. Pure colonies were cultivated in BHI (Brain Heart Infusion) broth medium (Himedia, India) supplemented with 5% (w/vol.) of NaCl, then stored in 20% of glycerol at -80°C, for further identification and characterization.

Phenotypic characterization

Gram-staining characteristics, cell morphologies, catalase and oxidase activities were determined by standard methods (Prescott et al., 2003).

Halotolerance of isolates was assayed by plating each of the isolates onto Columbia agar at pH 7.5 supplemented with NaCl to total concentrations of 0.5; 2; 3; 5; 10; 15; 20 or 25% (w/vol.). Cultures were incubated in aerobic conditions at 30°C for up to 3 days. Alkalitolerance or pH requirement of all isolates was estimated by plating each isolate onto Columbia agar at pH 4.5; 7.5 or 9.2 supplemented with NaCl to a total concentration of 5% (w/vol.) and incubated at 30°C for up to 3 days.

The ability to grow at different temperature was evaluated by plating each isolate onto Columbia Agar at pH 7.5 supplemented with NaCl to total concentrations of 5% (w/vol.) and incubated at 10; 30 and 55°C. Growth was determined by visual observation.

Determination of extracellular hydrolytic activities was performed as follow: proteolytic activity of each isolate was screened qualitatively as described previously (Sadfi-Zouaoui et al., 2008). Starch hydrolysis was tested according to Cowan (1991). DNase activity was revealed as described by Jeffries et al. (1957). Lipolytic and cellulolytic activities were revealed according to the methods described by Sierra (1957) and Sadfi-Zouaoui et al. (2008), respectively.

DNA extraction and rep-PCR genomic fingerprinting

Total DNA was extracted as described by Gevers et al. (2001). For $(GTG)_5$ and BOXA1R PCR the following primers were used $(GTG)_5$ (5'-GTG GTG GTG GTG GTG-3') and BOXA1R (5'-CTA CGG CAA GGC GAC GCT GACG- 3') (Versalovic et al., 1994).

PCR amplifications were performed with a DNA thermal cycler Gene AmpR PCR System 2700 (Applied Biosystems, Foster City, USA). The PCR products were separated by electrophoresis on 1.5% agarose gels at 35 V and 4°C for 18 h.

The rep-PCR profiles were visualized after staining with Ethidium bromide under ultraviolet light, followed by digital image capturing using a CCD Camera 570 LTV (GEL SMART, France). The resulting fingerprints were analysed using BioNumerics software package version 6.1 (Applied Maths, Sint-Martens-Latem, Belgium). The similarity among the digitized profiles was calculated using the Pearson correlation coefficient, and an average linkage (UPGMA) dendrogram was derived from the profiles. A clustering level of 80% was regarded as significant grouping (Gevers et al., 2001).

16S rRNA gene sequencing

The 16S rRNA gene was amplified using FD1 (CCGAATTCGTCGA CAACAGAGTTTGATC-CTGGCTCAG) and rP2 (CCCGGGATCCA AGCTTACG GCTACCTTGT-TACGACTT) primers (Weisburg et al., 1991) locally synthesized by the Functional genomics platform UATRS/ CNRST, using the Polyplex II synthesizer. PCR were conducted in a thermal cycler Gene AmpR PCR System 2700 (Applied Biosystems, Foster City, USA): under the following conditions: 4 min at 96°C (initial denaturation), 35 cycles of 10 s at 96°C (denaturation), 40 s at 52°C (annealing), 2min at 72°C (extension), and one final step of 4 min at 72°C (extension cycle).

The 16S rRNA gene of the isolate was amplified by adding 5 µL DNA (30 ng/µL) extract to a thermocycler microtube containing 48.8 µl sterile distilled water, 7 µL 10XPCR buffer (Promega), 2.1 µl $MgCl_2$ (50 mM), 5.6 µl dNTP (10mM), 0.35 µl primers (100 µM), and 0.8 µl 5 U Taq polymerase/µl (Promega).

PCR products were purified using ExoSAP-IT reagent (USB Corporation, Cleveland, Ohio) following the manufacturer's instructions. The enzymatically cleaned PCR products were used as a template for the cycle sequencing reaction.

Forward and reverse sequencing were performed using Big Dye Terminator version 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions.

DNA sequencing was performed on an ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems) using the POP-7 polymer and ABI PRISM Genetic Analyzer Data Collection and ABI PRISM Genetic Analyzer Sequencing Analysis software.

Preliminary identification was performed by FASTA search of the NCBI database and a more precise identification was performed by phylogenetic analysis with type strains of the nearest neighbours. Isolates were regarded as belonging to a species when sequence similarity with the species type strain was at least 99% and to a genus when sequence similarity with a type strain was at least 97%.

Nucleotide sequence accession numbers

The 16S rRNA gene sequences, determined in this study, have been deposited in the Genbank database under accession numbers JN208023-JN208135.

RESULTS

Physico-chemical parameters of sampling sites

The average salt concentration of our samples varied from 30 g/L in sediment from the marsh to 100 g/L in the salterns. The pH was 7.7 in salterns and 8.45 in marsh (Table 1). The average temperature of the site, measured in May, 2006, was 34.8°C. According to the

Table 1. Distribution of the isolates in the protected complex of Lower Loukkos (Larache, Morocco).

