



CHAPTER IV

RESULTS AND DISCUSSION

1. Samples collection, isolation, pH measurement and primary screening of actinomycetes

1.1 Samples collection

Eighty mangrove soils samples were collected from provinces around the inner gulf of Thailand; Samut prakarn (30 samples), Samut songkram (10 samples), Samut sakorn (30 samples) and Petchaburi (10 samples), and screened for *Actinomycetes*. Mangrove soils around the inner gulf of Thailand are lush green and preserved therefore, they are an interesting niche environment to isolate novel antimicrobial producing actinomycetes.

1.2 Isolation and pH measurement of actinomycetes

A total of 50 actinomycetes were isolated. The isolates were maintained on YMA slant and kept at 4 °C. Sources of mangrove soils, isolation date, pH, and isolation number of actinomycetes are shown in Table 4.1. The pH of the soil samples ranges from 4.49-8.20. The isolated actinomycetes could grow in widely range of pH, and most isolates were isolated from the soil samples contained pH about 8. Scheme 4.1 shows diagram of studies on the 50 isolates

1.3 Primary screening of antimicrobial producing actinomycetes

Antimicrobial activities of the 50 actinomycetes were tested. Thirty isolates showed antimicrobial activity. Four isolates showed inhibitory activity against *E. coli* ATCC 25922, 15 isolates against *M. luteus* ATCC 9341, 21 isolates against *B. subtilis*

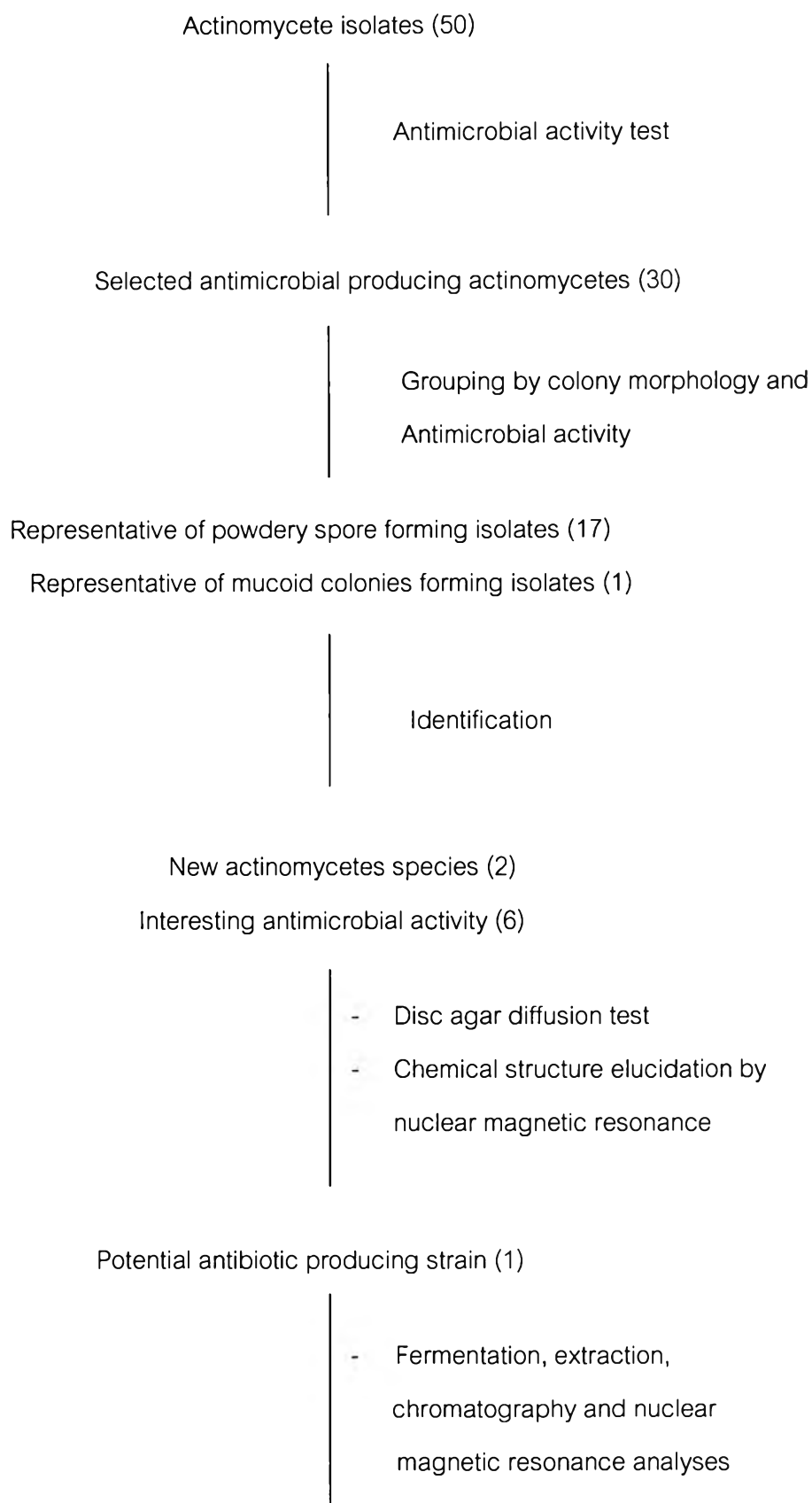
ATCC 16633, 5 isolates against *C. albicans* ATCC 10231, 2 isolates against *Ps.aeruginosa* ATCC 27853, 23 isolates against *S. aureus* ATCC 6538P. As mentioned, the most of powdery spore forming isolates could exhibit antimicrobial activities against Gram-positive bacteria and some of them could do against Gram-negative bacteria and yeast. The mucoid colonies forming isolates showed no antimicrobial activities against the tested microorganisms as shown in Table 4.2.

Table 4.1 Source of mangrove soil samples, isolation date, sample pH, and sample number that contained antimicrobial producing actinomycetes

Locations	Isolation date	pH	Sample No.
Baan-Leam, Phetchaburi	3 Jul 2006	8.2	PH2-1
Muang, Samut songkram	3 Jul 2006	7.44	SAM2-1, SAM2-2
Bangpu, Samut prakarn	3 Jul 2006	8.12	SMP2-1
Bangpu, Samut prakarn	3 Jul 2006	7.84	SMP3-1
Muang, Samut sakorn	28 Aug 2006	7.98	A5-8-1
Muang, Samut sakorn	3 Jul 2006	7.97	A10-4-2
Muang, Samut sakorn	14 Jul 2006	8.05	A10-7-1
Muang, Samut sakorn	28 Nov 2006	7.54	B5-2-1
Muang, Samut sakorn	28 Nov 2006	7.6	B5-6-1
Muang, Samut sakorn	28 Nov 2006	7.5	B5-8-1
Muang, Samut sakorn	28 Aug 2006	7.8	B10-3-1, B10-3-2 B10-3-3, B10-3-4 B10-3-5
Muang, Samut sakorn	28 Nov 2006	7.74	B10-4-1
Muang, Samut sakorn	28 Nov 2006	7.65	B10-8-3
Muang, Samut sakorn	28 Aug 2006	7.50	B15-2-1
Muang, Samut sakorn	28 Aug 2006	7.70	B15-4-1
Muang, Samut sakorn	29 Aug 2006	7.47	C10-2-1
Muang, Samut sakorn	29 Aug 2006	7.66	C10-6-1
Muang, Samut sakorn	29 Aug 2006	7.96	D5-7-1, D5-7-2
Muang, Samut sakorn	29 Aug 2006	7.92	D10-1-1
Muang, Samut sakorn	29 Aug 2006	7.23	D10-3-1
Muang, Samut sakorn	29 Aug 2006	7.39	D10-5-1
Muang, Samut sakorn	29 Aug 2006	7.43	D10-7-1, D10-7-2
Muang, Samut sakorn	18 Aug 2006	7.82	D10-9-1, D10-9-2 D10-9-5, D10-9-6
Pra-pa dang, Samut prakarn	29 Nov 2006	4.82	J4-2
Pra-pa dang, Samut prakarn	29 Nov 2006	5.22	J5-1
Pra-pa dang, Samut prakarn	29 Nov 2006	5.18	J 6-1
Pra-pa dang, Samut prakarn	29 Nov 2006	6.25	J8-1

Table 4.1 Source of mangrove soil samples, isolation date, sample pH, and sample number that contained antimicrobial producing actinomycetes

Locations	Date of isolation	pH	Isolation no.
Pra-pa dang, Samut prakarn	29 Nov 2006	4.49	J10-2, J10-3
Pra-pa dang, Samut prakarn	29 Nov 2006	5.50	J12-1
Pra-pa dang, Samut prakarn	29 Nov 2006	5.56	J14-1, J14-2
Pra-pa dang, Samut prakarn	29 Nov 2006	5.82	J15-1, J15-2
Pra-pa dang, Samut prakarn	29 Nov 2006	5.60	J16-1, J16-2
Pra-pa dang, Samut prakarn	29 Nov 2006	5.40	J17-2, J17-3
Pra-pa dang, Samut prakarn	29 Nov 2006	5.63	J18-1
Pra-pa dang, Samut prakarn	29 Nov 2006	5.35	J19-1



Scheme 4.1 Screening, identification, fermentation and chromatographic analyzes.

