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Full Length Research Paper

Isolation, characterization and antimicrobial activity of halophilic bacteria in foreshore soils

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Halophilic bacteria are commonly found in natural environments containing significant concentration of NaCl. Two hundred halophilic bacteria were isolated. Among which, 63 strains were tested for the antimicrobial activity against 10 pathogenic bacteria, three yeast and nine pathogenic fungi. Of these isolates, only 12 strains showed activity against the test microorganisms, namely *Pseudomonas aeruginosa*, *Serratia marcescens*, *Enterobacter cloacae*, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Escherichia coli*, *Bacillus subtilis*, *Candida albicans*, *Colletotrichum gloeosporioides*, *Fusarium solani* and *Alternaria alternata.* These 12 active isolates were identified to be of the genera, *Streptomyces* (seven strains), *Microbacterium* (one strain), *Micrococcus* (one strain), *Bacillus* (one strain), *Planococcus* (one strain) and *Marinobacter* (one strain). Seven strains were active against pathogenic bacteria and five strains against pathogenic fungi. Two strains SC-2 and SC-13 were found to have a broad spectrum of activity against all tested pathogenic bacteria and yeasts but not against pathogenic fungi. The strains SC-2 and SC-13 were closely related to *Microbacterium oxydans* (99%) and *Streptomyces fradiae* (99%) respectively. These findings show that the foreshore soil of Daecheon Beach and Saemangeum Sea of Korea represents an untapped source of bacterial biodiversity, and also that most actinobacterial isolates are capable of antibacterial and antifungal metabolite production.

Key words: Culturable diversity, halophilic bacteria, foreshore soil.

INTRODUCTION

Halophilic bacteria are adapted to high osmolarity and can grow in high saline environments. These bacteria are categorized as slightly, moderately and extremely halophiles, according to the extent of their halotolerant characteristics (Larsen, 1986; Ventosa et al., 1998, Ventosa, 2006). These bacteria were isolated from saline environments such as ocean, salt lakes and salt fields (Mimura and Nagata, 2000; Carrasco et al., 2006; Yuan et al., 2007; Fukushima et al., 2007). The ecological characteristics (diversity, distribution and composition) of halophic bacteria in saline and hypersaline habitats have been extensively investigated (Jiang et al., 2007; Mancinelli, 2005; Oren, 2002a, b). Halophilic bacteria are able to grow at higher saline environments because they synthesize compatible solutes in cells or possess the transporters that take them up in the medium. Such osmoregulatory solutes as potassium ion, glutamate, proline, ectoine, betaine in bacteria have been reported (Galinski, 1993). In foreshore soils, the microorganisms

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Table 1. Samples used in this study.

Code	Sample	Site
A	Foreshore soil	Deacheon, Chungnam
B	Foreshore soil	Saemangeum, Jeonbuk

are adapted to live in extreme saline environment. Foreshore soil is enriched with salts and nutrients which provides a conducive environment for the growth of halophilic microorganisms. Therefore, in this study, samples were collected from the foreshore soil in order to determine the halophilic bacterial diversity associated with marine environment.

Halophilic bacteria provide a high potential for biotechnological applications for at least two reasons: (1) their activities in natural environments with regard to their participation in biogeochemical processes of C, N, S and P, the formation and dissolution of carbonates, the immobilization of phosphate and the production of growth factors and nutrients (Rodriguez-Valer, 1993); and (2) their nutritional requirements are simple. Majority of them can use a broad range of compounds as their sole carbon and energy source. Most of them can grow at high salt concentrations, minimizing the risk of contamination (Birbir and Ilgaz, 1996). Moreover, several genetic tools developed for the nonhalophilic bacteria can be applied to the halophiles, and hence their genetic manipulation seems feasible (Ventosa et al., 1998). Halophilic bacteria are used for the production of compatible solutes, hydrolytic enzymes, exopolysaccharides, osmoprotectants, stabilizers of enzymes and cells (Margesin and Schinner, 2001; Lee et al., 2003; Birbir and Ilgaz, 1996; Onishi, 1970; Onishi et al., 1983, 1991; Oren, 1999). Some of them are used for the degradation of polluting industrial residues or toxic chemicals and for enhancing oil-recovery processes (Ventosa and Nieto, 1995; Birbir and Ilgaz, 1996).

In this study, the halophilic bacteria diversity of foreshore soil (Daecheon Beach, Chungnam Province and Saemangeum Sea, Jeonbuk Province in Korea) and antimicrobial activity of halophilic bacterial isolates were examined.

The aim of this study was to evaluate halophilic actinobacterial diversity in foreshore soil and to test the isolates for antagonistic activity. To our knowledge, this is the first extensive report on halophilic actinobacteria isolation, exhibiting antimicrobial activity from foreshore soil, Korea.