Characteristic	Sediment from region of Larache		
	Strain numbers	**Salterns**	**Marsh**
Number of samples		2	5
Number of isolates		17	107
Salinity (‰)		100	30
pH		7.7 [a]	8,45 [a]
Temperature (°C) (May2006)		34.9 [a]	34.8 [a]
CFU/g		5.10^{5} [a]	2.10^{7} [a]
Species identified (Number of isolates) Gram-positive			
Bacillus aquimaris (32)	B619, B628, B593, B597 , B591, B687, B682, B600, B675, **B677**, B573, B575, B592, B576, B697, B676, B636, B674, B685, B589, B683, B631, B585, B580, B701, B634, B700, B681, B667, B686, B627, B656,	(5)	(27)
*Bacillus aquimaris/ Bacillus vietnamensis**(2)	B620, B588		(2)
Bacillus megaterium (9)	B574, B577, B581, B586, B590, B626, B572, B583, B666,		(9)
Bacillus megaterium/Bacillus flexus(1)*	B599		(1)
Bacillus oceanisediminis (8)	B616, B587, B618, B584, B637, B655, B648, B645		(8)
Bacillus hwajinpoensis (7)	B651, B652, B690, B650, B646, B707, B691		(7)
Bacillus safensis (6)	B693, B582, B679, B629, B669, B630		(6)
*Bacillus safensis/ Bacillus pumilis**(1)	B668		(1)
Bacillus simplex (3)	B621, B622, B624		(3)
*Bacillus muralis/ B. simplex /B. macroides**(1)	B692		(1)
Bacillus thioparans (2)	B649, B698		(2)
Bacillus cereus (2)	B613, B702		(2)
Bacillus jeotgali (1)	B670		(1)
*Bacillus boroniphilus / Bacillus jeotgali**(3)	B614, B704, **B653**		(3)
*Bacillus boroniphilus/ B. jeotgali / B. selenatarsenatis**(1)	B671		(1)
Bacillus niabensis(1)	B579		(1)
Bacillus licheniformis (1)	B699		(1)
Bacillus flexus (1)	B625		(1)
Bacillus gibsonii (1)	B578		(1)
Bacillus clausii (1)	B623		(1)
Jeotgalibacillus campisalis (2)	B639 , B640		(2)
Planococcus rifietoensis (2)	B654, B688	(1)	(1)
Staphylococcus warnerii(2)	B635, B684		(2)
Thalassobacillus devorans (1)	B596	(1)	
Jeotgalibacillus salarius (1)	B657	(1)	
Staphylococcus hominis (1)	B644		(1)
Bacillus sp. * (9)	B632, B633, B598, B658, B617, B615 B647, B703, B673	(1)	(8)
Gram-negative			
Vibrio alginolyticus(2)	B641, B708		(2)
Vibrio atypicus (1)	B696		(1)
Vibrio fluvialis (1)	B664		(1)
Vibrio azureus (1)	B705		(1)

Table 1. Contd

*Vibrio azureus / Vibrio rotiferianus** (1)	B694		(1)
Salinivibrio costicola subsp. *costicola* (2)	B680, B689	(2)	
Salinivibrio costicola subsp. *Vallismortis* (1)	B659	(1)	
Halomonas salina (1)	B594	(1)	
*Halomonas salina / Halomonas halophila** (1)	B595	(1)	
Halomonas sacchare vitans (1)	B661	(1)	
Psychrobacter piscatorii (1)	B706		(1)
Photobacterium halotolerans (1)	B643		(1)
Vibrio sp.* (3)	B642, B672, B663		(3)
Photobacterium sp.* (1)	B665		(1)
Salinivibrio sp.*(1)	B638	(1)	
Halomonas sp.* (1)	B660	(1)	
Marinobacterium sp.*(1)	B678		(1)
Pseudoalteromonas sp.* (1)	B662		(1)

[a] The pH and salt values are the mean of three determinations per sample. All strains identified in this study were given B numbers and are preserved at Moroccan Coordinated Collections of Micro-organisms (CCMM).Strain with (*)should be characterized further using other methods. In bold isolates not showing any extracellular hydrolytic enzyme activity.

classification of saline waters, the marsh could be placed in Biotope 2 (30 g/L) and the salterns in biotope 3 (100 g/L) (Ricard, 1977).

Isolation and phenotypic characterisation

The number of heterotrophic aerobic or facultative anaerobic bacteria that grew on Columbia agar containing different concentrations of salt, varied from 10^5 to 10^7 colony forming units/g (CFU/g) (Table 1).

From the 10^{-3} dilution plates, a total of 124 isolates that exhibited different colony characteristics (size, pigmentation, opacity, texture, form, elevation, margin, and surface) were selected for further analysis. Of these, 56 isolates (44.8 %) were cream-colored. The others (55.2%) formed yellow, white, or orange colonies on Columbia agar supplemented with salt. 102 isolates (82.3 %) were Gram positive of which 92 (90.0 %) were endospore forming, while 22 isolates (17.8 %) were Gram negative (Table 2). Most (60.5%) of the isolates were catalase positive and oxidase negative. All of the isolates grew aerobically within 24 to 48 h at 30°C on Columbia agar supplemented with 5% NaCl and at pH 7.5.

Genomic profiling and 16S rDNA sequencing

Clustering of halophilic isolates

Firstly, all the 124 isolates (102 Gram positive and 22 Gram negative bacteria) were subjected to BOXA1R-PCR and $(GTG)_5$-PCR fingerprinting with the aim to determine their genotypic diversity and to select representative strains from each cluster for further analysis. The resulting electrophoretic patterns were used to construct dendrograms based on a Pearson correlation (Data not shown). In all these dendrograms small clusters with limited number of isolates can be distinguished at 80% similarity. Bandings patterns of the isolates generated both by $(GTG)_5$ and BOXA1R-PCR, were complex producing a large number of polymorphic bands of variable intensity. Very few significant groupings were observed among the Moroccan halophilic isolates, implying that they represented a large diversity with few duplicates.

16S rRNA gene sequencing analysis

The 124 isolates were subjected to 16S rRNA gene sequence analysis. For all but four of isolated strains, the 16S rDNA sequences determined in this study comprised 600 to 1500 nucleotides (Table 2). There are widely accepted criteria for delineating species in current bacteriology, stating that strains with a sequence similarity greater than or equal to 97% may be considered a genus level match. A species level match is based on a similarity greater than or equal to 99% (Drancourt et al., 2000).

