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Research Article

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Antioxidant, Anti-inflammatory, and Antibacterial Activities of Actinobacteria Isolated from Marine Sediment

Grace Choi[1*], Myeong Seok Lee[1], Yun Gyeong Park[1], Il-Whan Choi[2] and Dae-Sung Lee[1]

Abstract

Secondary metabolites associated with bacteria possess various biological activities such as antioxidant and antibacterial effects. Here, we isolated approximately 90 strains from marine sediments and obtained 7% *Actinobacteria* among them based on biological activity. The genus *Streptomyces* is considered as promising source of bioactive secondary metabolites. Several *Streptomyces* strains and other strains showed antioxidant, anti-inflammatory, and antibacterial activities. Most strains (those selected for biological activity in this study) showed antibacterial effects against *Staphylococcus aureus* KCTC1927 and *Salmonella typhimurium* KCTC1925. Extracts of *Streptomyces* sp. SCS525 and *Planomicrobium* sp. SCS1153 showed strong antioxidant activity in DPPH and ABTS assays. *Streptomyces sp*. SCS525 and SCS538, *Arthrobacter* sp. SCS553, *Microbacterium* sp. SCS1115, and *Planomicrobium* sp. SCS1153 showed anti-inflammatory effects in NO and PGE_2 production experiments. Thus, *Actinobacteria* isolated from marine sediments possess promising biological activities. However, fractionation and further characterization of active compounds from these strains are needed for their optimum utilization.

Keywords

Actinobacteria; Marine sediment; Antioxidant; Anti-inflammatory; Antibacterial

Introduction

The phylum *Actinobacteria* is one of the dominant bacterial phyla that produce many antibiotics [1,2]. *Actinobacteria* are widely distributed in intertidal zones, sea water, sponges, and marine sediment [3,4]. *Actinobacteria* inhabiting marine environments such as sea sediments have gained considerable attention because they are considered more challenging to culture than their terrestrial relatives [5]. They have special growth requirements and media composition. Furthermore, several *Actinobacteria* genera produce novel secondary metabolites with bioactivities [6]. *Actinobacteria* are considered the most economical and biotechnologically important prokaryotes that produce several secondary metabolites with significant biological activities. Among *Actinobacteria*, *Streptomyces* are an important industrial group of organisms that have been widely explored for a broad range of biologically active compounds [6]. Also, new isolation methods for *actinobacteria* from the natural environment are being tried, and these methods will contribute to finding new active secondary metabolites [7]. Here we explored *Actinobacteria* from marine sediments and isolated useful marine *Actinobacteria.* These Actinomycetes strains could be potential sources for pharmacological compounds and need to be researched further for the development of novel bioactive compounds.

Material and Methods

Isolation of bacterial strains from marine sediments

Marine sediments were collected from Songnim shore (36°00′42.7″N 126°39′41.8″E) of Janghang, Seocheon, Chungcheong in the South Korea (May 2016). The sediments were dried and diluted 20-fold with sterile sea water. The diluted sediment suspension (100 µl) was spread on a marine agar 2216 (Difco Laboratories, Detroit, MI, USA). Single bacterial colonies were isolated on a marine agar and cultured at 27°C for 2 weeks; they were further cultured in 5 L marine broth 2216 (Difco) at 27°C for 7 days for 16S rRNA sequencing and solvent extraction. Stocks of all cultures were maintained at -80°C in culture medium containing 15% glycerol.

Species identification, and solvent extraction of bacterial strains

For species identification, chromosomal DNA was isolated from the isolated pure strains using a LaboPass™ tissue genomic DNA isolation kit (Cosmogenetech, Daejeon, South Korea). PCR was used to amplify the 16S rRNA genes using the primers 27F and 1492R [8]; the products were purified using a LaboPass™ PCR purification kit (Cosmogenetech) according to the manufacturer's protocol and sequenced using a capillary electrophoresis instrument (Applied Biosystems 3730XL, CA, USA). Similarities between the 16S rRNA gene sequence of the pure isolated bacteria and those of other previously described bacteria were determined by performing BLAST searches of the GenBank [9]. After species identification, the selected *Actinobacteria* strains were cultured in marine broth (Difco) for solvent extraction. These SCS (Seocheon sediment) strains were cultured at 27°C with shaking at 175 rpm. At the end of the culture period (day 7), the culture broth was extracted twice with the same volume of ethyl acetate (EtOAc). The EtOAc fractions of the strain culture broth were combined and dried using a vacuum evaporator (Rotavapor R-100, Büchi, Flawil, Switzerland). As mentioned in Table 1, the 16S rRNA gene sequence of these SCS strains is accessible under the Genbank accession number KY996369, KF881296, MN339851, KU714873, KY386372, DQ365561, and NR025011. Also, these seven SCS strains were registered in the Marine Bio-Resource Information System (MBRIS) of the National Marine Biodiversity Institute of Korea (MABIK) and are available for distribution to related researchers.