MATERIALS AND METHODS

Collection of samples

The sampling sites are located in Chungnam and Jeonbuk Provinces in Korea. In Chungnam Province, the sample was collected from the foreshore soil of Daecheon Beach. In Jeonbuk Province, samples were collected from the foreshore soil of Saemangeum Sea (Table 1). The samples were stored at 4°C until they reached the laboratory.

Media and isolation of halophilic bacteria

The procedure of heat treatment used was previously described by Seong et al. (1993). For the isolation, 10 ml of sterile 1/4 strength Ringer's solution (2.25 g sodium chloride, 0.105 g potassium chloride, 0.12 g calcium chloride and 0.05 g sodium bicarbonate in 1 L distilled water) was added to 1 g soil sample. The tenfold diluents were shaken on reciprocal shaker for 20 min and then incubated at 50°C for 15 min. All media contained Difco Bacto agar (18 g l^{-1}) at pH 7.0. Aliquots (0.2 ml) of each dilution were spread on media plates using 6 selected media (Table 2) and were incubated at 30°C for two to four weeks. Distinct colonies were selected and purified by repeated streaking on the same media. The purified isolates were stored in 20% glycerol at -80°C.

Cultivation of strains

Sixty three (63) strains were selected using the morphological differences based on visible examination of the growth characteristics (aerial mycelium, substrate mycelium and diffusible figments). For DNA extraction, the selected strains were cultured in Bennett's Broth and M1-M4 (Broth) media for 7 days at 30°C.

DNA extraction

Total DNA was extracted from one week-old cultures using the method described previously (Cho et al., 2006). Cells were taken into Eppendorf tube containing 200 µl of STES buffer (500 mM NaCl, 200 mM Tris-HCl (pH 7.6), 10 mM EDTA, 1% SDS) and glass beads. The mixture was vortexed for 15 min by TOMY micro tube mixer (TOMY, Japan). The suspension was supplemented with 100 µl lysozyme (50 mg/ml) and incubated for 2 h at 37°C, then lysed completely by adding 100 µl TE buffer (pH 8.0). An equal volume of phenol/chloroform/isoamyl-alcohol (24:25:1, v/v) was added to the supernatant and centrifuged at 5,000 rpm for 15 min. After repetition of the above step, it was treated with 3 µl of RNase A (10 mg/ml). Total DNA in the supernatant was precipitated by adding 0.6 volume of isopropanol, collected by centrifugation and the precipitate was washed twice with 70% aqueous ethanol, then dried in air for 20 min and dissolved in 50 µl distilled water for 1 h at 65°C. The size and amount of extracted DNA were determined by Agarose gel (1%). Extracted DNA was stored at -20°C until needed.

PCR amplification and sequencing of 16S rDNA

PCR amplification of 16S rDNA was carried out using two universal primers, 27f (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 1492r (5′-GGY TAC CTT GTT ACG ACT T-3′), which were also used for sequencing. The PCR consisted of an initial denaturation step at 95°C for 3 min, which was followed by 30 cycles of 95°C for 1 min, 55°C for 40 min and 72°C for 1 min. Sequencing was performed using the service of Solgent Co. (Korea).

Phylogenetic analysis

The 16S rDNA sequences of the isolates were compared with the sequences available by the BLAST search in the NCBI, GenBank

Table 2. Composition of six different media.

Media	Composition	Reference
M1	6 mL 100% glycerol, 1 g arginine, 1 g K_2HPO_4, 0.5 g $MgSO_4$, 18 g agar, and 1 L of natural seawater	(Mincer et al., 2002)
M2	0.1 g L-asparagine, 0.5 g K_2HPO_4, 0.001 g $FeSO_4$, 0.1 g $MgSO_4$, 2 g peptone, 4 g sodium propionate, 20 g NaCl, 18 g agar, and 1 L of water	(Zhang et al., 2006)
M3	2 g peptone, 0.1 g asparagine, 4 g sodium propionate, 0.5 g K_2HPO_4, 0.1 g $MgSO_4$, 0.001 g $FeSO_4$, 5 g glycerol, 20 g NaCl, 18 g agar, and 1 L of water	(Webster et al., 2001)
M4	4 g yeast extract, 15 g soluble starch, 1 g K_2HPO_4, 0.5 g $MgSO_4$, 20 g NaCl, 18 g agar, and 1 L of water	(Webster et al., 2001)
SCA	10 g soluble starch, 0.3 g vitamin-free casein, 2.0 g potassium nitrate, 2.0 g NaCl, 0.05 g $MgSO_4$, 0.02 g $CaCO_3$, 0.01 g ferric sulphate, 5.45 g potassium phosphate, 18 g agar and 1 L of water	This study
R2A	0.5 g yeast extract, 0.5 g proteose peptone, 0.5 g casamino acids, 0.5 g dextose, 0.5 g soluble starch, 0.3 g sodium pyruvate, 0.3 g dipotassium phosphate, 0.05 g magnesium sulphate, 15.0 g agar and 1 L of water	This study