The 16S rRNA gene sequence analysis of 91 Gram positive isolates (89%) and 14 (63%) Gram negative isolates showed that there was a high similarity (≥ 99%) between the test strains and their closest phylogenetic relative, which may indicate that 16S rRNA gene sequence data are helpful for identification of halophilic bacteria at the species level (Table 2). Several isolates have only moderate 16S rDNA sequence similarity (97 to

Table 2. 16S rRNA sequencing analysis of isolates from marsh and salterns sediments.

B-number	N° of bp sequenced	Similarity with nearest type strain (%)	Tentative identification based on nearest neighbor	Accession number
B573	710	100	*Bacillus aquimaris*	JN208023
B575	697	100	*Bacillus aquimaris*	JN208024
B701	1325	99.3	*Bacillus aquimaris*	JN208054
B656	1319	99.1	*Bacillus aquimaris*	JN208040
B597	1403	99.3	*Bacillus aquimaris*	JN208032
B682	771	100	*Bacillus aquimaris*	JN208047
B681	652	99.7	*Bacillus aquimaris*	JN208046
B585	1288	99.7	*Bacillus aquimaris*	JN208027
B685	1515	99.3	*Bacillus aquimaris*	JN208049
B631	1390	99.3	*Bacillus aquimaris*	JN208037
B683	1386	99.1	*Bacillus aquimaris*	JN208048
B627	1415	99.4	*Bacillus aquimaris*	JN208035
B700	724	99.9	*Bacillus aquimaris*	JN208053
B634	775	99.9	*Bacillus aquimaris*	JN208038
B619	734	99.9	*Bacillus aquimaris*	JN208034
B593	1321	99.6	*Bacillus aquimaris*	JN208031
B591	1312	99.5	*Bacillus aquimaris*	JN208029
B687	1334	99.5	*Bacillus aquimaris*	JN208051
B576	1288	99.5	*Bacillus aquimaris*	JN208025
B592	1349	99.3	*Bacillus aquimaris*	JN208030
B675	744	100	*Bacillus aquimaris*	JN208043
B636	766	99.9	*Bacillus aquimaris*	JN208039
B667	1301	99.2	*Bacillus aquimaris*	JN208041
B580	1305	99.5	*Bacillus aquimaris*	JN208026
B676	716	99.9	*Bacillus aquimaris*	JN208044
B674	641	99.8	*Bacillus aquimaris*	JN208042
B628	721	100	*Bacillus aquimaris*	JN208036
B589	1305	99.6	*Bacillus aquimaris*	JN208028
B697	1292	99.6	*Bacillus aquimaris*	JN208052
B600	784	99.5	*Bacillus aquimaris*	JN208033
B677	733	100	*Bacillus aquimaris*	JN208045
B686	767	100	*Bacillus aquimaris*	JN208050
B588	1293	99.5/ 99.4	*Bacillus aquimaris/ Bacillus vietnamensis*	-
B620	1396	99.0/ 99.1	*Bacillus aquimaris/ Bacillus vietnamensis*	-
B574	1408	99.9	*Bacillus megaterium*	JN208056
B581	1396	99.9	*Bacillus megaterium*	JN208058
B577	1332	100	*Bacillus megaterium*	JN208057
B586	1314	99.9	*Bacillus megaterium*	JN208060
B666	1297	99.8	*Bacillus megaterium*	JN208063
B590	1323	100	*Bacillus megaterium*	JN208061
B572	1433	99.2	*Bacillus megaterium*	JN208055
B583	1516	99.9	*Bacillus megaterium*	JN208059
B626	1389	100	*Bacillus megaterium*	JN208062
B599	642	100/ 100	*Bacillus megaterium/ Bacillus flexus*	-
B655	1502	99.4	*Bacillus oceanisediminis*	JN208071
B587	1395	99.9	*Bacillus oceanisediminis*	JN208065
B648	1502	99.4	*Bacillus oceanisediminis*	JN208070
B645	1502	99.4	*Bacillus oceanisediminis*	JN208069