Table 4.2 Antimicrobial activity of the isolated actinomycetes

Isolate No.	Inhibitory activities					
	<i>E. coli</i>	<i>M.luteus</i>	<i>B. subtilis</i>	<i>C. albicans</i>	<i>Ps.aeruginosa</i>	<i>S. aureus</i>
	ATCC 25922	ATCC 9341	ATCC 16633	ATCC 10231	ATCC 27853	ATCC 6538P
PH2-1	-	-	-	-	-	-
SAM2-1	-	-	-	-	±	+++
SAM2-2	-	-	-	-	-	+++
SMP2-1	-	-	-	-	-	-
SMP3-1	±	+	+	-	-	+
A5-8-1	-	-	-	-	-	-
A10-4-2	-	-	-	-	-	-
A10-7-1	-	-	-	-	-	-
B5-2-1	-	-	-	-	-	-
B5-6-1	-	-	-	-	-	-
B5-8-1	-	-	-	-	-	-
B10-3-1	-	±	±	-	-	++++
B10-3-2	-	-	±	-	-	±
B10-3-3	-	-	-	-	-	-
B10-3-4	-	++++	++++	-	-	-
B10-3-5	-	-	-	-	-	-
B10-4-1	-	-	-	-	-	-
B10-8-3	-	-	-	-	-	-
B15-2-1	-	-	-	-	-	-
B15-4-1	-	-	-	-	-	±
C10-2-1	-	+++	++	++	-	+++
C10-6-1	-	++	+++	-	-	+++
D5-7-1	-	-	-	-	++	-
D5-7-2	±	-	-	-	-	-
D10-1-1	-	±	-	-	-	++++
D10-3-1	-	-	-	-	-	-
D10-5-1	-	+	-	-	-	-
D10-7-1	-	-	±	-	-	-
D10-7-2	++++	++++	+	-	-	++++

Table 4.2 Antimicrobial activity of the isolated actinomycetes

Isolate No.	Inhibitory activities					
	<i>E. coli</i> ATCC 25922	<i>M.luteus</i> ATCC 9341	<i>B. subtilis</i> ATCC 16633	<i>C. albicans</i> ATCC 10231	<i>Ps.aeruginosa</i> ATCC 27853	<i>S. aureus</i> ATCC 6538P
D10-9-1	-	-	+	-	-	-
D10-9-2	-	-	-	-	-	-
D10-9-5	-	-	-	-	-	-
D10-9-6	-	-	-	-	-	-
J4-2	-	-	-	-	-	-
J5-1	-	-	±	±	-	-
J6-1	-	-	-	-	-	+
J8-1	-	-	++++	-	-	++++
J10-2	-	-	-	-	-	-
J10-3	-	-	-	-	-	++++
J12-1	-	-	++	-	-	++
J14-1	-	±	+	-	-	±
J14-2	-	-	+	-	-	++++
J15-1	+	++++	++++	-	-	++++
J15-2	-	++++	±	±	-	++++
J16-1	-	++++	++	-	-	++
J16-2	-	+++	++	-	-	++
J17-2	-	-	+	-	-	++++
J17-3	-	-	-	-	-	-
J18-1	-	++++	±	+	-	++++
J19-1	-	++++	+	+	-	+

++++, 25.1 mm; +++, 20.1-25.0 mm; ++, 15.1-20.0 mm; +, 10.1-15.0 mm; ±, 0 -10.0 mm; -, no activity

2. Identification and characterization of the isolated actinomycetes

All 50 isolates were identified and characterized based on their morphological, cultural, physiological and biochemical characteristics as shown in Tables 4.3-4.5.

2.1 Morphological and cultural characterizations

Spore morphology and pigment production of the 50 actinomycetes isolated grown on YMA medium at 30°C for 14 days are shown in Table 4.3. The spore chains were rectiflexibles and spirals. There are a lot of colors of the spore such as yellowish brown, white, brown, grayish white, orange, gray, etc. Moreover, some isolates could produce many colors of soluble pigment in the medium. Morphological and cultural characteristics of the isolates on different media incubated for 14 days are shown in Table 4.4. Most isolates grew better on YMA and oat meal agar medium than tyrosine, glycerol-asparagine agar and inorganic salt-starch agar. They showed different colors both on the top and the bottom of the colonies are on different agar media. Colony color and colonial appearance on YMA under light-microscope are shown in Table 4.5. Colonial appearance and scanning electron micrograph of representative strains are shown in figure 4.1-4.11. Thirty-eight isolates were identified as *Streptomyces* (Holt, 1989) based on powdery colony with spiral spore chain (5 isolates), and with rectiflexible spore chain (33 isolates). The color of substrate mycelium and aerial mycelium were variety; example, white, gray, red, olive, yellowish white, beige, etc. Spore Twelve isolates were identified as *Micromonospora* (Holt, 1989) (Kawamoto, 1989) based on mucoid colony with vegetative mycelium, but no aerial hyphae. Spore of them were borne singly on the substrate hyphae having approximately diameter of 0.5-0.6 μm . The colors of the substrate mycelium were yellowish white to vivid orange and turned to brownish black to black after sporulation. The representative strains, 17 of powdery colonies isolates exhibited antimicrobial activity and 1 isolate of mucoid were selected for further studies. The chemotaxonomic and molecular characteristics including the phylogenetic analyses based on their 16S rDNA sequence of the selected strains were carried out.

Table 4.3 Spore morphology and pigment production of the isolates grown on YMA medium for 14 days

Isolate no.	Spore morphology	Spore color	Soluble pigment
PH2-1	Single spore	Yellowish brown	-
SAM2-1	Rectiflexibiles	White	-
SAM2-2	Rectiflexibiles	White	-
SMP2-1	Single spore	Brown	Pale brown
SMP3-1	Rectiflexibiles	Grayish white	-
A5-8-1	Rectiflexibiles	Grayish white	-
A10-4-2	Single spore	Orange	-
A10-7-1	Spiral	Gray	-
B5-2-1	Single spore	Orange	-
B5-6-1	Spiral	White	-
B5-8-1	Single spore	Orange	Brown
B10-3-1	Rectiflexibiles	White	-
B10-3-2	Rectiflexibiles	White	-
B10-3-3	Single spore	Black	Pale yellow
B10-3-4	Spiral	White	Orange yellow
B10-3-5	Rectiflexibiles	White	-
B10-4-1	Single spore	Orange	-
B10-8-3	Single spore	Orange	Pale brown
B15-2-1	Rectiflexibiles	Yellowish white	Yellowish orange
B15-4-1	Rectiflexibiles	White	Yellowish white
C10-2-1	Rectiflexibiles	Light yellowish brown	-
C10-6-1	Rectiflexibiles	White	-
D5-7-1	Rectiflexibiles	White rose	-
D5-7-2	Rectiflexibiles	Grayish white	-
D10-1-1	Spiral	Grayish white	-
D10-3-1	Rectiflexibiles	Gray	-
D10-5-1	Spiral	White rose	-
D10-7-1	Rectiflexibiles	White-rose	-
D10-7-2	Spiral	Gray	-
D10-9-1	Rectiflexibiles	Grayish white	Pale brown
D10-9-2	Rectiflexibiles	White	Yellowish white

Table 4.3 Spore morphology and pigment production of the isolates grown on YMA medium for 14 days

Isolate no.	Spore morphology	Spore color	Soluble pigment
D10-9-5	Rectiflexibiles	Grayish white	-
D10-9-6	Single spore	Orange	Orange
J4-2	Single spore	Black	-
J5-1	Rectiflexibiles	White	-
J6-1	Rectiflexibiles	White	-
J8-1	Rectiflexibiles	Yellowish grey	-
J10-2	Single spore	Brown	-
J10-3	Single spore	Brownish gold	Brownish gold
J12-1	Rectiflexibiles	White	-
J14-1	Rectiflexibiles	White	-
J14-2	Rectiflexibiles	White	-
J15-1	Spiral	Olive	Orange
J15-2	Rectiflexibiles	White	-
J16-1	Rectiflexibiles	White	-
J16-2	Rectiflexibiles	White	-
J17-2	Rectiflexibiles	Grayish white	-
J17-3	Rectiflexibiles	Black	-
J18-1	Rectiflexibiles	White	-
J19-1	Rectiflexibiles	White	-

Table 4.4 Morphological and cultural characteristics of the isolates on different media incubated for 14 days

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
PH2-1	YM	+++	Yellowish brown	Orange	Dark yellowish
	Tyrosine	+++	Orange	Orange	brown
	Oatmeal	+++	Black	Black	Orange
	Asparagine	+++	Yellowish brown	White	Black
	Inorg. salt	+++	Orange	Yellowish white	Yellowish brown Orange
SAM2-1	YM	+++	White	Pale reddish yellow	Light reddish yellow
	Tyrosine	++	White	White	White
	Oatmeal	++	White	White	White
	Asparagine	++	White	White	White
	Inorg. salt	+++	White	White	White
SAM2-2	YM	+++	White	Yellowish white	Orange yellow
	Tyrosine	+++	Yellowish white	White	Pale yellow
	Oatmeal	++	Yellowish white	Yellowish white	Yellowish white
	Asparagine	+++	White	White	White
	Inorg. salt	+++	White	White	Yellowish white
SMP2-1	YM	+++	Brown	Orange	Dark yellowish brown
	Tyrosine	++	Orange	Orange	Orange
	Oatmeal	+++	Black	Black	Black
	Asparagine	+++	Dark orange	Orange	Orange
	Inorg. salt	+++	Orange	Orange	Orange
SMP3-1	YM	+++	Grayish white	Rose	Strong yellow
	Tyrosine	+++	Gray	Rose	Rose
	Oatmeal	+++	Gray	Rose	Rose
	Asparagine	+++	Purplish pink	White	Pink
	Inorg. salt	+++	Vivid red purple	Vivid red purple	Vivid red purple

Table 4.4 Morphological and cultural characteristics of the isolates on different media incubated for 14 days

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
A5-8-1	YM	+++	Grayish white	Yellowish white	Yellowish white
	Tyrosine	+++	Gray	Gray	Bluish gray
	Oatmeal	+++	Gray	Light bluish gray	Grayish white
	Asparagine	+++	White	White	Pale beige
	Inorg. salt	+++	White	Grayish white	Grayish white
A10-4-2	YM	+++	Orange	Orange	Orange
	Tyrosine	+	Orange	Orange	Orange
	Oatmeal	+++	Orange	Orange	Orange
	Asparagine	+	Orange	Orange	Orange
	Inorg. salt	-	-	-	-
A10-7-1	YM	+++	Gray	Light bluish gray	Pale beige
	Tyrosine	+++	Grayish white	Gray	Bluish gray
	Oatmeal	+++	Grayish white	Light bluish gray	Grayish white
	Asparagine	+++	Grayish white	White	Pale beige
	Inorg. salt	+++	White	Grayish white	Grayish white
B5-2-1	YM	+++	Orange	Orange	Orange
	Tyrosine	+++	Brown	Orange	Orange
	Oatmeal	+++	Gold	Gold	Brown
	Asparagine	++	Brown	Orange	Brown
	Inorg. salt	++	Orange	Orange	Orange
B5-6-1	YM	+++	White	White	Pale beige
	Tyrosine	+++	White	Pale beige	Pale beige
	Oatmeal	+++	White	White	Pale beige
	Asparagine	++	White	White	Yellowish white
	Inorg. salt	+++	White	White	White
B5-8-1	YM	+++	Orange	Orange	Orange
	Tyrosine	+++	Orange	Orange	Orange
	Oatmeal	+++	Brown	Orange	Orange
	Asparagine	++	Orange	Orange	Orange
	Inorg. salt	+	Orange	Orange	Orange