***Corresponding author:** Grace Choi, Department of Genetic Resources, Marine Biotechnology Research Division, National Marine Biodiversity Institute of Korea, Chungcheongnam-do, Republic of Korea, Tel: +82-41-950-0913; Fax: +82-41-950-0911; E-mail: gchoi@mabik.re.kr

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Table 1: Strain information.

Strain	Species (similarity with similar species, %)	GenBank accession no.	Similar species (accession no.)
SCS358	*Streptomyces* sp. (100)	MT950756	*Streptomyces* sp. Zah8 (KY996369)
SCS525	*Streptomyces* sp. (99.91)	MK402126	*Streptomyces* sp. SCC23 (KF881296)
SCS532	*Streptomyces* sp. (100)	MT950757	*Streptomyces* sp. MGB 2782 (MN339851)
SCS538	*Streptomyces* sp. (99.90)	MT950758	*Streptomyces* sp. MM108 (KU714873)
SCS553	*Arthrobacter* sp. (99.42)	MT950764	*Arthrobacter* sp. R-67793 (KY386372)
SCS1115	*Microbacterium phyllosphaerae* (100)	MT950762	*Microbacterium phyllosphaerae* GH06 (DQ365561)
SCS1153	*Planomicrobium koreense* (99.76)	MT951156	*Planomicrobium koreense* JG07 (NR025011)

Free radical scavenging assays for antioxidant activity

The 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay was performed according to the method described by Blois [10]. Briefly, 100 µl of extracts obtained from seven strains at various concentrations (from 20 to 1200 µg/ml) and 100 µl of 0.15 mM DPPH were dispensed into 96-well plates. The mixture was shaken carefully and left in the dark at 25°C for 30 min, after which absorbance (SpectraMax M2e; Molecular devices, San Jose, CA, USA) was measured at 516 nm with ascorbic acid (1.25 µg/ml-10 µg/ml) as a positive control. The DPPH scavenging activity was expressed as the half-maximal inhibitory concentration (IC_{50}). The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABST) assay was performed according to the method described by Re et al. [11]. Briefly, 7 mM ABTS and 2.45 mM potassium peroxodisulfate were mixed and incubated at 25°C for 16 h. Next, 100 µl of this mixture and 100 µl of extract obtained from seven strains at various concentrations (1 µg/ml-200 µg/ml) were dispensed into a 96-well plate, and the absorbance of the reaction mixture was measured at 734 nm (SpectraMax M2e; Molecular devices, San Jose, CA, USA). Ascorbic acid (1.25 µg/ml-20 µg/ml) was used as a positive control.

Nitric oxide assay and PGE_2 measurement

After the cells (5×10^5 cells/ml) were treated with 100 ng/ml LPS alone or with 0.1 µg/ml-300 µg/ml extracts of the SCS strains in 24-well plates for 24 h, 100 µl of each culture medium was mixed with the same volume of Griess reagent for nitrite quantitation. Nitrite levels were determined at 540 nm using an enzyme-linked immunosorbent assay plate reader (SpectraMax M2e; Molecular devices, San Jose, CA, USA). RAW264.7 cells were cultured in six-well plates (5×10^5 cells/ml) and incubated with extracts of the SCS strains in the presence or absence of LPS (100 ng/ml) for 24 h. PGE_2 production in macrophage culture medium was quantified using Enzyme Immunoassay (EIA) kits according to the manufacturer's instructions (Cayman Chemical, Ann Arbor, MI, USA).

Minimum inhibitory concentration

The antibacterial activity of the extracts from seven SCS strains was tested in a range of 64 µg/ml-1024 µg/ml against *Staphylococcus aureus* KCTC 1927, *Bacillus cereus* ATCC 14579, *Escherchia coli* KCTC 1682, and *Salmonella typhimurium* KCTC 1925. All strains were grown at 37°C, except for *B. cereus*, which was grown at 30°C in nutrient agar (Difco). Antibacterial activity was determined when the density of the growth control reached an absorbance of 0.150-0.200 at 600 nm (SpectraMax M2e; Molecular devices, San Jose, CA, USA). Each pathogenic microorganism was seeded in 96-well plates at 100 µl per well and incubated for 24 h. The extracts were then inoculated and incubated in 96-well plates at 30°C for *B. cereus* and at 37°C for the other pathogens. Growth density was checked every 6 h (0 h-42 h) at 600 nm.