Table 2. Contd

B637	1323	99.7	*Bacillus oceanisediminis*	JN208068
B618	1393	99.9	*Bacillus oceanisediminis*	JN208067
B584	1382	99.9	*Bacillus oceanisediminis*	JN208064
B616	1347	100	*Bacillus oceanisediminis*	JN208066
B690	778	99.6	*Bacillus hwajinpoensis*	JN208077
B646	1493	99.0	*Bacillus hwajinpoensis*	JN208073
B651	1360	99.8	*Bacillus hwajinpoensis*	JN208075
B691	1411	99.9	*Bacillus hwajinpoensis*	JN208078
B707	1363	99.7	*Bacillus hwajinpoensis*	JN208079
B652	1304	99.8	*Bacillus hwajinpoensis*	JN208076
B650	599	99.5	*Bacillus hwajinpoensis*	JN208074
B582	1394	100	*Bacillus safensis*	JN208080
B693	1403	100	*Bacillus safensis*	JN208085
B679	1377	100	*Bacillus safensis*	JN208084
B669	1308	100	*Bacillus safensis*	JN208083
B630	1382	99.9	*Bacillus safensis*	JN208082
B629	1379	99.9	*Bacillus safensis*	JN208081
B668	1364	99.9/ 99.9	*Bacillus safensis/ Bacillus pumilis*	-
B624	1348	99.8	*Bacillus simplex*	JN208088
B622	1348	99.9	*Bacillus simplex*	JN208087
B621	1320	99.9	*Bacillus simplex*	JN208086
B692	1308	99.9/ 99.8/ 99.8	*Bacillus muralis/ B. simplex /B. macroides*	-
B649	1477	99.3	*Bacillus thioparans*	JN208089
B698	1393	99.4	*Bacillus thioparans*	JN208090
B613	1403	99.9	*Bacillus cereus*	JN208091
B702	1313	99.8	*Bacillus cereus*	JN208092
B670	1445	99.9	*Bacillus jeotgali*	JN208093
B653	1327	99.9/99.9	*Bacillus boroniphilus /Bacillus jeotgali*	-
B614	665	99.7/99.7	*Bacillus boroniphilus / Bacillus jeotgali*	-
B704	1352	99.9/ 99.9	*Bacillus boroniphilus / Bacillus jeotgali*	-
B671	712	100/100/100	*Bacillus boroniphilus/ B. jeotgali / B. selenatarsenatis*	-
B579	1297	99.2	*Bacillus niabensis*	JN208097
B699	693	99.9	*Bacillus licheniformis*	JN208094
B625	1401	99.9	*Bacillus flexus*	JN208072
B578	1409	99.3	*Bacillus gibsonii*	JN208095
B623	679	99.9	*Bacillus clausii*	JN208096
B640	703	99.9	*Jeotgalibacillus campisalis*	JN208108
B639	1334	99.8	*Jeotgalibacillus campisalis*	JN208107
B654	1504	99.6	*Planococcus rifietoensis*	JN208109
B688	1331	99.9	*Planococcus rifietoensis*	JN208110
B684	1297	99.5	*Staphylococcus warneri*	JN208112
B635	1332	99.6	*Staphylococcus warneri*	JN208111
B596	1415	99.6	*Thalassobacillus devorans*	JN208114
B657	1504	99.2	*Jeotgalibacillus salarius*	JN208115
B644	1280	99.9	*Staphylococcus hominis*	JN208113
B633	1516	98.1/ 97.6	*Bacillus aquimaris/Bacillus vietnamensis*	JN208102
B598	1384	98/ 97.5	*Bacillus aquimaris/Bacillus vietnamensis*	JN208098
B658	1503	98.2/ 97.6	*Bacillus aquimaris/Bacillus vietnamensis*	JN208104
B632	1379	98.3/97.7	*Bacillus aquimaris/Bacillus vietnamensis*	JN208101
B673	1246	98.7	*Bacillus salmonicida*	JN208105

Table 2. Contd.

B617	423	97.4	*Bacillus hwajipoensis*	JN208100
B615	786	98.7	*Bacillus hwajinpoensis*	JN208099
B647	737	98.5	*Bacillus hwajinpoensis*	JN208103
B703	1261	98.7/98.2	*Bacilus nealsonii/ Bacillus circulans*	JN208106
B641	1377	99.7	*Vibrio alginolyticus*	JN208116
B708	1239	99.2	*Vibrio alginolyticus*	JN208117
B696	705	99.9	*Vibrio atypicus*	JN208118
B664	1505	100	*Vibrio fluvialis*	JN208119
B705	1301	99.1	*Vibrio azureus*	JN208120
B694	1518	99.5/ 99.5	*Vibrio azureus / Vibrio rotiferianus*	-
B689	1329	99.9	*Salinivibrio costicola* subsp. *costicola*	JN208122
B680	733	100	*Salinivibrio costicola* subsp. *costicola*	JN208121
B659	1334	99.2	*Salinivibrio costicola* subsp. *vallismortis*	JN208123
B594	1338	99.9	*Halomonas salina*	JN208124
B595	425	100/ 100	*Halomonas salina / Halomonas halophila*	-
B661	435	99.0	*Halomonas saccharevitans*	JN208125
B706	1245	99.8	*Psychrobacter piscatorii*	JN208126
B643	730	99.2	*Photobacterium halotolerans*	JN208127
B663	1495	98.0	*Vibrio navarrensis*	JN208129
B642	402	97.1	*Vibrio navarrensis*	JN208128
B672	1302	98.7	*Vibrio vulnificus*	JN208130
B665	1313	98.3	*Photobacterium halotolerans*	JN208131
B638	701	98.4	*Salinivibrio costicola* subsp. *vallismortis*	JN208132
B660	1493	98.2	*Halomonas saccharevitans*	JN208133
B678	1277	98.8	*Marinobacterium maritimum*	JN208134
B662	1493	97.9	*Pseudoalteromonas prydzensis*	JN208135

98.9%) to their GenBank best match sequences from taxonomically well determined bacteria species, indicating that they may be potential new species. Based on the mentioned criteria and phylogenetic analysis with related type strains, our results revealed that the Gram positive bacteria were dominated by genus *Bacillus* (89%) and the others were assigned to *Jeotgalibacillus, Planococcus*, *Staphylococcus* and *Thalassobacillus*. The Gram negative isolates were dominated by genus *Vibrio* (41%) and the others were assigned to *Halomonas*, *Psychrobacter, Marinobacterium*, *Pseudoalteromonas*, *Salinivibrio* and *Photobacterium* (Tables 1 and 2).

Influence of NaCl, pH and Temperature on growth of isolated strains

The growth of identified species was tested on Columbia Agar containing 0.5 to 25% NaCl, at pH 4.5 to 9.2 or at temperatures of 10 to 55°C. The growth of tested strains was inhibited by 25% NaCl in the medium. The optimal pH and temperature for growth of all isolates were determined as 7.5 and 30°C respectively. All but four of the strains: *H. salina* (B594), *H. salina*/*Halomonas halophila* (B595), *Halomonas* sp. (B660) and *Halomonas saccharevitans* (B661) were able to grow in the range of 0.5 to 5% NaCl. Of 124 strains, 86 (69%) tolerate 10% NaCl, 36 (29%) tolerate 15% and 11 (8.9 %) were able to grow in the presence of 20% NaCl. Only 3 strains *Bacillus muralis* / *Bacillus simplex* / *Bacillus macroides* (B692), *Staphylococcus warnerii* (B684) and *Staphylococcus hominis* (B644) were able to grow at pH 4.5. Of 124 strains, 16 failed to grow at pH 9.2, 46 failed to grow at 10°C and only 15 isolates were able to grow at 55°C (Table 3).

