

Isolation of Marine Actinomycetes with Antimicrobial Activity from East Coast of Korea

Il-Shik Shin*, Jung-Mo Lee and Uk-Yeon Park¹

Faculty of Marine Bioscience & Technology, Kangnung National University,
Kangnung 210-702, Korea

¹Department of Food & Life Science, Kangwon Provincial University,
Kangnung 210-804, Korea

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The marine actinomycetes are very interesting microorganisms for finding of new antibiotics, because they are inhabited in marine environmental conditions, such as salinity, organic material and low concentration of nutrient components that are differ from those of land. In this study, the actinomycetes producing antibiotics from sea waters and bottom deposit samples collected at east coast of Korea were investigated to invent new antibiotics. Nine actinomycetes strains having the antimicrobial activity of 431 actinomycetes were selected from sea waters and bottom deposit samples. Among the 9 strains, the strain having the highest antimicrobial activity was classified to *Streptomyces* sp. and named *Streptomyces* sp. NS 13239.

Key words: Marine actinomycetes, Antimicrobial activity, Antibiotics, *Streptomyces* sp.

Introduction

Although the actinomycetes have been much concerned to source of antibiotics, most of strains were isolated from soil. There are little papers about producing antibiotics of marine actinomycetes. It is supposed that the microflora of actinomycetes in marine environment is very different from those in land because the environmental conditions of marine for habitat of microorganisms are different from those of land. It is known that some marine actinomycetes existed in coastal area are originated from soil by influx of fresh water. It is expected that there are some actinomycetes accommodating marine environment and producing novel antibiotics (Tsukamoto et al., 1998). Although it is uncertain that the origin of the marine actinomycetes is marine or land, many marine actinomycetes are certainly inhabiting marine environment (Choi and Park, 1993; Takizawa et al., 1993; Weyland, 1969, 1981). Therefore, the marine actinomycetes are very interesting microorganisms

for searching the novel antibiotics (Okami et al., 1976).

In this study, we isolated the marine actinomycetes with antibacterial activity from east coast of Korea, and examined the morphological, cultural and physiological characteristics.

Materials and Methods

Sea water and bottom deposit

For isolating the marine actinomycetes with antibacterial activity, sea water and bottom deposit were collected 4 times at Sokcho, Namae, Jumunjin and Samchuk in east coast of Korea from Aug. 1998 to Jun. 1999. The samples were transferred to laboratory in 4°C of ice box. The temperature of sea water were 18~24°C.

Isolation of the marine actinomycetes

The isolation of the marine actinomycetes was carried out by method of David (1976). The collected samples were diluted by sterilized sea water and 0.1 mL of diluted samples were smeared on Starch casein agar (David, 1976) for first isolation of the marine actinomycetes. After incubating at 28°C for 7 days,

Corresponding author: shinis@kangnung.ac.kr

the colonies supposed marine actinomycetes were streaked on Modified Bennett's agar (David, 1976) and then cultured purely on the same agar slant.

Isolation of the marine actinomycetes with antibacterial activity

Using the sterile technique, each isolate was made by single streak inoculation on the surface of LB agar (Difco co.) plate so as to divide the plate and incubated at 28°C for 7 days. After incubation, each of 4 test cultures (*Escherichia coli* ATCC 25922, *Vibrio parahaemolyticus* ATCC 2210001, *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633) were inoculated on the bottom of plate drawn with 4 lines perpendicular to the growth of the antibiotic-producing isolate and incubated at 37°C for 24 hours.

Cultural characteristics of isolated marine actinomycetes

The isolates were inoculated to Waksman and International Streptomyces Project (ISP) medium by Shirling and Gottlieb (1966) method, and then incubated at 28°C for 7 days for observation of color of colony, hyphae, spore and reverse side.

Morphological characteristics of isolated marine actinomycetes

The isolated marine actinomycetes were incubated on Modified Bennett's agar at 28°C for 14 days by inclined coverslip method and the morphological characteristics of isolates were observed with light microscope (Williams et al., 1989).

Physiological characteristics of isolated marine actinomycetes

The starch hydrolysis, litmus milk reactions and gelatin hydrolysis of the isolates were examined by method of Gordon and Mihm (1957). The melanin pigment production and carbon utilization of carbohydrates of the isolates were examined by method of Shirling and Gottlieb (1966).

Results and Discussions

Isolation of marine actinomycetes

The marine actinomycetes isolated from east coast of Korea were shown in Table 1. One hundred seventy five and 256 marine actinomycetes were isolated from

Table 1. Detection of antibiotics producing actinomycetes

Sampling site	No. of isolates		No. of antibiotics-producing actinomycetes
	Sea water	Bottom deposit	
Sokcho	29	80	3
Namae	65	69	1
Jumunjin	47	59	3
Samchuk	34	48	2
Total	175	256	9
	431		

sea water and bottom deposit, respectively.