Results

Species identification and solvent extraction

We isolated and sorted *Streptomycete* sp., *Arthrobacter* sp., *Microbacterium* sp., and *Planomicrobium* sp. from marine sediments for biological activities (Table 1). Most strains belonged to the class *Actinomycetes* or *Bacilli*. We cultured each strain in 5 L for extraction and performed crude extracts using ethyl acetate of same volume. The ethyl acetate extracts were assessed for antioxidant, anti-inflammatory, and antibacterial activities.

Antioxidant activity

The crude extracts obtained from SCS strains were tested for their antioxidant activity by DPPH and ABTS assays. The crude extract of strain SCS525 showed strong antioxidant activity as 2 µg/ml (IC_{50} of radical scavenging) as shown in the ABTS assay results (Table 2). Among the seven SCS strains, strains SCS525 and SCS1153 showed higher radical activity in the DPPH and ABTS assays than the others (Table 2).

Anti-inflammatory activity

The cytotoxicity of the extracts of the SCS strains was examined using RAW 264.7 cells to determine the optimal concentration (effective at providing anti-inflammatory effect with minimum toxicity). No cytotoxicity was observed with the concentration of the SCS extracts (<300 µg/ml) (Figure 1a). To investigate the anti-inflammatory effects of the SCS strains in LPS-stimulated RAW 264.7 cells, the amount of NO in the culture medium was quantified. NO production significantly increased following stimulation with LPS; however, pretreatment with SCS538, SCS553, SCS1115, and SCS1153 significantly inhibited the NO production in a dose-dependent manner (Figure 1b). Pretreatment with SCS525, SCS538, SCS553, SCS1115, and SCS1153 (30 µg/ml-300 µg/ml) significantly inhibited PGE_2 production in a dose-dependent manner (Figure 1c).

Antibacterial activity

Streptomyces spp. SCS358, SCS525 and SCS532; *Arthrobacter* sp. SCS553; *Microbacterium* sp. SCS1115; and *Planomicrobium* sp. SCS1153 showed antibacterial activity at a concentration of 512 µg/

Table 2: Free radical-scavenging activity of the crude extracts obtained from the SCS strains measured using the DPPH and ABTS assays.

Sample	IC_{50} (mg/ml) of DPPH	IC_{50} (mg/ml) of ABTS
Ascorbic acid (Positive control)	0.007 ± 0.000	0.016 ± 0.000
SCS358	0.703 ± 0.006	0.013 ± 0.002
SCS525	0.516 ± 0.002	0.002 ± 0.000
SCS532	0.66 ± 0.007	0.05 ± 0.001
SCS538	0.61 ± 0.001	0.012 ± 0.000
SCS553	0.845 ± 0.001	>2.5
SCS1115	1.035 ± 0.003	0.043 ± 0.000
SCS1153	0.526 ± 0.004	0.005 ± 0.000