Screening of extracellular hydrolytic enzymes

All 124 isolates obtained in this study were plated on solid media containing different substrates for the detection of amylolytic, lipolytic, proteolytic, DNase and cellulolytic activities. All of the 102 Gram-positive bacteria screened, except 2 isolates *Bacillus aquimaris* (B677) and *Bacillus boroniphilus* / *Bacillus jeotgali* (B653), showed at least one extracellular hydrolytic enzyme activity (Table 4). Of the 22 Gram-negative isolates, 15 showed at least one of the extracellular hydrolytic

Table 3. Growth of identified halophilic bacteria under different physico-chemical conditions.

Strain (Number)	B-number	NaCl (w/v)					pH			T (°C)		
		0.5%	5%	10%	15%	20%	4,5	7,5	9,2	10	30	55
Bacillus aquimaris(32)	B573, B575	+	++	++	+	-	-	+	+	-	++	-
	B701	+	++	++	+	-	-	+	+	+	++	-
	B682, B686, B656	+	++	+	+	-	-	+	+	+	++	-
	B597	+	++	+	+	-	-	+	+	-	++	w
	B681	+	++	+	+	-	-	+	+	+	++	+
	B585	+	++	+	w	-	-	+	+	+	++	-
	B685	+	++	w	w	-	-	+	+	-	++	-
	B631	+	++	++	-	-	-	+	+	-	++	-
	B683	+	++	+	-	-	-	+	-	-	++	-
	B700	+	++	+	-	-	-	+	+	+	++	-
	B591	+	++	+	-	-	-	+	+	++	++	-
	B634, B619	+	++	+	-	-	-	+	+	-	++	-
	B593	+	++	+	-	-	-	+	+	+	++	-
	B687, B576, B627	+	++	+	-	-	-	+	+	+	++	-
	B592	+	++	-	-	-	-	+	-	+	++	-
	B675, B667	+	++	-	-	-	-	+	-	-	++	-
	B636, B677	+	++	-	-	-	-	+	+	w	++	-
	B580, B676	+	++	-	-	-	-	+	+	+	++	-
	B674, B589	+	++	-	-	-	-	+	+	++	++	-
	B628	+	++	-	-	-	-	+	+	-	++	+
	B697	+	++	-	-	-	-	+	-	-	++	-
	B600	+	++	-	-	-	-	+	w	+	++	-
Bacillus aquimaris / B. vietnamensis (2)	B588	+	++	+	-	-	-	+	+	++	++	-
	B620	+	++	+	-	-	-	+	+	w	++	-
Bacillus megaterium (9)	B574	+	++	+	-	-	-	+	+	+	++	+
	B581, B577, B586, B666, B583	+	++	+	-	-	-	+	+	+	++	-
	B590	+	++	+	-	-	-	+	+	-	++	-
	B572	+	++	+	-	-	-	++	++	-	++	-
	B626	+	++	-	-	-	-	+	+	+	++	-
Bacillus megaterium/ B. flexus(1)	B599	+	++	+	-	-	-	+	+	-	++	-
Bacillus oceanisediminis(8)	B655	+	++	+	+	-	-	+	+	+	++	-
	B587	+	++	+	+	+	-	+	+	+	++	-
	B648	+	++	+	w	-	-	+	+	+	++	-
	B645, B618	+	++	+	-	-	-	+	+	-	++	-
	B637	+	++	+	-	-	-	+	+	w	++	-
	B584	+	++	+	-	-	-	+	+	+	++	+
	B616	+	++	-	-	-	-	+	+	+	++	-
Bacillus hwajinpoensis(7)	B690	+	++	+	+	-	-	+	+	+	++	-
	B646	+	++	+	-	-	-	+	+	-	++	+
	B651, B691	+	++	+	-	-	-	+	+	++	++	-
	B707	+	++	w	-	-	-	+	+	-	++	-
	B652	+	++	-	-	-	-	+	+	++	++	-

Table 3. Contd.