Table 4.4 Morphological and cultural characteristics of the isolates on different media incubated for 14 days

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
B10-3-1	YM	+++	White	White	Dull reddish yellow
	Tyrosine	+++	Pale yellow green	White	Brownish white
	Oatmeal	+++	White	Pale beige	Pale beige
	Asparagine	+++	Yellowish white	Yellowish white	Yellowish white
	Inorg. salt	+++	Yellowish white	Yellowish white	Yellowish white
B10-3-2	YM	+++	White	Yellowish white	Brownish gold
	Tyrosine	+++	Pale yellow	Pale yellow	Brownish white
	Oatmeal	+++	White	Yellowish white	Pale beige
	Asparagine	+++	White	White	White
	Inorg. salt	+++	White	White	Yellowish white
B10-3-3	YM	+++	Black	Pale orange	Pale orange
	Tyrosine	+++	Orange	Orange	Orange
	Oatmeal	+++	Black	Black	Black
	Asparagine	+++	Orange	Orange	Orange
	Inorg. salt	+++	Orange	Orange	Orange
B10-3-4	YM	+++	Pale purplish pink	White	Pale yellow
	Tyrosine	+++	White	White	Yellowish white
	Oatmeal	+++	White	Grayish white	Pale beige
	Asparagine	+++	Yellowish white	Yellowish white	White
	Inorg. salt	+++	White	White	White
B10-3-5	YM	+++	Grayish white	Grayish white	Gray
	Tyrosine	+++	Pale grayish white	Grayish white	Yellowish white
	Oatmeal	+++	Gray	White	Gray
	Asparagine	+++	Grayish white	Grayish white	Yellowish white
	Inorg. salt	+++	Reddish gray	White	Grayish white

Table 4.4 Morphological and cultural characteristics of the isolates on different media incubated for 14 days

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
B10-4-1	YM	+++	Orange	Orange	Orange
	Tyrosine	-	-	-	-
	Oatmeal	+++	Orange	Orange	Orange
	Asparagine	-	-	-	-
	Inorg. salt	-	-	-	-
B10-8-3	YM	+++	Orange	Orange	Brown
	Tyrosine	-	-	-	-
	Oatmeal	+++	Brown	Orange	Brownish orange
	Asparagine	-	-	-	-
	Inorg. salt	-	-	-	-
B15-2-1	YM	+++	Yellowish white	Pale yellow	Orange yellow
	Tyrosine	+++	White	White	Pale yellow
	Oatmeal	+++	White	Yellowish white	Orange yellow
	Asparagine	-	-	-	-
	Inorg. salt	+++	Pale yellow	White	Pale yellow
B15-4-1	YM	+++	White	White	Yellowish white
	Tyrosine	+++	White	White	Pale yellow
	Oatmeal	+++	White	White	Yellowish white
	Asparagine	-	-	-	-
	Inorg. salt	+++	White	White	Pale yellow
C10-2-1	YM	+++	Light yellowish brown	Brownish white	Brownish white
	Tyrosine	+++	Grayish white	White	Yellowish white
	Oatmeal	+++	White	White	White
	Asparagine	++	White	White	White
	Inorg. salt	+++	White	White	White
C10-6-1	YM	+++	White	Pale yellow	Grayish yellow
	Tyrosine	-	-	-	-
	Oatmeal	+++	Grayish white	White	Grayish white
	Asparagine	++	White	White	White
	Inorg. salt	-	-	-	-

Table 4.4 Morphological and cultural characteristics of the isolates on different media incubated for 14 days

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
D5-7-1	YM	+++	White rose	White rose	Yellowish white
	Tyrosine	++	Yellowish white	Yellowish white	white
	Oatmeal	+++	Grayish white	Rose	Dark red
	Asparagine	+++	Pale gray	Pale gray	Dull reddish yellow
	Inorg. salt	+++	Pale gray	Pale gray	White
D5-7-2	YM	+++	Grayish white	Grayish white	Yellow
	Tyrosine	++	Pale yellowish pink	White	Yellowish white
	Oatmeal	+++	Grayish white	Gray	Pale red
	Asparagine	+++	Gray	Gray	Grayish yellow
	Inorg. salt	+++	White	Yellow red	Red
D10-1-1	YM	+++	Grayish white	White	Grayish brown
	Tyrosine	-	-	-	-
	Oatmeal	+++	Gray	Gray	Gray
	Asparagine	+	Gray	Gray	Dark gray
	Inorg. salt	+++	Grayish white	White	Yellowish white
D10-3-1	YM	+++	Yellowish white	Yellowish white	Yellowish white
	Tyrosine	++	White	White	White
	Oatmeal	+++	White	White	White
	Asparagine	-	-	-	-
	Inorg. salt	+++	Pale reddish yellow	White	Light reddish yellow
D10-5-1	YM	+++	White rose	White	Yellowish white
	Tyrosine	++	Grayish white	Grayish white	Grayish white
	Oatmeal	+++	Pale gray	Gray	Grayish white
	Asparagine	+++	Grayish white	Grayish white	Gray
	Inorg. salt	+++	Grayish white	Grayish white	Red

Table 4.4 Morphological and cultural characteristics of the isolates on different media incubated for 14 days

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
D10-7-1	YM	+++	White-rose	Yellowish white	Yellowish white
	Tyrosine	+++	Yellowish white	Yellowish white	Yellowish white
	Oatmeal	+++	Grayish red	White-rose	Grayish red
	Asparagine	+++	Grayish red	Grayish red	Dark gray
	Inorg. salt	+++	Grayish white	Red	Red
D10-7-2	YM	+++	Gray	Gray	Orange yellow
	Tyrosine	-	-	-	-
	Oatmeal	+++	Gray	Gray	Pale pink
	Asparagine	+	Grayish white	Grayish white	Grayish white
	Inorg. salt	+++	Grayish white	Yellow red	Red
D10-9-1	YM	+++	Grayish white	Yellowish white	Grayish brown
	Tyrosine	+++	Grayish white	White	Yellowish white
	Oatmeal	+++	White	White	Yellowish white
	Asparagine	+++	White	White	Yellowish white
	Inorg. salt	+++	White	White	Yellowish white
D10-9-2	YM	+++	Yellowish white	Yellowish white	Yellowish white
	Tyrosine	-	-	-	-
	Oatmeal	+++	White	White	White
	Asparagine	-	-	-	-
	Inorg. salt	-	-	-	-
D10-9-5	YM	+++	Grayish white	Grayish white	Yellowish white
	Tyrosine	++	Yellowish white	White	Yellowish white
	Oatmeal	+++	Light medium gray	Grayish white	Medium gray
	Asparagine	+++	Light medium gray	Grayish white	Yellowish white
	Inorg. salt	+++	Brownish gray	White	Yellowish gray

Table 4.4 Morphological and cultural characteristics of the isolates on different media incubated for 14 days

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
D10-9-6	YM	+++	Brown	Orange	Brownish orange
	Tyrosine	++	Brown	Orange	Brown
	Oatmeal	+++	Brown	Orange	Orange
	Asparagine	+++	Brown	Orange	Brownish orange
	Inorg. salt	+++	Brown	Orange	Brownish orange
J4-2	YM	+++	Black	Orange yellow	Black
	Tyrosine	++	Brown	Orange	Brown
	Oatmeal	+++	Black	Orange	Black
	Asparagine	+++	Black	Orange	Black
	Inorg. salt	+++	Brownish black	Orange	Black
J5-1	YM	+++	White	Yellowish white	Light yellowish brown
	Tyrosine	-	-	-	-
	Oatmeal	-	-	-	-
	Asparagine	-	-	-	-
	Inorg. salt	-	-	-	-
J6-1	YM	+++	White	Orange	Orange
	Tyrosine	+++	White	White	Yellowish white
	Oatmeal	+++	White	Orange	Pale beige
	Asparagine	+++	White	Orange white	Orange white
	Inorg. salt	+++	White	Orange white	White
J8-1	YM	+++	Yellowish grey	Yellowish grey	Brownish gold
	Tyrosine	+	White	White	White
	Oatmeal	+++	White	White	Yellowish white
	Asparagine	+	White	White	Brownish gold
	Inorg. salt	-	-	-	-

Table 4.4 Morphological and cultural characteristics of the isolates on different media incubated for 14 days

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
J10-2	YM	+++	Brown	Orange	Orange
	Tyrosine	-	-	-	-
	Oatmeal	+++	Brown	Orange	Black
	Asparagine	-	-	-	-
	Inorg. salt	-	-	-	-
J10-3	YM	+++	Brownish gold	Brownish gold	Brownish gold
	Tyrosine	++	Pale beige	Pale beige	Pale beige
	Oatmeal	-	-	-	-
	Asparagine	-	-	-	-
	Inorg. salt	-	-	-	-
J12-1	YM	+++	White	Yellowish white	Yellowish white
	Tyrosine	++	White	White	White
	Oatmeal	++	White	White	White
	Asparagine	-	White	White	White
	Inorg. salt	-	White	Yellowish white	Yellowish white
J14-1	YM	+++	White	White	Yellowish white
	Tyrosine	++	White-vivid red	White	White
	Oatmeal	-	-	-	-
	Asparagine	-	-	-	-
	Inorg. salt	-	-	-	-
J14-2	YM	+++	White	Orange	Orange
	Tyrosine	+++	White	Pale orange	Pale orange
	Oatmeal	+++	White	Pale orange	Pale orange
	Asparagine	++	Pale orange	Pale orange	Pale orange
	Inorg. salt	++	White	Pale orange	Pale orange
J15-1	YM	+++	Olive	Olive	Olive
	Tyrosine	+++	Greenish gray	Gray	Greenish gray
	Oatmeal	+++	Olive	Olive	Brownish gold
	Asparagine	+++	Pale olive	Pale olive	Greenish gray
	Inorg. salt	+++	Olive	Olive	Greenish gray