database (http://www.ncbi.nlm.nih.gov). The sequences were proofread, edited and merged into comparable sequences using the PHYDIT program version 3.0 (available at http://plaza.snu.ac.kr/~jchun/phydit/). The sequences were aligned together with those of reference taxa retrieved from public databases. The evolutionary distances were generated based on parameter model (Jukes and Cantor, 1969) and phylogenetic trees were constructed by using the neighbor-joining method (Saitou and Nei, 1987).

Antimicrobial activity

A total of 63 strains were tested for their ability to inhibit growth of indicator microorganisms which were obtained from Korean Collection for Type Cultures (KCTC), Culture Collection of Antimicrobial Resistant Microorganisms (CCARM) and Korean Agricultural Culture Collection (KACC).

The indicator bacterial strains *(Pseudomonas aeruginosa* CCARM 0222, *Serratia marcescens* CCARM 0255, *Enterobacter cloacae* CCARM 0250, *Staphylococcus aureus* CCARM 0203, *Salmonella typhi* CCARM 0242, *Klebsiella pneumoniae* CCARM 0015, *Micrococcus luteus* CCARM 0022, *Escherichia coli* CCARM 0227, *Bacillus subtilis* CCARM 0003 and *Staphylococcus aureus* CCARM 3089) and yeast strains (*Candida albicans* KCTC 7270, *C. albicans* KCTC 7965 and *C. albicans* KCTC 7753) were used. The pathogenic fungi used in the bioassay included *Phytophthora capsici* KACC 40476, *Colletotrichum gloeosporioides* KACC 40003, *Fusarium solani* KACC 41093, *Sclerotinia scleotiorum* KACC 41065, *Rhizoctonia solani* KACC 40113, *Alternaria alternata* KACC 42131, *Alternaria mali* KACC 40846, *Fusarium oxyporum* KACC 41083 and *Pyricularia grisea* KACC 40414 (Table 3). For the test of antimicrobial activities against selected pathogens, the strains were grown in GSS medium (1% soluble starch, 2% glucose, 2.5% soybean meal, 0.4% beef extract, 2% NaCl, 0.025% potassium dihydrogen phosphate and 0.2% calcium carbonate; pH 7.2). The shake flasks were cultivated at 30°C for 10 days with 180 rpm. Both the biomass and culture filtrates were tested for inhibition of bacterial pathogens for selected strains.

For testing of antimicrobial activity, the paper disks with each extract solution (60 µl) were placed on the test media, which had been inoculated with the test organisms. Plates were incubated for 24 h at 37°C in the case of bacteria and 28°C for 4 days in the case of fungi. The antimicrobial activities were then determined by measuring the respective zones of inhibition in mm.

RESULTS

Isolation of halophilic bacteria and effect of isolation media on the recoverability of marine bacteria

Significant differences in the total number of isolates recovered were observed among 6 different media (Table 2). Two hundred bacterial strains were selected from isolation plates. SCA media produced the highest recovery with 71 isolates, followed by M1 (64 isolates), M3 (21 isolates), M4 (19 isolates), R2A (13 isolates) and M2 (12 isolates). Sixty three strains were selected using the morphological differences based on visible examination of the growth characteristics, and were used for further analysis. The 16S rRNA genes of the 63 strains were sequenced and the genera of specific strains were determined after BLAST analysis. It was found that the marine bacteria diversity recovered in the different media also varied considerably. M1 and M4 had the best recoverability, with 5 of the total 11 genera recovered; 4 genera were recovered from SCA and M2; and only 2 genera were recovered from M2 and R2A. Among 6 different isolation media tested, halophilic actinobacteria were recovered on 5 media only, producing a total of 20 strains with different morphologies. SCA produced the highest recovery with 9 isolates, followed by M1 (7 isolates), M4 (2 isolates), M3 (1 isolate) and R2A (1 isolate). No halophilic actinobacteria were isolated from M2 media. *Streptomyces* was recovered only on two media, which was the dominant genus of halophilic actinobacteria.

Table 3. Antimicrobial activity of the tested isolates.