Bacillus safensis(6)	B650	+	++	-	-	-	-	+	-	+	++	-
	B582	+	++	++	+	-	-	+	+	+	++	+
	B693	+	++	+	+	-	-	+	+	+	++	+
	B679	+	++	+	+	-	-	+	+	+	++	++
	B669	+	++	+	+	-	w	+	+	+	++	++
	B630	+	++	+	-	-	-	+	+	+	++	+
	B629	+	++	++	-	-	-	++	++	+	++	+
B. safensis/B. pumilis(1)	B668	+	++	+	+	-	-	+	+	-	++	+
Bacillus simplex (3)	B624	+	++	+	-	-	-	+	+	+	++	-
	B622	+	++	-	-	-	-	+	-	-	++	-
	B621	+	++	-	-	-	-	+	+	+	++	-
B. muralis/ B. simplex / B. macroides (1)	B692	+	++	-	-	-	+	+	+	+	++	-
Bacillus thioparans (2)	B649	+	++	+	w	-	-	+	+	++	++	-
	B698	+	++	-	-	-	-	+	-	-	++	-
Bacillus cereus(2)	B613	+	++	-	-	-	-	+	+	+	++	-
	B702	+	++	-	-	-	-	+	+	-	++	-
Bacillus jeotgali (1)	B670	+	++	-	-	-	-	+	-	-	++	-
Bacillus boroniphilus /B. jeotgal l (3)	B614, B704	+	++	-	-	-	-	+	+	-	++	-
	B653	+	++	+	-	-	-	+	-	-	++	-
B. boroniphilus / B. jeotgali / B. selenatarsenatis (1)	B671	+	++	-	-	-	-	+	w	-	++	-
Bacillus niabensis(1)	B579	+	++	+	-	-	-	+	+	+	++	-
Bacillus licheniformis(1)	B699	+	++	+	+	-	-	+	+	+	++	+
Bacillus flexus(1)	B625	+	++	+	+	-	-	+	+	+	++	+
Bacillus gibsonii(1)	B578	+	++	++	+	-	-	+	+	+++	++	-
Bacillus clausii(1)	B623	+	++	-	-	-	-	+	-	+	++	-
Jeotgalibacillus campisalis(2)	B640	+	++	+	-	-	-	+	+	++	++	-
	B639	+	++	+	-	-	-	+	+	+	++	-
Planococcus rifietoensis(2)	B654	+	++	+	+	-	-	+	+	++	++	-
	B688	+	++	+	+	-	-	+	+	+	++	-
Staphylococcus warnerii(2)	B684	+	++	+	+	+	+	+	+	+	++	-
	B635	+	++	+	+	+	-	+	+	++	++	-
Thalassobacillus devorans(1)	B596	+	++	+	-	-	-	+	+	-	++	-
Jeotgalibacillus salarius(1)	B657	+	++	+	+	-	-	+	+	-	++	-
Staphylococcus hominis(1)	B644	+	++	-	-	-	+	+	+	w	++	-
Bacillus sp.(9)	B633	+	++	-	-	-	-	+	+	-	++	-
	B598	+	++	+	-	-	-	+	+	-	++	-

Table 3. Contd.

Bacillus sp.(9)	B658	+	++	+	w	-	-	+	+	-	++	+++
	B632	+	++	-	-	-	-	+	+	+	++	-
	B617	+	++	++	w	-	-	+	+	-	++	-
	B615	+	++	++	+	+	-	+	+	+	++	-
	B647	+	++	+	+	-	-	+	+	++	++	-
	B703	+	++	-	-	-	-	+	-	-	++	-
	B673	+	++	-	-	-	-	+	+	-	++	-
Vibrio alginolyticus(2)	B641	+	++	++	-	-	-	+	++	-	++	-
	B708	+	++	+	-	-	-	+	-	-	++	-
Vibrio atypicus (1)	B696	+	++	++	-	-	-	+	++	-	++	-
Vibrio fluvialis(1)	B664	+	++	w	-	-	-	+	+	-	++	-
Vibrio azureus(1)	B705	+	+	++	-	-	-	+	+	++	++	w
Vibrio azureus / V.rotiferianus(1)	B694	+	++	++	+	-	-	+	-	-	++	-
Salinivibrio costicola subsp. *costicola* (2)	B689	+	++	+++	+++	+	-	+	++	++	++	-
	B680	+	++	++	++	-	-	+	+	-	++	-
Salinivibrio costicola subsp. *vallismortis*(1)	B659	+	++	+	+	+	-	+	+	+++	++	-
Halomonas salina(1)	B594	-	+	++	+	+	-	+	+	++	++	-
Halomonas salina / H. halophila(1)	B595	-	+	++	+	+	-	+	+	++	++	-
H. saccharevitans(1)	B661	-	+	+	+	+	-	+	+	+	++	-
Psychrobacter piscatorii(1)	B706	+	+	+	-	-	-	+	-	+++	++	-
Photobacterium halotolerans (1)	B643	+	++	+	-	-	-	+	+	++	++	-
Vibrio sp.(3)	B672, B663	+	++	+	-	-	-	+	+	++	++	-
	B642	+	++	-	-	-	-	+	+	-	++	w
Photobacterium sp.(1)	B665	+	++	-	-	-	-	+	+	++	++	-
Salinivibrio sp.(1)	B638	+	++	++	+	+	-	+	+	+++	++	-
Halomonas sp.(1)	B660	-	++	+	+	+	-	+	+	-	++	-
Marinobacterium sp.(1)	B678	+	++	-	-	-	-	+	+	-	++	-
Pseudoalteromonas sp.(1)	B662	+	++	+	+	w	-	+	-	-	++	-

Symbols: +, positive growth; w, weak growth; -, no growth.

activities tested (Table 4). Among the 124 isolates tested, only the *S. warnerii* isolate showed lipase activity.

Four combined hydrolytic activities were detected in 12 strains (Table 4). In addition, 28 strains showed three combined hydrolase activities and 40 strains were able to produce two extracellular enzymes (Table 4).

DISCUSSION

Until now,no studies have addressed the characterization of halophilic bacteria in protected ecosystems in Morocco. Based on 16S rRNA gene sequencing and phenotypic properties we identified and characterized 124 moderately halophilic and halotolerant bacteria in sediments from a marsh and salterns in a protected wetland located in the Northwest of Morocco (Table 1).

Cultivation conditions

Halophilic bacteria were present in all sediment samples analysed. Their counts on Columbia supplemented with salt varied from 5×10^5 CFU/g in salterns to 2×10^7 CFU/g in the salt marsh which is in accordance with the counts previously reported in saline sediments (Sass et al., 2008). The distribution of the total isolates is reported in Table 1. The number of genera and species

Table 4. Hydrolytic exoenzymes activities of moderately halophilic species isolates.