Table 4.4 Morphological and cultural characteristics of the isolates on different media incubated for 14 days

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
J15-2	YM	+++	White	White	Yellowish white
	Tyrosine	+++	White	White	White
	Oatmeal	+++	White	White	White
	Asparagine	+++	White	White	Pale beige
	Inorg. salt	+++	White	Yellowish white	Yellowish white
J16-1	YM	+++	White	Yellowish white	Pale greenish yellow
	Tyrosine	-	-	-	-
	Oatmeal	+++	White	White	White
	Asparagine	-	-	-	-
	Inorg. salt	+++	White	White	Yellowish white
J16-2	YM	+++	White	White	Yellowish white
	Tyrosine	+	Yellowish white	White	White
	Oatmeal	+++	White	White	White
	Asparagine	+	White	White	Yellowish white
	Inorg. salt	+++	White	White	Yellowish white
J17-2	YM	+++	Grayish white	Yellowish white	Yellowish white
	Tyrosine	+++	Grayish brown	Grayish brown	Pale beige
	Oatmeal	+++	Gray	Dark brown	Black
	Asparagine	+	Grayish brown	Grayish brown	Brown
	Inorg. salt	+++	Grayish white	Yellowish white	Pale beige
J17-3	YM	+++	Black	Orange	Black
	Tyrosine	+++	Dark brown	Dark brown	Gray
	Oatmeal	+++	Black	Orange	Black
	Asparagine	+++	Brown	Orange brown	Brown
	Inorg. salt	+++	Orange	Orange	Pale beige

Table 4.4 Morphological and cultural characteristics of the isolates on different media incubated for 14 days

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
J18-1	YM	+++	White	White	Yellowish white
	Tyrosine	+	White	White	White
	Oatmeal	+++	White	White	Yellowish white
	Asparagine	++	White	White	White
	Inorg. salt	++	White	White	White
J 19-1	YM	+++	White	White	Yellowish white
	Tyrosine	-	-	-	-
	Oatmeal	+++	Grayish white	White	White
	Asparagine	-	-	-	-
	Inorg. salt	+++	White	White	Yellowish white

YM, Yeast extract-Malt extract agar; Tyrosine, Tyrosine agar; Oatmeal, Oatmeal agar (Difco);

Asparagine, Glycerol-asparagine agar; Inorg. salt, Inorganic salt-starch agar

+++ , good; ++ , moderate; + , poor; - , no growth

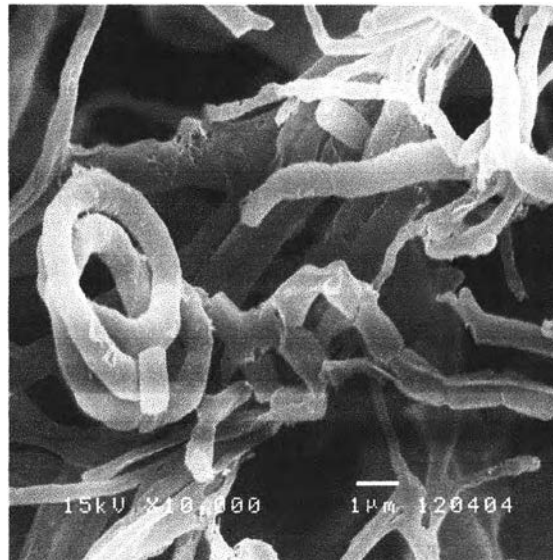


Figure 4.1 Scanning electron micrograph of *Streptomyces* sp. SAM2-1 grown on YMA medium (14 days)

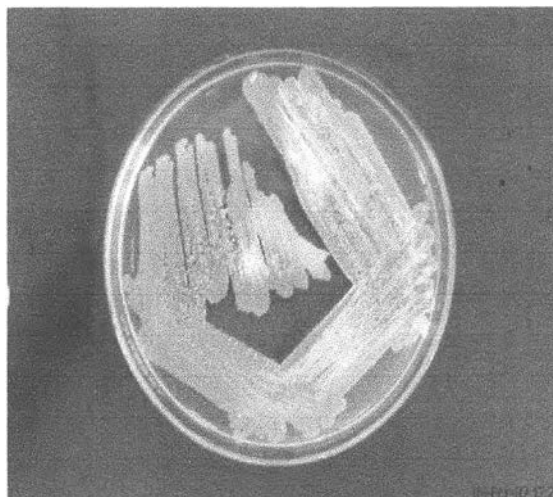
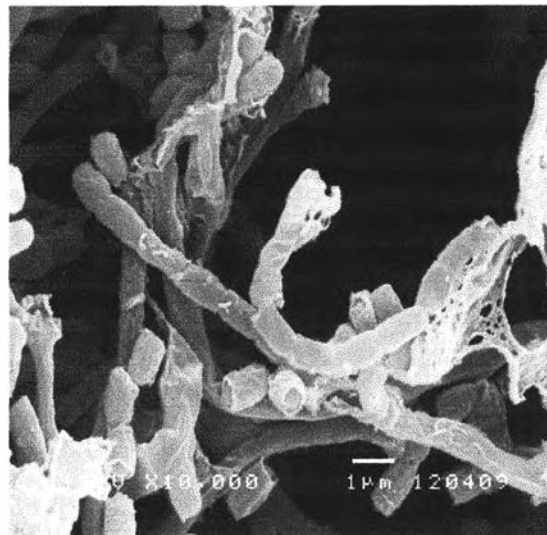


Figure 4.2 Scanning electron micrograph and colonial appearance of *Streptomyces* sp. SMP3-1 grown on YMA medium (14 days)

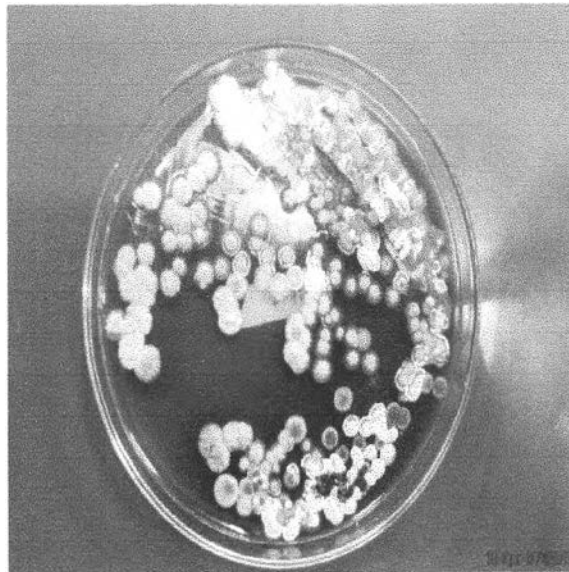
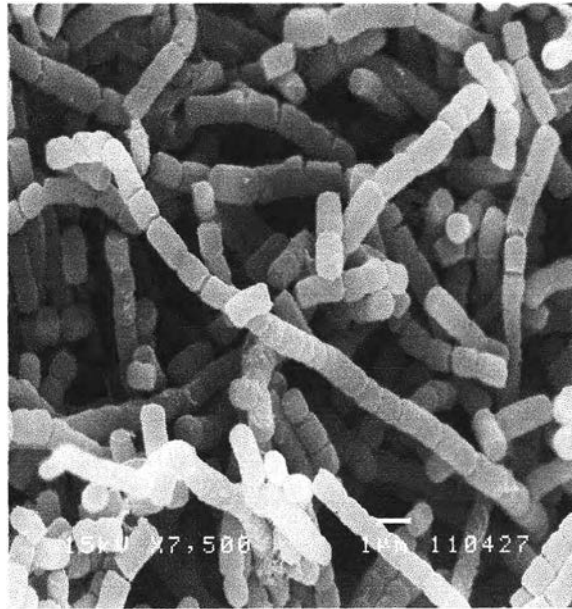


Figure 4.3 Scanning electron micrograph and colonial appearance of *Streptomyces* sp. B15-4 grown on YMA medium (7 days)

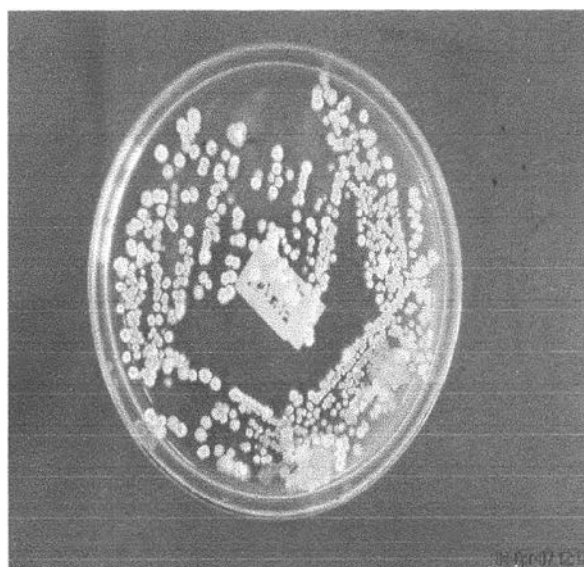
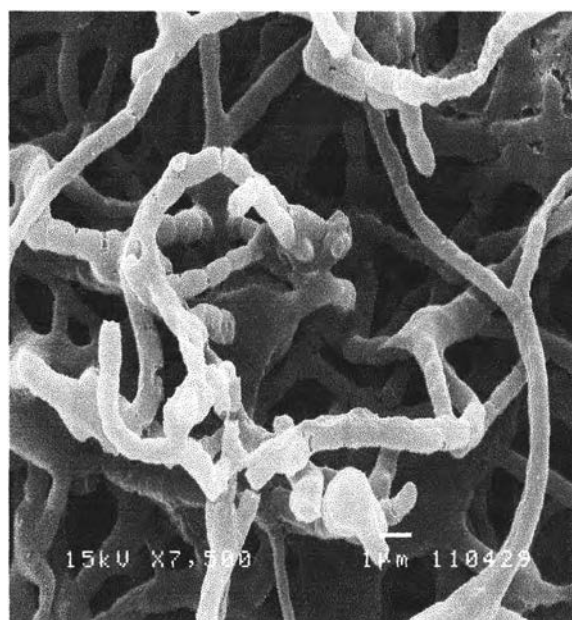