Test organisms	SC-2	SC-13	SC-19	SC-20	SC-27	SC-31	M1C-6	M1C-15	M1A-1	M4B-1	M4C-1	M4A-2
Candida albicans 7270	+++	+++	+	-	-	-	-	-	-	-	-	-
Candida albicans 7965	++	++	+	-	-	-	-	-	-	-	-	-
Candida albicans 7753	++	++	-	-	-	-	-	-	-	-	-	-
Pseudomanas aeruginosa 0222	++	++	-	-	-	-	-	-	-	-	-	-
Serratia marcescens 0255	+	+	-	-	-	-	-	-	-	-	-	-
Enterobacter cloacae 0250	++	++	-	-	-	-	-	-	-	-	-	-
Staphylococcus aureus 0203	++	+++	-	-	+	-	+	-	-	-	-	-
Salmonella typhi 0242	++	++	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae 0015	++	++	-	-	-	-	-	-	-	-	-	-
Micrococcus luteus 0022	++	+++	++	++	++	++	++	-	-	-	-	-
Escherichia coli 0227	++	++	-	-	-	-	-	-	-	-	-	-
Bacillus subtilis 0003	++	++	-	-	-	-	-	-	-	-	-	-
Staphylococcus aureus 3089	+	+	-	-	-	-	-	-	-	-	-	-
Collectotrichum gloeosporioides 40003	-	-	-	-	-	-	-	++	+	-	-	-
Alternaria alternata 42131	-	-	-	-	-	-	-	++	++	-	-	-
Fusarium solani 41093	-	-	-	-	-	-	-	-	-	+	+	+

The antimicrobial activity is expressed by the diameter of inhibition zone, (-) No activity observed; (+), up to 7 mm inhibition zone; (++), 10 mm inhibition zone; (+++), 15 mm inhibition zone.

Phylogenetic analysis based on 16S rDNA sequences

Phylogenetic analysis based on 16S *rDNA* gene sequences revealed that 63 isolated strains could be further affiliated with 3 diverse phylogenetic groups, e.g. 23 strains of Firmicutes, 20 strains of Proteobacteria and 20 strains of Actinobacteria. *Bacillus* was the dominant genus within phylum Firmicutes (22 isolates), and only one isolate belonged to genus *Planococcus* (Figure 1). Eighteen strains belonging to genus *Bacillus* shared 99% similarity of the 16S rDNA sequences with previously published species. Only two strains showed 96 to 98% 16S rDNA similarity with known species, and thus would represent novel species (Table 4). Among 20 strains of Proteobacteria, seven strains belong to genus *Pseudomonas*, seven strains belongs to genus *Halomonas*, four strains belong to genus *Yangia*, one strain belongs to genus *Marinobacter* and one strain belongs to genus *Psychrobacter*. Only three strains showed 98% 16S rDNA similarity with known species, and thus would represent novel species (Table 5). Among 20 strains of Actinobacteria, fifteen belongs to genus *Streptomyces*, three strains belong to genus *Micrococcus*, one strain belongs to genus *Microbacterium* and one strain belongs to genus *Isoptericola*. Only six strains showed 96 to 98% 16S rDNA similarity with known species, and thus would represent novel species (Table 6).

The proteobacterial isolates mainly belonged to the genera *Pseudomonas* (7 isolates), *Halomonas* (7 isolates) and *Yangia* (4 isolates). Bacteria belonging to genera *Marinobacter* (1 isolate) and *Psychrobacter* (1 isolate) were found as minor groups (Figure 2). Of the 20 isolated actinobacteria, 15 belongs to the genus *Streptomyces*, which was the dominant actinobacterial genus, and 5 isolates belongs to 3 other actinobacterial genera, e.g *Micrococcus* (3 strains), *Microbacterium* (1 strain) and *Isoptericola* (1 strain) (Figure 3).

Antimicrobial activity

When 63 isolated strains were tested for antimicrobial activity, only 12 strains showed activity against tested microorganisms. Seven