Species (Number of strains)	Hydrolytic activity					
	Number of strains	Amylases	Lipases	DNases	Proteases	Cellulases
Gram-positive (102)						
Bacillus aquimaris (32)	(3)	+	-	+	+	+
	(7)	+	-	+	-	+
	(1)	+	-	+	+	-
	(1)	++	-	-	+	-
	(1)	-	-	+	+	-
	(4)	+	-	+	-	-
	(4)	+	-	-	-	+
	(7)	+	-	-	-	-
	(2)	-	-	-	-	+
	(1)	-	-	-	+	-
	(1)	-	-	-	-	-
Bacillus aquimaris / Bacillus vietnamensis (2)	(1)	+	-	+	+	+
	(1)	-	-	+	+	+
Bacillus megaterium (9)	(5)	+	-	+	+	+
	(1)	+	-	-	+	+
	(1)	+	-	+	+	-
	(2)	-	-	+	+	+
Bacillus megaterium / Bacillus flexus(1)	(1)	+	-	-	-	-
Bacillus oceanisediminis (8)	(2)	+	-	+	-	+
	(1)	+	-	-	-	+
	(3)	+	-	+	-	-
	(1)	-	-	-	+	+
	(1)	-	-	+	-	-
Bacillus hwajinpoensis (7)	(2)	+	-	+	+++	-
	(1)	-	-	+	+	-
	(2)	-	-	+	-	-
	(1)	-	-	-	+++	-
	(1)	w	-	-	-	-
Bacillus safensis (6)	(1)	+	-	+	+	w
	(3)	+	-	+	+	-
	(1)	-	-	+	++	-
	(1)	-	-	+	-	-
Bacillus safensis/ Bacillus pumilis(1)	(1)	-	-	+	++	-
Bacillus simplex(3)	(1)	+	-	+	-	-
	(2)	+	-	-	-	+
Bacillus muralis/ Bacillus simplex /Bacillus macroides(1)	(1)	-	-	-	-	+

Table 4. Contd.

Bacillus thioparans (2)	(1)	+	-	+	-	-
	(1)	-	-	+	-	-
Bacillus cereus (2)	(1)	+	-	-	-	-
	(1)	-	-	-	+	-
Bacillus jeotgali (1)	(1)	-	-	+	++	-
Bacillus boroniphilu /B. jeotgali(3)	(1)	+	-	-	+	-
	(1)	+	-	+	-	-
	(1)	-	-	-	-	-
Bacillus boroniphilus/ B.jeotgali/ B. selenatarsenatis (1)	(1)	+	-	+	-	-
Bacillus niabensis (1)	(1)	+	-	-	-	+
Bacillus licheniformis (1)	(1)	w	-	+	-	+
Bacillus flexus (1)	(1)	+	-	w	+	+
Bacillus gibsonii(1)	(1)	-	-	+	-	-
Bacillus clausii (1)	(1)	+	-	-	-	+
Jeotgalibacillus campisalis (2)	(1)	+	-	+	-	-
	(1)	+	-	-	-	-
Planococcus rifietoensis (2)	(1)	+	-	+	-	+
	(1)	-	-	++	w	-
Staphylococcus warneri (2)	(2)	-	++	-	-	-
Thalassobacillus devorans (1)	(1)	-	-	++	-	-
Jeotgalibacillus salarius (1)	(1)	-	-	++	-	-
Staphylococcus hominis (1)	(1)	+	-	+	+	+
Bacillus sp. (9)	(1)	+	-	+	+++	-
	(2)	+	-	+	-	-
	(1)	-	-	+	+	-
	(2)	-	-	+	-	-
	(1)	+	-	-	-	-
	(1)	-	-	+	-	-
	(1)	-	-	+	-	-
Gram-negative (22)						
Vibrio alginolyticus (2)	(2)	+	-	-	-	+
Vibrio atypicus (1)	(1)	+	-	-	-	+
Vibrio fluvialis (1)	(1)	-	-	-	-	+
Vibrio azureus (1)	(1)	-	-	-	-	+
Vibrio azureus / Vibrio rotiferianus (1)	(1)	+	-	+	-	+
Salinivibrio costicola subsp. *costicola* (2)	(2)	-	-	+	+	-
Salinivibrio costicola subsp. *vallismortis* (1)	(1)	+	-	-	+	-
Halomonas salina (1)	(1)	-	-	-	-	-
Halomonas salina / Halomonas halophila (1)	(1)	-	-	-	-	-
Halomonas saccharevitans (1)	(1)	-	-	-	-	-
Psychrobacter piscatorii (1)	(1)	-	-	-	-	-
Photobacterium halotolerans (1)	(1)	-	-	+	+	+

Table 4. Contd

	(1)	+	-	+	-	+
Vibrio sp. (3)	(1)	+	-	-	-	+
	(1)	-	-	+	-	+
Photobacterium sp. (1)	(1)	+	-	+	-	+
Salinivibrio sp. (1)	(1)	-	-	+	+++	+
Halomonas sp. (1)	(1)	-	-	-	-	-
Marinobacterium sp. (1)	(1)	-	-	-	-	-
Pseudoalteromonas sp. (1)	(1)	-	-	-	-	-

Symbols: +, exoenzyme production; ++ or +++ overproduction; w, weak production; - , no production.

found decreased from marsh to salterns, which is in agreement with the principle that increasing salinity decreases the microbial diversity of the ecosystem (Ventosa et al., 1998). Moreover, in the salt sediment, the type and the amount of substrate (e.g. Carbon substrate) in soil affect abundance of some bacterial phyla and only few of them can degrade different substrates and use their metabolic to do so (Fernández -Luqueño et al., 2008). Among the 124 isolates obtained, 107 originated from marsh (3% NaCl) and only 17 were from salterns (10% NaCl). In this study, 12 genera and 30 species were recovered, which indicates that there is a substantial diversity for the cultured strains within the bacteria that were isolated from marsh and salterns sediments. It should be emphasized that the same selection, that is, sampling strategy and growth conditions, was applied to isolate all these bacteria and the strains were selected in part because they had different colony and cell morphologies. One might assume that had the chemi-cal composition of sediment been determined, the kind of pollutants been known such as polycyclic aromatic hydrocarbons (PAHs) (Fernández -Luqueño et al., 2008, 2011) and selective conditions been varied, e.g. other growth media than Columbia agar or growth temperature used, the diversity would be even higher. The phenotypic analysis of the isolates demonstrated that the culturable fraction of the microbial community was largely dominated by Gram positive bacteria in all the samples studied.