Figure 4.4 Scanning electron micrograph and colonial appearance of *Streptomyces* sp. C10-6 grown on YMA medium (7 days)

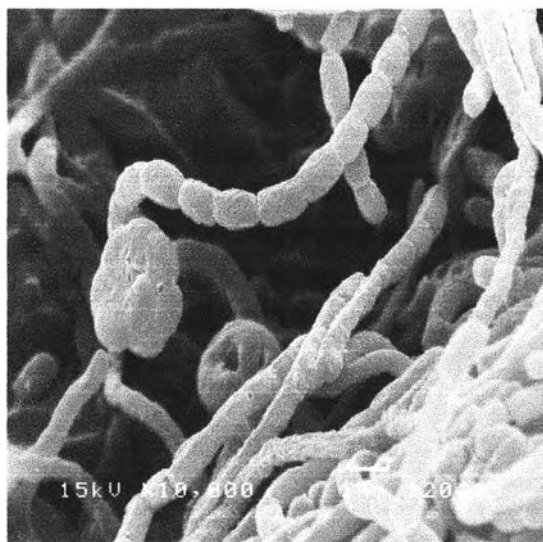


Figure 4.5 Scanning electron micrograph of *Streptomyces* sp. D10-5 grown on YMA medium (7 days)

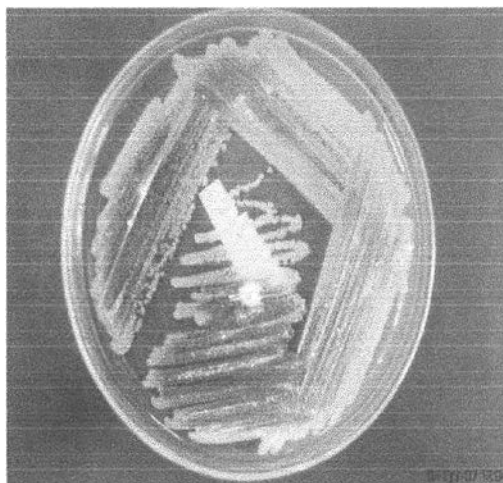
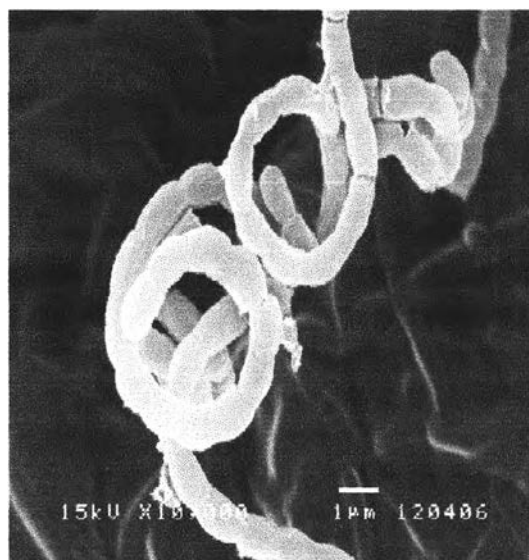


Figure 4.6 Scanning electron micrograph and colonial appearance of *Streptomyces* sp. D10-7-2 grown on YMA medium (7 days)

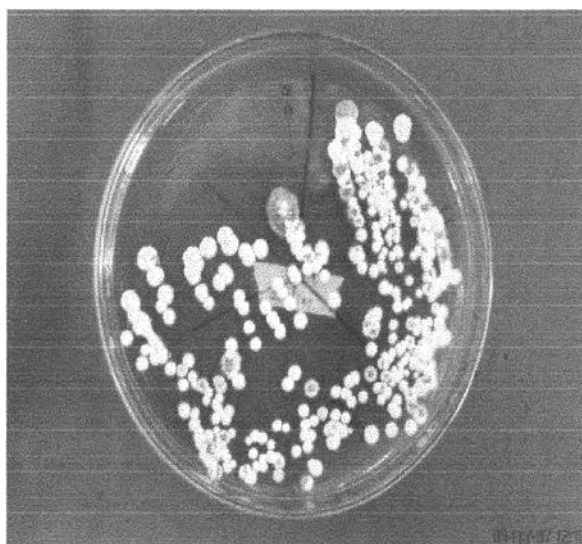
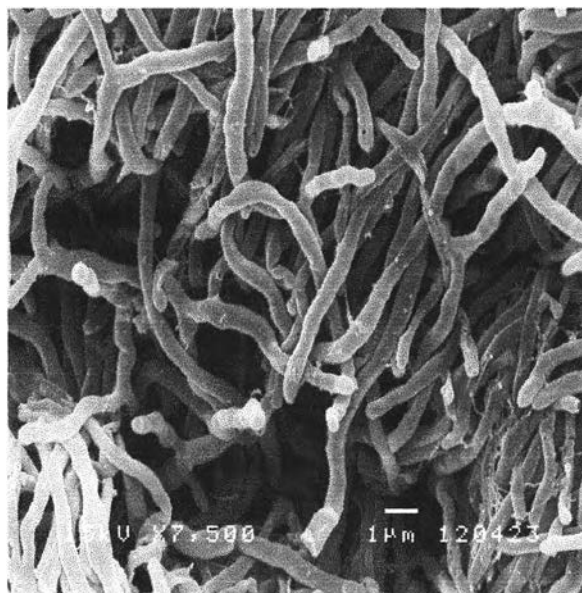


Figure 4.7 Scanning electron micrograph and colonial appearance of *Streptomyces* sp. J8-1 grown on YMA medium (7 days)

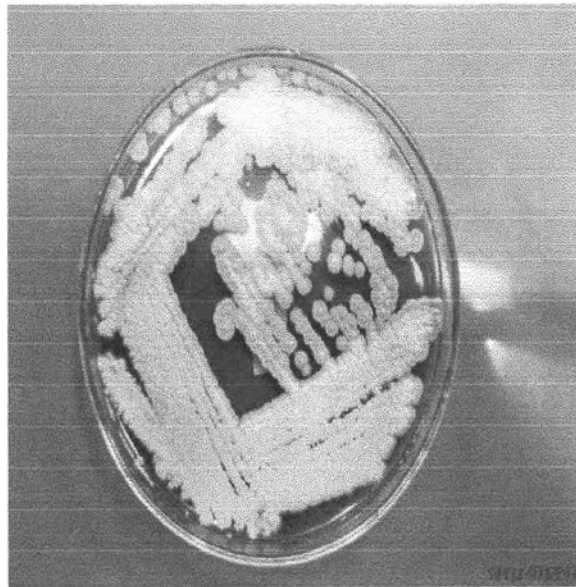
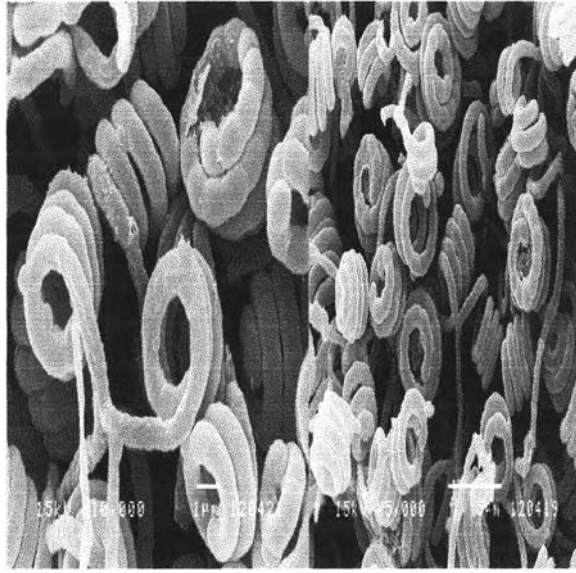


Figure 4.8 Scanning electron micrograph and colonial appearance of *Streptomyces* sp. J15-1 grown on YMA medium (7 days)

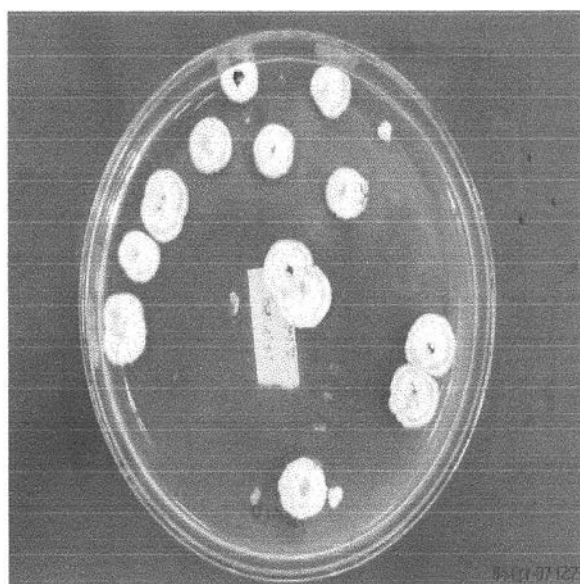
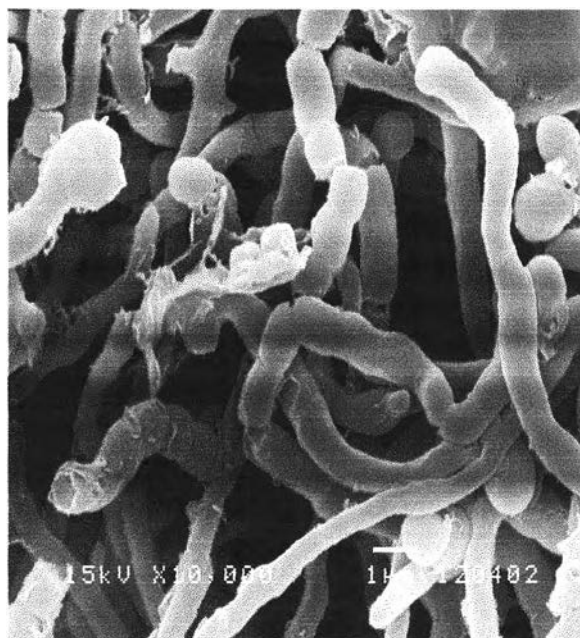