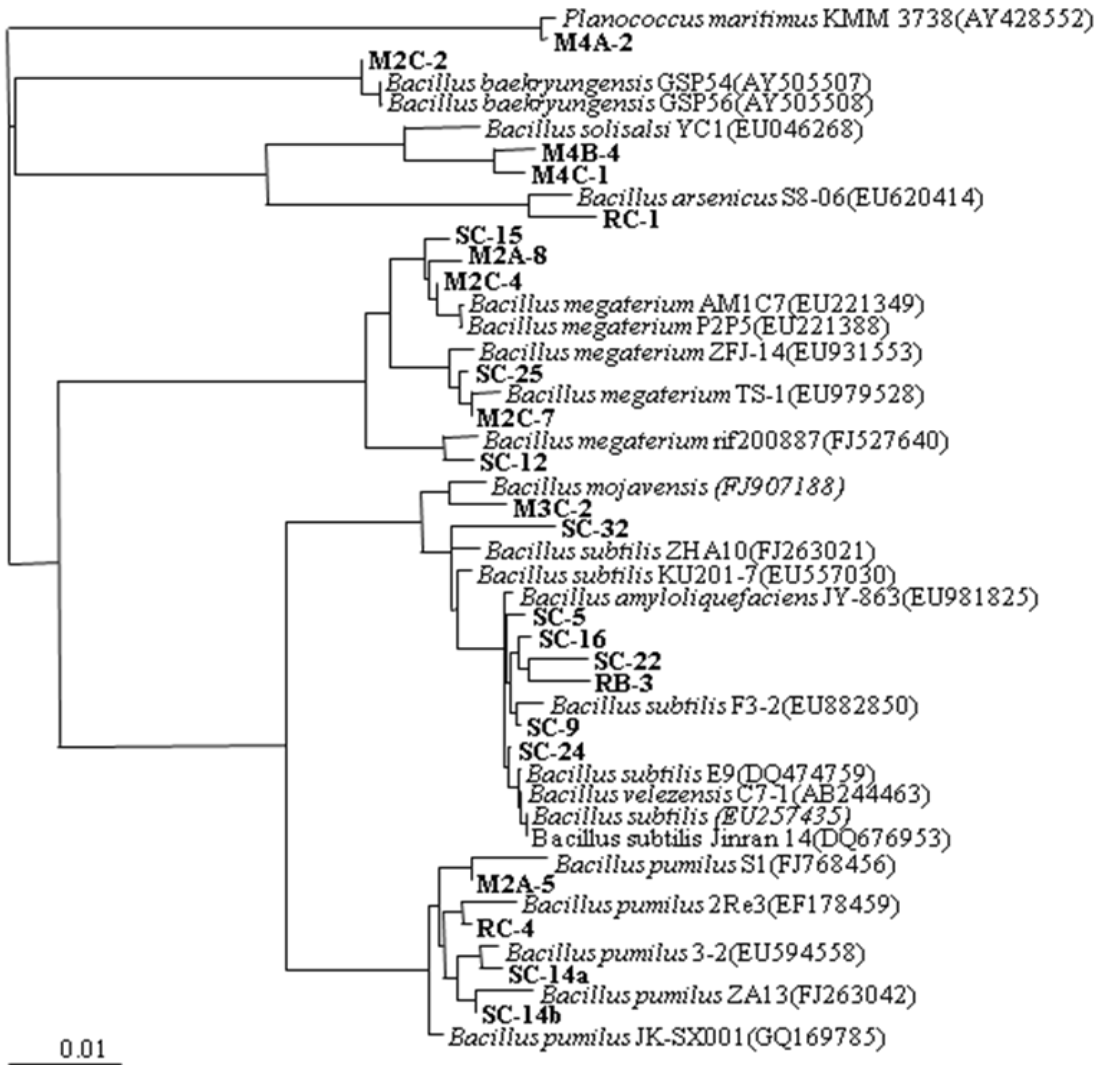

Figure 1. Phylogenetic tree based on 16S rDNA sequences showing the relationships among the representatives of *Bacillus, Planococcus* and their phylogenetic relatives. The strains isolated in this study are indicated in bold.

strains (SC-2, SC-13, SC-19, SC-20, SC-27, SC-31 and M1C-6) showed inhibitory activity against pathogenic bacteria and yeasts. All 7 strains were active against *Micrococcus luteus* CCARM 0022. A high level of antimicrobial activity was revealed in two strains. Strains SC-2 and SC-13 showed activity against all the tested pathogenic bacteria and yeasts, but not against fungi. Strains SC-2 and SC-13 were closely related to *Microbacterium oxydans* (99%) and *Streptomyces fradiae* (99%) respectively. Strain SC-19 was active against *C. albicans* KCTC 7270*, C. albicans* KCTC 7965 and *M. luteus* CCARM 0022 and was closely related to *Streptomyces viridobrunneus* (99%). Strains SC-20 *(Streptomyces drozowiczii*) and SC-31 *(Streptomyces griseus)* were active against *M. luteus* CCARM 0022. Strains SC-27 and M1C-6 showed activity against *S. aureus* CCARM 3089 and *M. luteus* CCARM 0022. Five isolates displayed inhibitory activity against three pathogenic fungi, including *C. gloeosporioides, F. solani* and *A. alternata*. Three stains: M4B-1, M4C-1 and M4A-2 were active against *F. solani.* Two strains: M1C-15 and M1A-1 showed activity against *C. gloeosporioides* and *A. alternate* (Table 3). These reports strongly suggest that foreshore soil samples can be the potent source of antimicrobial compounds.