Diversity recovered

Phylogenetic analysis based on 16S rDNA sequences reveals that among the 12 genera obtained from saline sediments the dominant genus is *Bacillus* with 93 strains representing 14 species indicating that *Bacillus* is well adapted to saline sediments. All other genera recovered are represented by four or less strains (Tables 1 and 2). In a previous study, it has been reported that Gram-positive bacteria assigned to *Bacillus* were extensively represented in saline soils (Ventosa et al., 2008). Most of them were classified as halotolerant micro-organisms, being able to grow, in most cases in presence of NaCl up to 25% (Kushner, 1985).

In this study, the use of 16S rDNA sequences as a main tool to identify halophilic bacteria demonstrate that this method is helpful since 96 isolates (77.5%) were identified at the species level with ≥ 99% similarity with their relative reference strains. Moreover, it turned out that four of the strains recovered in this study belonged to *Bacillus oceanisediminis* (Zhang et al., 2010), *Jeotgalibacillus salarius* (Yoon et al., 2010), *Vibrio atypicus* (Wang et al., 2010) and *Psychrobacter piscatorii* (Yumoto et al., 2010) which were described in 2010 as new species, and *B. oceanisediminis* is one of the dominating species (Table 2).

Based on 16S rDNA sequence comparison and phylogenetic inference, five dominant phylotypes were determined. They belonged to *B. aquimaris* (32 isolates), *Bacillus megaterium* (9 isolates), *B. oceanisediminis* (8 isolates), *Bacillus hwajinpoensis* (7 isolates), *Bacillus safensis* (6 isolates). Nevertheless, there are some physiological differences between strains belonging to the same species, such as tolerance of NaCl, temperature and pH for growth and production of hydrolytic enzymes. This observation is valid even for phylotypes represented by four or less strains (Tables 3 and 4). This finding demonstrates that these phylotypes contain considerable genetic variation despite their high (≥ 99%) 16S rDNA sequence similarity, which is in agreement with previous reports (Adiguzel et al., 2009; Papalexandratou et al., 2009). This explains why we failed to cluster isolates belonging to the same phylotype using $(GTG)_5$-PCR and BOXA1R-PCR fingerprinting although it has been reported that rep-PCR has a higher taxonomic resolution than 16S rRNA gene sequencing (Ishii and Sadowsky, 2009).

Seventeen isolates assigned to *Bacillus* sp. (9 isolates), *Vibrio* sp. (3 isolates), *Halomonas* sp. (1 isolate),

Marinobacterium sp. (1 isolate), *Salinivibrio* sp. (1 isolate), *Photobacterium* sp. (1 isolate) and *Pseudoalteromonas* sp. (1 isolate) were identified as potentially novel species based on their 16S rDNA sequence analysis. These isolates need to be assessed by further characterization. The species identity of eleven other isolates B620, B588, B614, B704, B653, B599, B671, B668, B692, B595 and B694 remained unresolved as they are equally similar to two or three species with high percentage of similarity with the closest reference strains. In these cases the species identity needs to be clarified by another genomic method such as house-keeping gene sequencing and/or DNA/DNA hybridization.

Distribution of halophilic bacteria in different sediments examined

In this work we uncovered a rich biodiversity of cultural bacteria present in sediments from a marsh and two salterns situated in a Moroccan region with intense agricultural activity and raising livestock. The majority of the genera and species recovered in this study were from marsh sediments and have previously been described in saline environments except *B. safensis, Bacillus thioparans* and *B. simplex* (Ventosa et al., 1998; Okamoto et al., 2001; Yoon et al., 2001; 2003; 2004; 2010; Chookietwattana, 2003; Sánchez-Porro et al., 2003; Borsodi et al., 2005; Fan et al., 2006; Pérez-Ibarra et al., 2007; Ventosa et al., 2008; Parvathi et al., 2009; Sasaki et al., 2009; Zhang et al., 2010; Bassey and Grigson, 2011). This finding extends our knowledge of the bacterial diversity in saline environments and indicates that the groups obtained, including potential new species, are well adapted.

Our study showed that diverse and abundant bacterial isolates could secrete at least one of the extracellular enzymes screened indicating that the *in situ* microbiota may have developed genetic and physiological adaptivity for utilizing organic matter, especially in sediments from marsh, via exoenzymes production. Some strains even harboured all the extracellular hydrolytic enzymes screened except lipase. The microbial ecophysiology may present a good bio-indicator of terrestrial impact on marsh and salterns environment located in a Moroccan protected ecosystem. The diverse extracellular enzymes detected in the current study might also provide a resource for novel biocatalyst discovery and biotechnological application.

Conclusion

This is the first identification and characterization of the bacterial microflora in the Moroccan saline environment. Out of the 124 halophilic bacteria isolated in this study, we identified 11 isolates as belonging to the species *B. oceanisediminis*, *J. salaries, V. atypicus* and *P. piscatorii* that were described in 2010 as new species, 17 isolates in different genera represent potentially novel species, which means they should be characterized using other methods and 11 isolates belonged to different species should be clarified. The rich microbial diversity found, indicates that this is an important region for further investigation by combining different cultures conditions (media and growth temperature) and other molecular methods. The abundance of the genus *Bacillus* and their high diversity observed in this work point out the need for more extensive studies to understand their distribution and ecology in this protected environment and their relation to other microorganisms, e.g. halophilic Archaea.

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