Figure 4.9 Scanning electron micrograph and colonial appearance of *Streptomyces* sp. J17-2 grown on YMA medium (14 days)

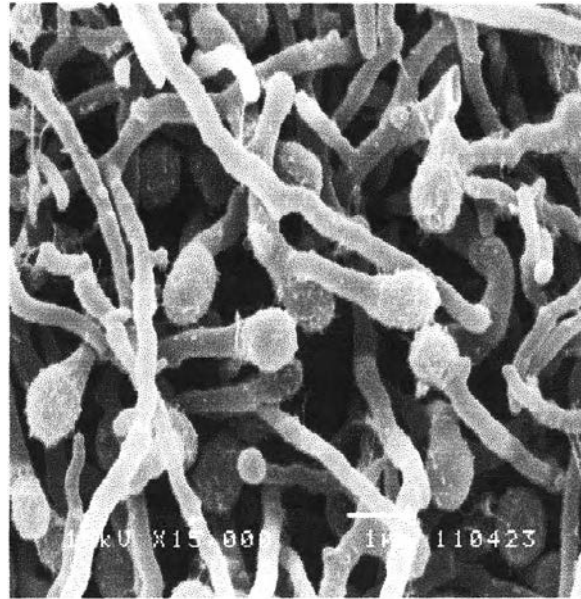


Figure 4.10 Scanning electron micrograph and colonial appearance of *Micromonospora* sp. B5-2-1 grown on YMA medium (7 days)

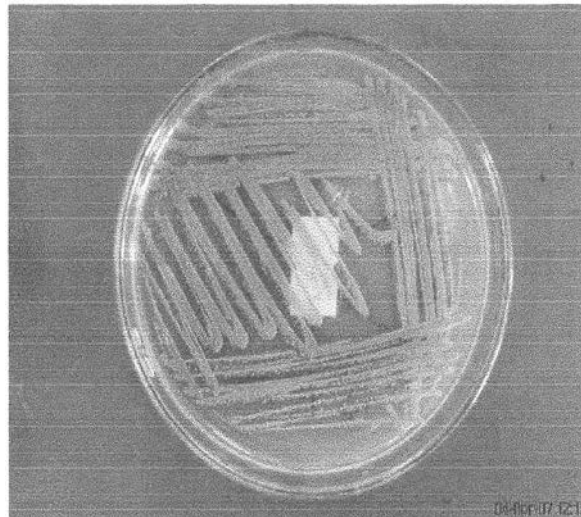
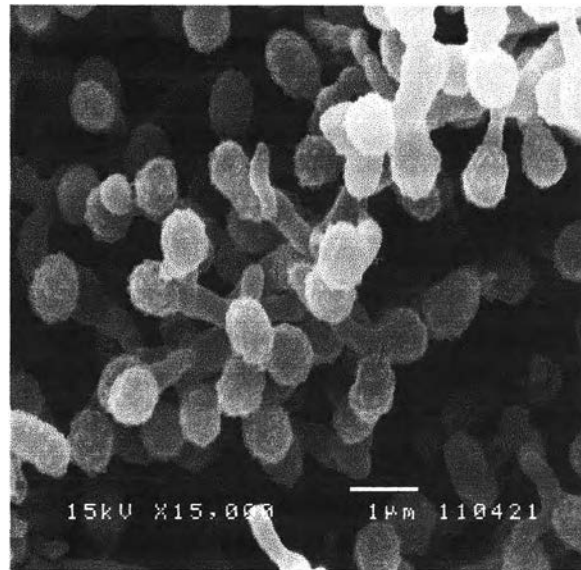


Figure 4.11 Scanning electron micrograph and colonial appearance of *Micromonospora* sp. B5-8-1 grown on YMA medium (7 days)

Table 4.5 Cultural characteristics of the isolates grown on YMA at 30°C for 14 days

Isolate no.	Colonial appearance	Colony color	
		Upper	Lower
PH2-1	Mucoid	Orange	Dark yellowish brown
SAM2-1	Powdery	Pale reddish yellow	Light reddish yellow
SAM2-2	Powdery	Yellowish white	Orange yellow
SMP2-1	Mucoid	Orange	Dark yellowish brown
SMP3-1	Powdery	Rose	Strong yellowish yellow
A5-8-1	Powdery	Yellowish white	Yellowish white
A10-4-2	Mucoid	Orange	Orange
A10-7-1	Powdery	Light bluish gray	Pale beige
B5-2-1	Mucoid	Orange	Orange
B5-6-1	Powdery	White	Pale beige
B5-8-1	Mucoid	Orange	Orange
B10-3-1	Powdery	White	Dull reddish yellow
B10-3-2	Powdery	Yellowish white	Brownish gold
B10-3-3	Mucoid	Pale orange	Pale orange
B10-3-4	Powdery	White	Pale yellow
B10-3-5	Powdery	White	Pale beige
B10-4-1	Mucoid	Orange	Orange
B10-8-3	Mucoid	Orange	Brown
B15-2-1	Powdery	Pale yellow	Orange yellow
B15-4-1	Powdery	White	Yellowish white
C10-2-1	Powdery	Brownish white	Brownish white
C10-6-1	Powdery	Pale yellow	Grayish yellow
D5-7-1	Powdery	White rose	Yellowish white
D5-7-2	Powdery	Grayish white	Redish yellow
D10-1-1	Powdery	White	Grayish brown
D10-3-1	Powdery	Yellowish white	Yellowish white
D10-5-1	Powdery	White	Yellowish white
D10-7-1	Powdery	Yellowish white	Yellowish white
D10-7-2	Powdery	Gray	Orange yellow
D10-9-1	Powdery	Yellowish white	Grayish brown
D10-9-2	Powdery	Yellowish white	Yellowish white

Table 4.5 Cultural characteristics of the isolates grown on YMA at 30°C for 14 days

Isolate no.	Colonial appearance	Colony color	
		Top	Bottom
D10-9-5	Powdery	Grayish white	Yellowish white
D10-9-6	Mucoid	Orange	Brownish orange
J4-2	Mucoid	Orange yellow	black
J5-1	Powdery	Yellowish white	Light yellowish brown
J6-1	Powdery	Orange	Orange
J8-1	Powdery	Yellowish grey	Brownish gold
J10-2	Mucoid	Orange	Orange
J10-3	Mucoid	Brownish gold	Brownish gold
J12-1	Powdery	Yellowish white	Yellowish white
J14-1	Powdery	White	Pale yellow
J14-2	Powdery	Orange	Orange
J15-1	Powdery	Olive	Olive
J15-2	Powdery	White	Pale yellow
J16-1	Powdery	Yellowish white	Pale greenish yellow
J16-2	Powdery	White	Yellowish white
J17-2	Powdery	Yellowish white	Yellowish white
J17-3	Mucoid	Orange	black
J18-1	Powdery	White	Pale beige
J19-1	Powdery	White	Yellowish white

2.2 Physiological and biochemical characteristics

Most of 50 strains grew on YMA medium containing 2% and 4% NaCl, at pH 5.0, 7.0, 9.0, 10.0, and at 28°C or 45°C, whereas fewer strains grew in 6% NaCl, all strains could not grow at pH 4, and at 10°C. Their physiological characteristics of were shown in Table 4.6.

The biochemical characteristics of the 30 antimicrobial producing actinomycetes could not hydrolyse chitin. Most strains reduced nitrate, coagulated skim milk, hydrolysed starch and liquefied gelatin, whereas some strains formed melanin, but all strains did not produced H₂S (Table 4.7). Most strains used glucose, glycerol, L-arabinose, D-xylose, mannitol, fructose, sucrose, rhamnose, and raffinose, as single carbon sources (Table 4.8).

Table 4.6 Physiological characteristics of the antimicrobial producing actinomycetes isolated grown on YMA medium at various conditions

Isolate no.	NaCl			pH					Temperature		
	2%	4%	6%	4	5	7	9	10	10°C	28°C	45°C
PH2-1	-	+	+	-	±	+	+	±	-	+	+
SAM2-1	+	+	±	-	±	+	+	+	-	+	±
SAM2-2	+	+	-	-	-	+	+	+	-	+	±
SMP2-1	±	+	+	-	-	+	+	+	-	+	+
SMP3-1	-	+	-	-	-	-	+	+	-	+	+
A5-8-1	+	+	+	-	+	+	+	+	-	+	±
A10-4-2	-	-	-	-	-	+	+	-	-	+	+
A10-7-1	+	+	+	-	-	+	+	±	-	+	±
B5-2-1	±	+	+	-	±	±	+	±	-	+	+
B5-6-1	+	+	+	-	-	+	+	±	-	+	±
B5-8-1	-	+	±	-	-	+	+	-	-	+	+
B10-3-1	±	±	-	-	±	+	+	±	-	+	+
B10-3-2	-	-	-	-	±	+	+	-	-	+	±
B10-3-3	-	+	+	-	-	+	+	±	-	+	+
B10-3-4	+	+	±	-	-	+	+	+	-	+	+
B10-3-5	+	±	±	-	-	+	+	+	-	+	+
B10-4-1	-	-	+	-	-	+	+	-	-	+	±
B10-8-3	-	-	+	-	±	+	+	+	-	+	+
B15-2-1	+	+	+	-	-	+	±	±	-	+	±
B15-4-1	±	+	+	-	-	+	+	+	-	+	±
C10-2-1	+	-	-	-	-	+	+	-	-	+	±
C10-6-1	±	±	-	-	-	+	+	-	-	+	±
D5-7-1	+	+	+	-	-	+	+	±	-	+	+
D5-7-2	-	+	+	-	-	+	±	+	-	+	±
D10-1-1	+	+	+	-	+	+	±	+	-	+	+
D10-3-1	+	+	+	-	-	+	±	±	-	+	+
D10-5-1	+	+	+	-	+	+	+	+	-	+	+
D10-7-1	+	+	+	-	-	+	+	±	-	+	+
D10-7-2	-	+	+	-	-	±	+	+	-	+	+
D10-9-1	+	+	±	-	-	+	+	±	-	+	±