Based on the phylogenetic analysis of the 16S rDNA sequence, nine strains were assigned to the actinobacterial groups. Among these strains, seven

Table 4. Summary of information on 16S rDNA sequence determination of isolated strains belonging to Firmicutes.

Strain no.	Accession no.	Highest match	Similarity (%)
SC.5	EU257435	*Bacillus subtilis*	100
SC.9	EU882850	*Bacillus subtilis*	99
SC.12	FJ641033	*Bacillus megaterium*	99
SC.14.a	EU594558	*Bacillus pumilus*	99
SC.14.b	FJ263042	*Bacillus pumilus*	99
SC.15	EU221388	*Bacillus megaterium*	100
SC.16	EU981825	*Bacillus amyloliquefaciens*	99
SC.22	EU981825	*Bacillus amyloliquefaciens*	98
SC.24	DQ676957	*Bacillus subtilis*	99
SC.25	EU979528	*Bacillus megaterium*	99
SC.32	FJ263021	*Bacillus subtilis*	99
M2A.5	GQ169785	*Bacillus pumilus*	99
M2A.8	EU221388	*Bacillus megaterium*	99
M2C.2	AY505508	*Bacillus baekryungensis*	99
M2C.4	EU221388	*Bacillus megaterium*	99
M2C.7	EU979528	*Bacillus megaterium*	99
M3A.5	FJ435213	*Bacillus cereus*	98
M3C.2	FJ907189	*Bacillus mojavensis*	99
M4B.4	EU046268	*Bacillus solisalsi*	96
M4C.1	EU046268	*Bacillus solisalsi*	98
RB.3	EU981825	*Bacillus amyloliquefaciens*	99
RC.1	EU620414	*Bacillus arsenicus*	99
RC.4	EF178459	*Bacillus pumilus*	99
M4A.2	NR_025247	*Planococcus maritimus*	99

Table 5. Summary of information on 16S rDNA sequence determination of isolated strains belonging to Proteobacteria.

Strain no.	Accession no.	Highest match	Similarity (%)
M1A.1	NR_025799	*Marinobacter flavimaris*	99
M1C.38	DQ224384	*Pseudomonas stutzeri*	98
M1C.23	DQ288952	*Pseudomonas putida*	99
SC.3	FJ768951	*Pseudomonas stutzeri*	99
SC.10	NR_025972	*Pseudomonas balearica*	98
SC.11	FJ768951	*Pseudomonas stutzeri*	99
SC.17	EU440977	*Pseudomonas pseudoalcaligenes*	99
SC.18	EU440977	*Pseudomonas pseudoalcaligenes*	99
M3A.4	FJ613610	*Psychrobacter celer*	98
M2C.1	AY616755	*Halomonas variabilis*	99
M3A.2	AY245449	*Halomonas boliviensis*	99
M3A.3	AY616755	*Halomonas variabilis*	99
M3C.8	AY616755	*Halomonas variabilis*	99
M1A.4	AM229317	*Halomonas denitrificans*	99
M1C.10	EF144149	*Halomonas saccharevitans*	99
M1C.24	AM229317	*Halomonas denitrificans*	99
M1C.12	AJ877265	*Yangia pacifica*	99
M1C.14	AJ877265	*Yangia pacifica*	99
M1C.18	AJ877265	*Yangia pacifica*	99
M1C.20	AJ877265	*Yangia pacifica*	99

belongs to the genus *Streptomyces* and the remaining two strains belong to the genera *Mycobacterium* and *Micrococcus*, respectively (Figure 3). These results are in line with the previous studies by Manivasagan et al. (2009). A single isolate belonged to the genera *Marinobacter*, *Bacillus* and *Planococcus*, respectively. Seven isolates showed inhibitory activity against pathogenic bacteria and yeasts, whereas five isolates were active against fungi. A high level of antimicrobial activity was observed in two strains. The strains SC-2 and SC-13 were more active against all tested pathogenic bacteria and yeasts (Figure 4). The strains SC-2 and SC-13 were closely related to *M. oxydans* (99%) and *Streptomyces fradiae* (99%) (Table 6).

DISCUSSION

The data presented here show that the foreshore soil of Daechon Beach and Seamangeum Sea of Korea represent an untapped source of halophilic bacteria biodiversity. 6 different media were used for the isolation of halophilic bacteria. Among the tested media, SCA medium produced the highest recovery with 71 isolates. SCA is the modified medium in which 2% NaCl was added for the growth of halophilic bacteria. This shows that our modified medium applied in the present study have much better results in terms of the recovery of halophilic bacteria in comparison with other reported media (Mincer et al., 2002; Zhang et al., 2006; Webster et al., 2001). These results are in compliance with the findings of MacLeod (1965) and Oh et al. (1991) who stated that sodium is required for growth of marine bacteria which are indicative of highly evolved marine adaptations, such as a respiration-dependent sodium ion pump and/or a sodium-dependent membrane transport mechanism.