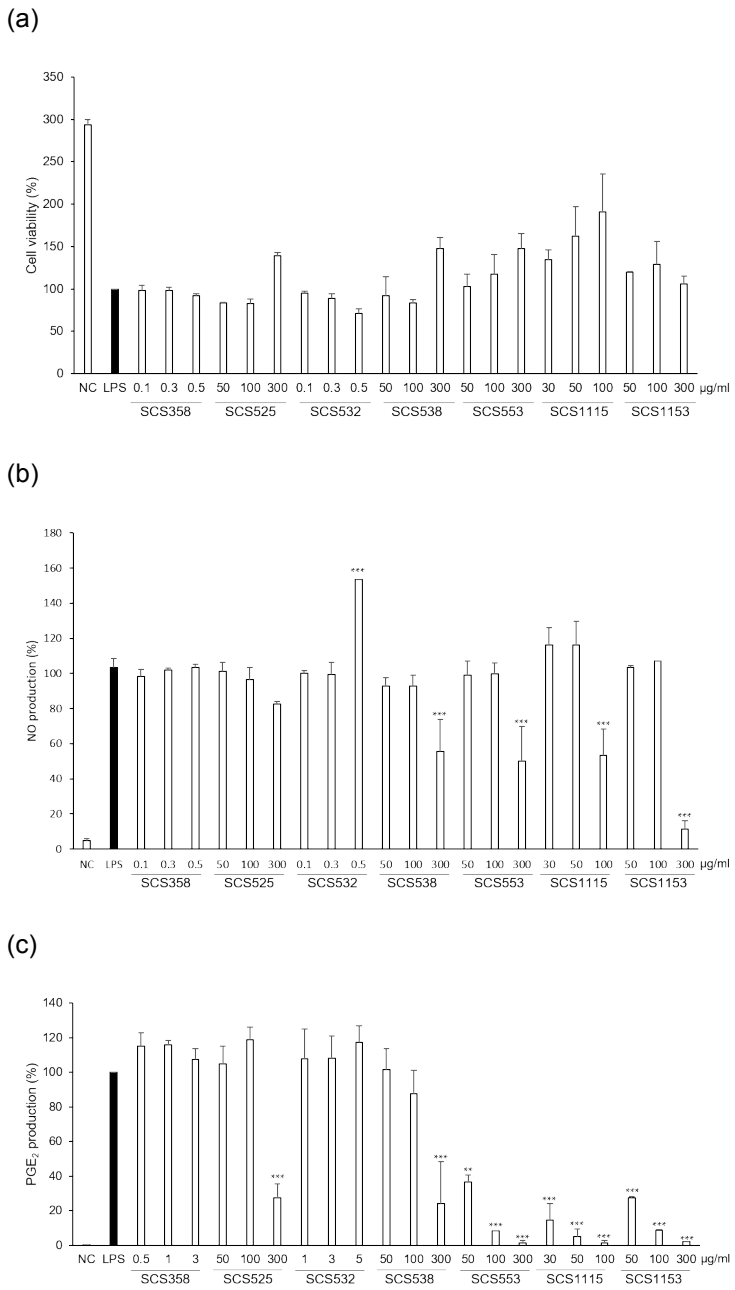

Figure 1: The production of nitric oxide (NO) and prostaglandin E_2 (PGE_2) of the SCS strains in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells, (a): RAW 264.7 macrophages were cultured with SCS strain extracts for 24 h, and cell viability was assessed based on intracellular dehydrogenase activity; (b): The levels of NO and PGE_2 in the medium were quantified using Griess reagent; (c): PGE_2 concentration was analyzed.

Table 3: Minimum inhibition concentration (MIC, μg/ml) of SCS strains against pathogenic bacteria.

Strain	*E. coli* (KCTC 1682)		*S. typhimurium* (KCTC 1925)		*S. aureus* (KCTC 1927)		*B. cereus* (ATCC 14579)	
	1st experiment	2nd experiment	1st experiment	2nd experiment	1st experiment	2nd experiment	1st experiment	2nd experiment
SCS358	1024	1024	512	512	256	256	1024	1024
SCS525	1024	1024	512	512	256	256	1024	1024
SCS532	1024	1024	512	512	256	256	1024	1024
SCS538	1024	1024	1024	1024	512	512	1024	1024
SCS553	1024	1024	512	512	256	256	1024	1024
SCS1115	1024	1024	512	512	256	256	1024	1024
SCS1153	1024	1024	512	512	512	512	1024	1024

ml against *S. typhimurium* KCTC 1925 (Table 3). All SCS strains show antibacterial activity against *S. aureus* KCTC 1927 under a concentration of 512 μg/ml (Table 3). In particular, *Streptomyces* spp. SCS358, SCS525, SCS532; *Arthrobacter* sp. SCS553; and *Microbacterium* sp. SCS1115 exhibited strong antibacterial activities (Table 3).

Discussion

In this study, we tested six *Actinobacteria* and one *Firmicutes* obtained from marine sediments and showed that they exhibited various bioactivities such as antioxidant, anti-inflammatory, and antibacterial activity. These findings suggest that further studies should be conducted to determine which single compound exhibits the corresponding active effect. *Streptomyces* sp. SCS525 and *Planomicrobium* sp. SCS1153 showed strong antioxidant activities in the DPPH and ABTS assays, and these bacteria showed anti-inflammatory and antibacterial activities.

In earlier studies, we isolated approximately 90 strains from marine sediments over 5 months. Of the 90 strains, 6 corresponding to 7% were *Streptomyces*. Ethyl acetate extract of *Streptomyces* species VITSTK7, isolated from the marine environment of the Bay of Bengal, exhibited 43.2% DPPH scavenging activity [12]. Similarly, antioxidant activity in three marine *Actinobacteria* isolated from the marine sediments of Nicobar Islands, whereas phenolic compounds extracted from *Streptomyces* sp. LK-3 exhibited 76% DPPH scavenging activity at 100 μg/ml [13]. The antioxidant and other bioactivities of *Streptomyces* have been reported in several articles. The usefulness of marine *Actinobacteria* has been proven in many research fields, and excellent substances with physiological activity are being discovered [14].

Conclusionss

Based on our results, we believe that further research is needed on the potential of the described strains as sources of novel antioxidant, anti-inflammatory, and antibacterial compounds.

Conflict of Interest

The authors have declared that no competing interests exist.

Acknowledgments

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Author Affiliation Top

[1]*Department of Genetic Resources, Marine Biotechnology Research Division, National Marine Biodiversity Institute of Korea, Chungcheongnam-do, Republic of Korea*

[2]*Department of Microbiology and Immunology, Inje University College of Medicine, Busan, Republic of Korea*