Table 4.6 Physiological characteristics of the antimicrobial producing actinomycetes isolated grown on YMA medium at various conditions

Isolate no.	NaCl			pH					Temperature		
	2%	4%	6%	4	5	7	9	10	10°C	28°C	45°C
D10-9-2	±	±	±	-	±	+	±	-	-	+	±
D10-9-5	+	+	+	-	-	+	+	+	-	+	+
D10-9-6	-	±	±	-	±	+	+	±	-	+	±
J4-2	-	+	+	-	±	+	+	±	-	+	+
J5-1	-	-	-	-	±	+	+	+	-	+	±
J6-1	+	+	+	-	+	+	+	+	-	+	+
J8-1	-	-	-	-	-	+	+	-	-	+	+
J10-2	-	±	±	-	±	±	+	+	-	+	+
J10-3	±	±	±	-	-	+	+	+	-	+	+
J12-1	+	+	+	-	+	+	+	+	-	+	+
J14-1	-	+	+	-	-	+	+	-	-	+	+
J14-2	-	+	+	-	-	+	+	+	-	+	+
J15-1	+	+	-	-	+	+	+	+	-	+	+
J15-2	+	+	+	-	-	+	+	+	-	+	+
J16-1	+	+	+	-	±	+	+	+	-	+	+
J16-2	+	+	+	-	-	+	+	±	-	+	+
J17-2	+	+	+	-	+	+	+	+	-	+	+
J17-3	±	+	+	-	-	+	+	±	-	+	+
J18-1	±	+	+	-	-	-	+	+	-	+	+
J19-1	-	+	+	-	-	+	+	-	-	+	+

Table 4.7 Biochemical characteristics of the antimicrobial producing actinomycetes isolated

Isolate no.	NO ₃ reduction	H ₂ S production	Melanin formation	Starch hydrolysis	Chitin hydrolysis	Gelatin liquefaction	Skim milk	
							Coagulation	Peptonization
SAM2-1	+	-	-	+-	-	+	+	-
SAM2-2	+	-	-	+-	-	+	+	+
SMP3-1	+	-	-	+	-	+	-	-
B10-3-1	+	-	-	+	-	+	+	+
B10-3-2	+	-	-	+	-	+	+	+
B10-3-4	+	-	-	+	-	+	-	-
B15-4-1	+	-	-	+	-	+	-	-
C10-2-1	+	-	-	+	-	+	+	+
C10-6-1	+	-	-	+	-	+	+	+
D5-7-1	+	-	-	+	-	+	+	+
D5-7-2	+	-	-	+	-	+	-	-
D10-1-1	+	-	-	+	-	-	+	+
D10-5-1	+	-	-	+	-	+	+	+
D10-7-1	+	-	-	+	-	-	+	+
D10-7-2	+	-	-	+	-	+	-	-
D10-9-1	+	-	+	+	-	+	-	+
J5-1	+	-	-	+	-	+	+	+
J6-1	+	-	-	+-	-	+	-	+
J8-1	+	-	-	+	-	+	+	+

Table 4.7 Biochemical characteristics of the antimicrobial producing actinomycetes isolated

Isolate no.	NO ₃ reduction	H ₂ S production	Melanin formation	Starch hydrolysis	Chitin hydrolysis	Gelatin liquefaction	Skim milk	
							Coagulation	Peptonization
J10-3	+	-	-	+	-	+	-	-
J12-1	+	-	-	+	-	+	+	+
J14-1	+	-	-	+	-	+	+	+
J14-2	+	-	-	+	-	+	+	+
J15-1	+	-	-	+	-	+	+	+
J15-2	+	-	-	+-	-	+	+	+
J16-1	+	-	-	+-	-	+	+	+
J16-2	+	-	-	+	-	+	+	+
J17-2	+	-	-	+	-	+	-	+
J18-1	+	-	-	+	-	+	+	+
J19-1	+	-	-	+	-	+	-	-

Table 4.8 Utilization of various carbon sources by the antimicrobial producing isolated actinomycetes at 30°C for 14 days

Isolate no.	None	Glucose	Glycerol	L-Arabinose	D-Xylose	Mannitol	Fructose	Sucrose	Rhamnose	Raffinose
SAM2-1	-	-	+	±	±	+	-	-	+	+
SAM2-2	-	-	+	+	+	±	+	±	±	+
SMP3-1	-	±	±	-	-	±	-	+	-	-
B5-2-1	±	±	±	±	±	-	-	±	±	-
B5-8-1	-	+	+	-	-	-	-	±	-	-
B10-3-1	-	+	+	-	-	±	-	-	+	+
B10-3-2	±	+	+	-	-	+	-	-	+	+
B10-3-4	-	+	+	-	-	±	±	-	+	+
B15-4-1	±	±	-	-	-	-	-	-	±	-
C10-2-1	-	-	±	-	+	-	-	-	-	-
C10-6-1	±	±	+	-	-	-	-	±	-	-
D5-7-1	±	+	+	+	-	±	-	+	+	-
D5-7-2	±	+	+	-	-	-	-	+	+	-
D10-1-1	±	+	±	+	+	+	+	-	-	+
D10-5-1	±	+	+	+	+	+	+	+	+	+
D10-7-1	±	+	+	-	-	+	+	+	+	-
D10-7-2	±	+	+	+	+	+	+	+	+	+
D10-9-1	-	-	-	+	+	+	+	+	-	-
D10-9-6	-	+	+	-	-	-	-	±	-	-
J5-1	-	-	+	-	-	+	-	-	+	-
J6-1	-	+	+	+	+	+	+	±	+	+
J8-1	-	+	±	+	+	+	+	±	+	+

Table 4.8 Utilization of various carbon sources by the antimicrobial producing isolated actinomycetes at 30°C for 14 days

Isolate no.	None	Glucose	Glycerol	L-Arabinose	D-Xylose	Mannitol	Fructose	Sucrose	Rhamnose	Raffinose
J10-3	-	+	+	-	-	-	±	-	+	-
J12-1	-	-	-	-	-	-	+	-	-	-
J14-1	-	-	+	-	-	-	-	-	-	-
J14-2	-	+	±	-	-	+	+	+	+	+
J15-1	-	+	+	+	+	+	+	-	+	-
J15-2	-	-	+	-	-	-	-	+	+	-
J16-1	-	+	±	-	-	+	+	-	-	-
J16-2	-	+	-	-	-	-	-	-	+	-
J17-2	-	+	±	+	+	-	-	+	+	-
J18-1	±	+	±	+	+	±	+	+	+	+
J19-1	±	+	+	+	+	+	+	-	+	+

Results were recorded as follows:

+, Positive when growth on tested carbon in basal medium is equal to or greater than growth on basal medium plus glucose; ±, doubtful when growth on tested carbon is significantly better than on the basal medium without carbon, but somewhere less than on the basal medium plus glucose; -, negative when growth is similar to or less than growth on basal medium without carbon (utilization is always recorded as negative if growth is not better than no carbon control).

2.3 Chemotaxonomic characteristics

Cell wall composition of the representative strains, isolate number SAM2-1, SMP3-1, B10-3-1, B10-3-2, B10-3-4, B15-4-1, C10-2, C10-6, D5-7-1, D10-1-1, D10-5-1, D10-7-2, J8-1, J15-1, J16-1, J17-2 and J18-1 were L-diaminopimelic acid (L-DAP) (Table 4.9), indicating that these strains have wall chemotype I as described by Lechevalier & Lechevalier (1970), which is the cell wall type of *Streptomyces* sp. as described by Schleifer & Kandler (1972). The major menaquinones of 4 tested strains were MK-9(H₆) (33.2%-67.6%), and MK-9(H₈) (32.4%-66.8%). G+C DNA contents of the 4 representative strains ranged 69-73.8 mol% which were similar to those of *Streptomyces* sp. (69-78 mol%).

Strain B5-8-1 contained meso-diaminopimelic acid, indicated that this strain had cell wall chemotype II, which was the cell wall type of *Micromonospora* as described by Schleifer & Kandler (1972). G+C DNA content of B5-8-1 strain was not determined, but normally G+C DNA contents of *Micromonospora* species were 71-73 mol%. The major menaquinone of *Micromonospora* were MK-9(H₄), MK-10(H₄).

Table 4.9 DNA G+C contents, Diaminopimelic acid type, and % of menaquinone of the representative strains

Isolate no.	G+C content (mol%)	Diaminopimelic acid type		% of Menaquinone	
		<i>meso</i> -DAP	L-DAP	MK-9(H ₆)	MK-9(H ₈)
SAM2-1		-	+	51.6	48.4
SMP3-1		-	+	33.2	66.8
B5-8-1		+	-		
B10-3-1		-	+		
B10-3-2		-	+	67.6	32.4
B10-3-4		-	+		
B15-4-1		-	+		
C10-2		-	+		
C10-6		-	+	43.4	56.6
D5-7-1	72.29	-	+		
D10-1-1	69.03	-	+		
D10-5-1	72	-	+		
D10-7-2		-	+		
J8-1	73.84	-	+		
J15-1		-	+		
J16-1		-	+		
J17-2		-	+		
J18-1	70.27	-	+		

2.4 16S rDNA sequence analysis and phylogenetic tree construction

2.4.1 16S rDNA amplification by PCR

Chromosomal DNAs of the representative strains (SAM2-1, SMP3-1, B15-4, D10-1, D10-5, J8-1, and J17-2) were used as DNA templates for 16S rDNA amplification by PCR. Primers of SAM2-1, B15-4, D10-1, D10-5, J8-1, and J17-2 strains were 357R, 802R, 1115R, 1541R and the primers of SMP3-1 was 27f, 530f, and 1114f. All of them clearly yield ~ 1.5 kb PCR product which their sizes corresponded well with the size of 16S rDNA. The amplified 16S rDNA fragment was digested with *EcoRI* and the restriction fragments were analyzed in 1% agarose gel electrophoresis.

2.4.2 16S rDNA sequence

The nucleotide sequences of 16S rDNA amplified fragment of the representative strain were determined by thermal cycle DNA sequencing method. The obtained nucleotide sequences are illustrated in APPENDIX C. The 16S rDNA sequence alignment results between the representative strains and those of all of validly described *Streptomyces* species.