Several studies have been made to describe the reliability of 16S rDNA sequence analysis for bacterial identification (Thurlow and Gillock, 2005). Therefore, sequencing of 16S rDNA was used as a tool for genotypic characterization of halophilic bacteria in the present study. Sequence analysis of the 16S rRNA gene is one of the most reliable methods to delineate phylogenetic relationships among bacteria. Even though the sequence of 16S rRNA gene is highly conserved among bacteria, however it still contains variable regions

Table 6. Summary information of 16S rDNA sequence determination of isolates belonging to Actinobacteria.

Strain no.	Accession no.	Highest match	Similarity (%)
M3A.1	FJ357615	*Micrococcus luteus*	99
M4B.1	EU438932	*Micrococcus luteus*	98
RB.2	FJ380993	*Micrococcus luteus*	99
SC.2	EU918731	*Microbacterium oxydans*	99
M4A.3	AY789835	*Isoptericola halotolerans*	98
M1C.2	FJ773992	*Streptomyces viridochromogenes*	99
M1C.6	EU570721	*Streptomyces anulatus*	99
M1C.7	EU570526	*Streptomyces microflavus*	98
M1C.11	FJ486289	*Streptomyces anulatus*	99
M1C.15	EU570682	*Streptomyces sampsonii*	99
M1C.16	EF192235	*Streptomyces griseus*	98
M1C.32	EU841652	*Streptomyces hawaiiensis*	99
M1C.34	FJ792550	*Streptomyces bacillaris*	99
SC.6	EU273549	*Streptomyces globisporus*	99
SC.8	EU841581	*Streptomyces aureus*	96
SC.13	DQ026630	*Streptomyces fradiae*	99
SC.19	AJ781372	*Streptomyces viridobrunneus*	99
SC.20	AM921646	*Streptomyces drozdowiczii*	98
SC.26	AB184793	*Streptomyces olivaceus*	100
SC.31	EU647493	*Streptomyces griseus*	99

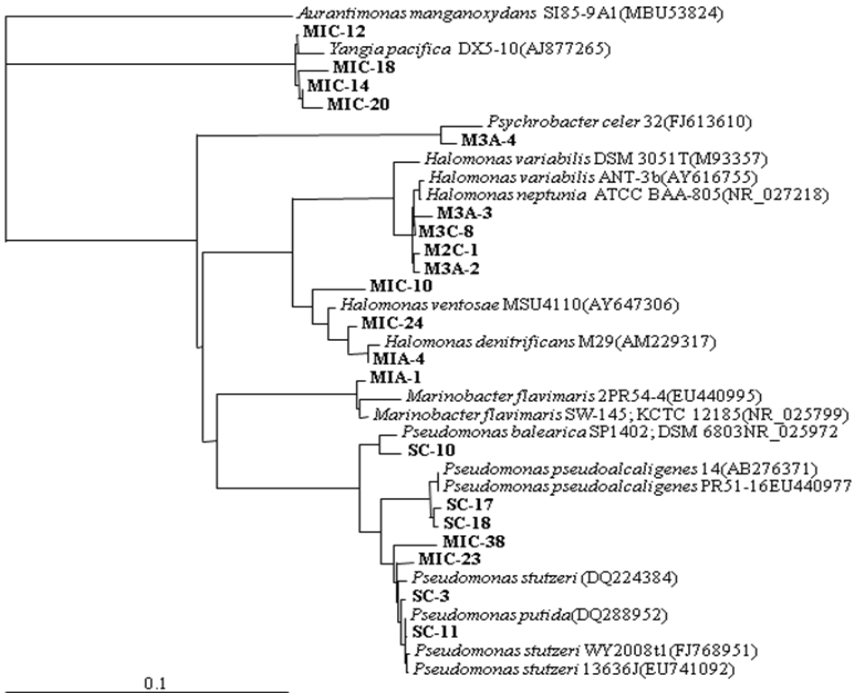

Figure 2. Phylogenetic tree based on 16S rDRNA gene sequences showing the relationships among the representatives of Proteobacteria and their phylogenetic relatives. The strains isolated in this study are indicated in bold.