The 431 isolates were classified to 34 group by morphology and color. Among them, 9 marine actinomycetes with antibacterial activity were obtained by determination of antimicrobial spectrum test (Table 2).

A marine actinomycetes with strong antibacterial activity against gram-positive bacteria such as *B. subtilis* and *S. aureus* was isolated and temporarily named to strain NS 13239.

Table 2. Antimicrobial activity of marine actinomycetes by perpendicular cross streak method

Strain code	Tested strain			
	<i>E. coli</i>	<i>V. parahaemolyticus</i>	<i>S. aureus</i>	<i>B. subtilis</i>
DB 1217	+	+	-	-
SS 11131	+	+	±	-
JB 13109	+	+	±	±
NS 13239	+	+	-	-
JS 13151	+	+	+	±
SB 14117	+	+	+	±
DS 11255	+	+	±	±
SB 14108	+	+	+	±
JB 12202	+	+	±	+

-, No growth; ±, Slight growth; +, Growth.

Cultural characteristics

The cultural characteristics of strain NS 13239 were shown in Table 3. The color of aerial hyphae was dark-brown or dark-green and the color of reverse side was variable with white, gray and yellow. The soluble pigments were not produced in all media.

Morphological characteristics

The vegetative and the aerial hyphae were formed and long. The spores with spheroidal shape were formed straightly on aerial hyphae (Fig. 1).

Table 3. Cultural characteristics of marine actinomycetes strain NS 13239

Culture media	Growth	Color or aerial mycelium	Soluble pigment	Reverse side
Tryptone-yeast agar	Scant	Dark yellow	None	Gray
Yeast extract-malt extract agar	Moderate	Dark green	None	Yellow
Inorganic salt-starch agar	Scant	Dark brown	None	White
Glycerine-asparagine agar	Moderate	Dark green	None	Dark yellow
Tyrosine agar	Scant	Dark brown	None	White
Pridham-Gottlieb's agar	Scant	Dark green	None	White gray
Sucrose-nitrate agar	Moderate	Dark green	None	Dark yellow
Glucose-asparagine agar	Moderate	Dark green	None	Yellow
Nutrient agar	Moderate	Dark green	None	Cream

Table 4. Physiological characteristics of marine actinomycetes strain NS 13239

Characteristics	Strain NS 13239
Starch hydrolysis	+
Tyrosinase	-
Liquefaction of gelatin	+
Peptonization of milk	-
Coagulation of milk	-
Melanin pigment production	-
Carbon utilization of Arabinose	-
Xylose	+
Galactose	-
Fructose	-
Glucose	+
Rhamnose	-
Maltose	-
Sucrose	-
Dextrin	+
Soluble starch	+
Glycerol	+

+, positive; -, negative.

Identification of the strain NS 13239

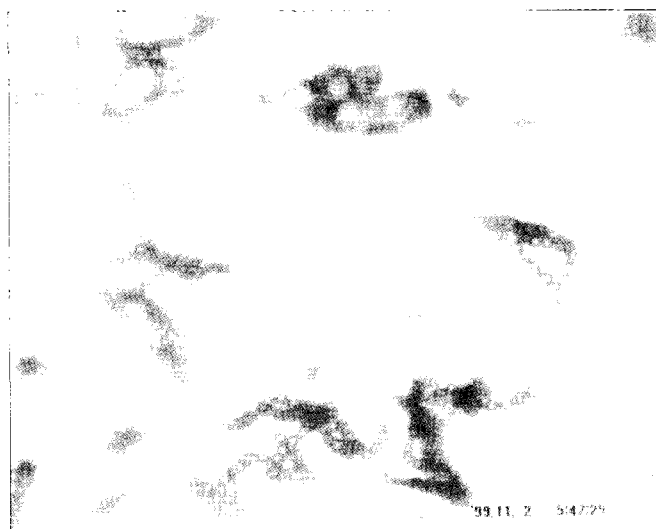
From cultural, morphological and physiological characteristics, the strain NS 13239 isolated from marine environment was identified *Streptomyces* sp. (Williams et al., 1989).

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($\times 400$)

Fig. 1. Morphological characteristics of marine actinomycetes strain NS 13239 cultured in Modified Bennett's medium at 28°C for 14 days.

Physiological characteristics

The physiological characteristics of the strain NS 13239 were shown in Table 4. The starch hydrolysis and gelatin hydrolysis were positive. The peptonization and coagulation of milk, and melanin pigment production were negative. The strain NS 1323 utilized xylose, glucose, dextrin and glycerol, but not arabinose, galactose, fructose, rhamnose, maltose and sucrose.

313~340.

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