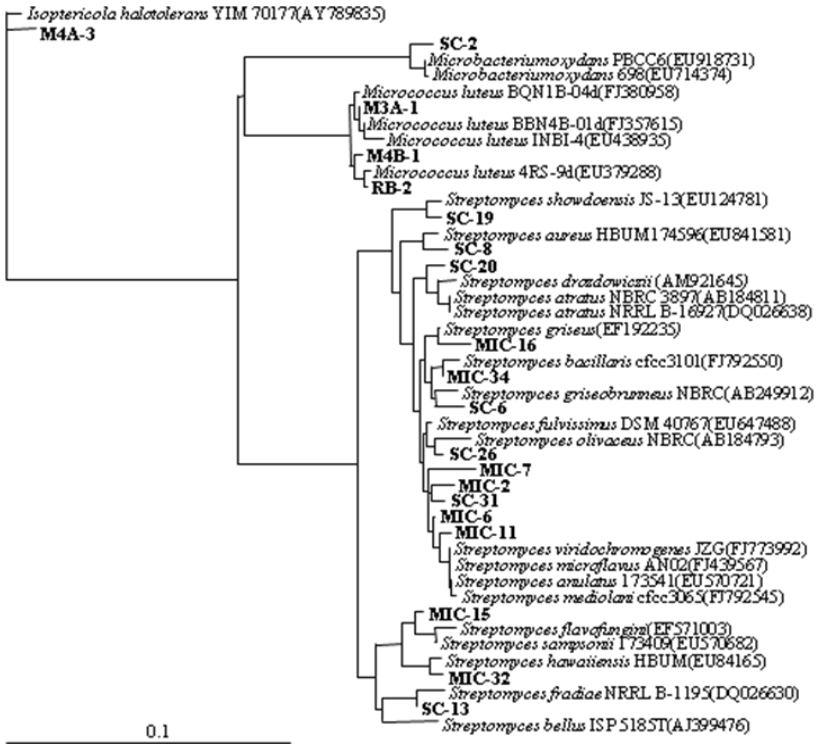

Figure 3. Phylogenetic tree based on 16S rRNA gene sequences showing the relationships among the representatives of Actinobacteria and their phylogenetic relatives. The strains isolated in this study are indicated in bold.

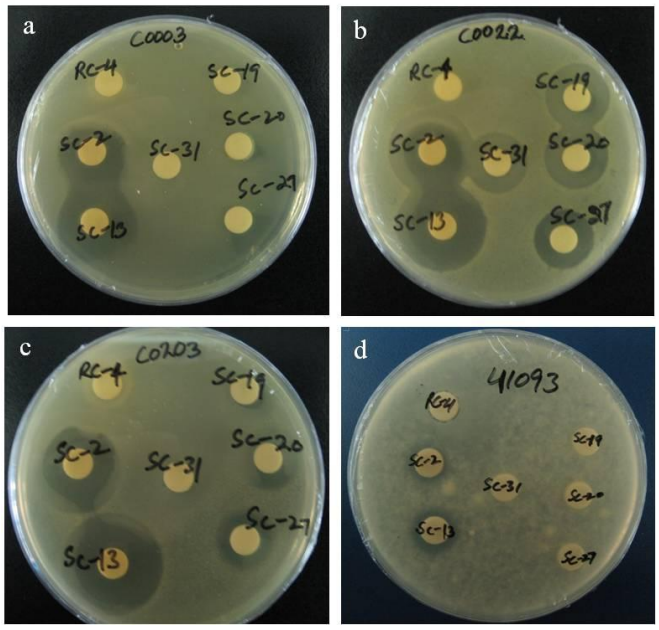

Figure 4. Strains SC-2 and SC-13 were found to have a broad spectrum of activity against tested pathogenic bacteria (a and b); against pathogenic yeasts (c) but did not show activity against pathogenic fungi (d).

and is thought to be less affected by horizontal gene transfer (Acinas et al., 2004). Automated DNA sequencing technology is the rapid and popular technique used in bacterial classification (Vandamme et al., 1996).

A total of 63 strains were assayed for antagonistic activity against indicator microorganisms and pathogenic fungi. Screening showed twelve strains with inhibitory activity against tested fungi, bacteria and yeast. Based on the phylogenetic analysis of the 16S rDNA sequence, nine strains were assigned to the actinobacterial groups. Among these strains, seven belongs to the genus *Streptomyces* and the remaining two belongs to the genera *Mycobacterium* and *Micrococcus*, respectively. These results are in line with the previous studies by Manivasagan et al. (2009). A single isolate belonged to the genera *Marinobacter*, *Bacillus* and *Planococcus*, respectively. Seven isolates showed inhibitory activity against pathogenic bacteria and yeasts, whereas five isolates were active against fungi. A high level of antimicrobial activity was observed in two strains. The strains SC-2 and SC-13 were more active against all tested pathogenic bacteria and yeasts. The strains SC-2 and SC-13 were closely related to *M. oxydans* (99%) and *S. fradiae* (99%). A species level match is based on a similarity greater than or equal to 99% (Drancourt et al., 2000).

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