2.4.3 16S rDNA sequence analysis and phylogenetic tree construction

The almost complete 16S rDNA sequences consisting of 1,070-1,542 nucleotides were determined for all of representative strains; 1,394 nt fragment was used for phylogenetic analysis of Figure 4.12, similarity percentage calculation, and compared with 16S rDNA database sequences of members of the family *Streptomycetaceae*. The phylogenetic tree was constructed from evolutionary distances by using neighbor-joining method in the MEGA software programme version 2.1.

Phylogenetic analysis of the almost complete 16S rDNA sequence of the tested organisms with the corresponding sequences of all the type strains of validly described *Streptomyces* species, selected sequences of *Actinoplanes*

species, and *Micromonospora* sp. as an outgroup as shown in Figure 4.12 Table 4.10 and Figure 4.13 Table 4.11, respectively. It is evident from figure 12 that SAM2-1, B15-4, D10-1, and J17-2 strains form distinct phyletic lines in the 16S *Streptomyces* rDNA tree

The two strains of *Streptomyces* (SAM2-1 and B15-4) shared 16S rDNA nucleotide similarities within the range 97.7%, values which correspond to 32 nucleotide differences. Strain SAM2-1 showed almost identical physiological and biochemical properties to B15-4.

The other two strains of *Streptomyces* (D10-1 and J17-2) showed identical 16S rDNA nucleotide sequences was supported by a high bootstrap value of 100%. These two strains shared a 16S rDNA similarity of 92.7%, which corresponds to the 102 nucleotide differences.

Compare similarity percentage of each strain (SAM2-1, B15-4, D10-1, and J17-2) with all the type strains they showed 98.4%, 98.3%, 94.7%, and 93.5% respectively so maybe indicated that the %similarity of the strains which lower than 97.5% is the new species.

From figure 13 that SMP3-1, D10-5, and J8-1 strains form distinct phyletic lines in the 16S *Streptomyces* rDNA tree. The two strains of *Streptomyces* sp. (SMP3-1 and D10-5) shared 16S rDNA nucleotide similarities within the range 93.5%, and J8-1 strain form subclade with *S. vayuensis* N2^T. When compare similarity percentage of each strain with all the type strains they presented %similarity lower than 97.0% they are novel species. However, if it was higher than 97.0%, the strains should be determined for the DNA-DNA hybridization experiments to confirm their taxonomic status as the novel species.

Figure 4.12 Unroot neighbor-joining tree base on nearly complete 16S rDNA sequences, showing the position of the representation *Streptomyces* strains in the *Streptomyces* tree.

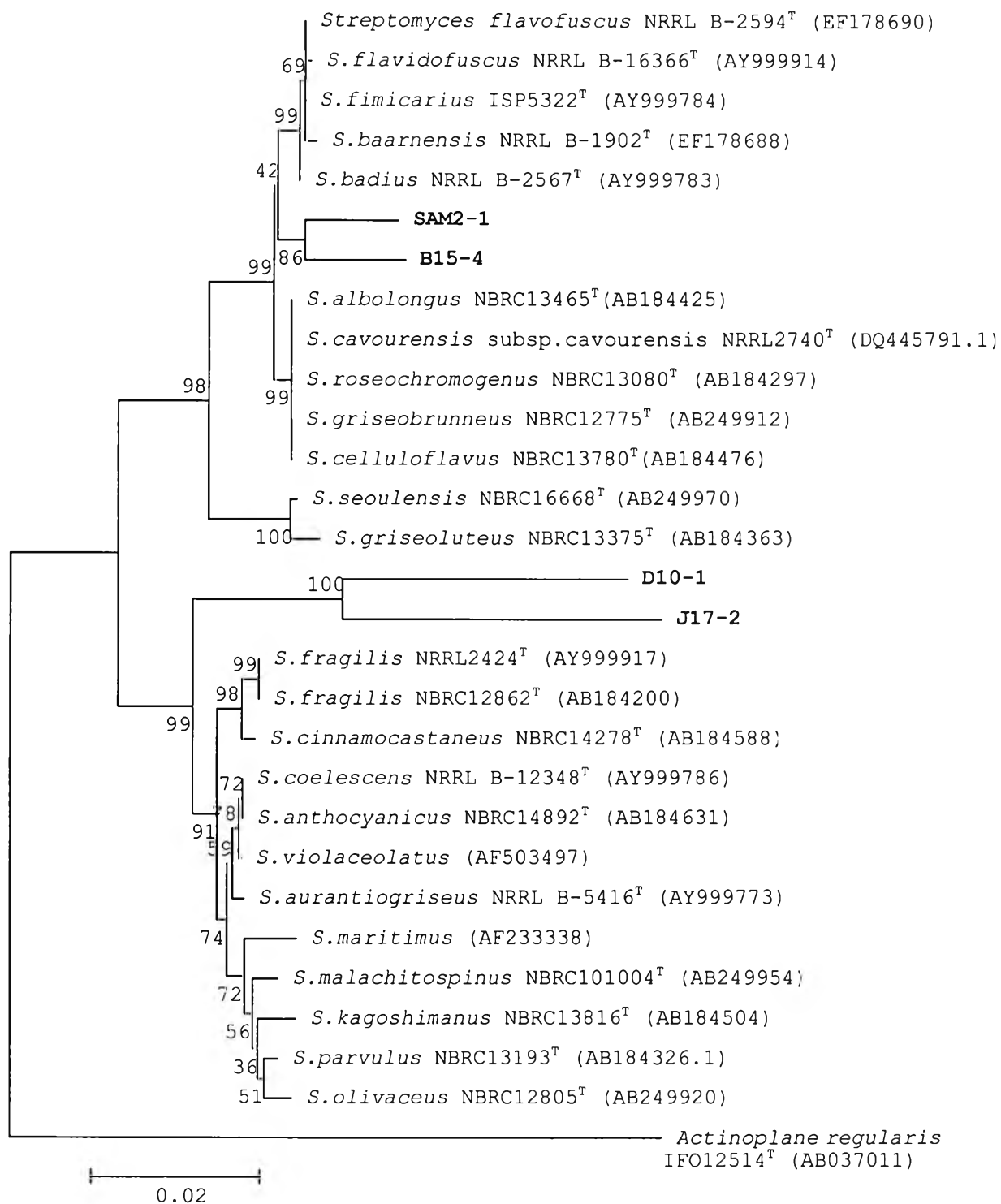


Table 4.10 Similarity percentage of the representative *Streptomyces* strains

Accession no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1.SAM2-1	100	98.3	98.4	98.3	98.2	98.3	97.7	98.2	98.2	98.2	98.2	98.2	94.8	95	95.4	94.7	94.7	95.4	94.6	95.5	90.9	87.9	95.1	96.5	95.1	96.1	95.1	95.4	90.9
2.EF178690	98.3	100	99.9	100	99.9	99.9	98	99.4	99.4	99.4	99.4	99.4	95.8	95.8	96.3	95.7	95.6	96.3	95.6	96.4	91.5	88.6	96.2	98	96.2	97.6	96.2	96.3	91.4
3.AY999783	98.4	99.9	100	99.9	99.8	99.9	98.1	99.5	99.5	99.5	99.5	99.5	95.9	95.9	96.4	95.8	95.7	96.4	95.7	96.5	91.4	88.6	96.2	98	96.2	97.5	96.2	96.4	91.3
4.AY999784	98.3	100	99.9	100	99.9	99.9	98	99.4	99.4	99.4	99.4	99.4	95.8	95.8	96.3	95.7	95.6	96.3	95.6	96.4	91.5	88.6	96.2	98	96.2	97.6	96.2	96.3	91.4
5.EF178688	98.2	99.9	99.8	99.9	100	99.8	97.9	99.3	99.3	99.3	99.3	99.3	95.7	95.7	96.2	95.5	95.5	96.1	95.5	96.2	91.4	88.6	96	97.9	96	97.4	96	96.1	91.2
6.AY999914	98.3	99.9	99.9	99.9	99.8	100	98	99.3	99.3	99.3	99.3	99.3	95.8	95.8	96.2	95.6	95.5	96.2	95.5	96.3	91.4	88.5	96.1	98	96.1	97.5	96.1	96.2	91.3
7.B15-4	97.7	98	98.1	98	97.9	98	100	98.3	98.3	98.3	98.3	98.3	94.5	94.7	95	94.4	94.3	95	94.3	95.1	90.7	87.5	94.7	96.5	94.9	96.1	94.7	95	90.8
8.DQ445791	98.2	99.4	99.5	99.4	99.3	99.3	98.3	100	100	100	100	100	95.8	95.9	96.4	95.7	95.7	96.4	95.7	96.4	91.4	89.1	96.1	98	96.2	97.6	96.1	96.4	91.6
9.AB249912	98.2	99.4	99.5	99.4	99.3	99.3	98.3	100	100	100	100	100	95.8	95.9	96.4	95.7	95.7	96.4	95.7	96.4	91.4	89.1	96.1	98	96.2	97.6	96.1	96.4	91.6
10.AB184425	98.2	99.4	99.5	99.4	99.3	99.3	98.3	100	100	100	100	100	95.8	95.9	96.4	95.7	95.7	96.4	95.7	96.4	91.4	89.1	96.1	98	96.2	97.6	96.1	96.4	91.6
11.AB184297	98.2	99.4	99.5	99.4	99.3	99.3	98.3	100	100	100	100	100	95.8	95.9	96.4	95.7	95.7	96.4	95.7	96.4	91.4	89.1	96.1	98	96.2	97.6	96.1	96.4	91.6
12.AB184476	98.2	99.4	99.5	99.4	99.3	99.3	98.3	100	100	100	100	100	95.8	95.9	96.4	95.7	95.7	96.4	95.7	96.4	91.4	89.1	96.1	98	96.2	97.6	96.1	96.4	91.6
13.AB184326	94.8	95.8	95.9	95.8	95.7	95.8	94.5	95.8	95.8	95.8	95.8	95.8	100	99.4	99.3	99.5	98.9	99.2	99.4	99.1	94.7	89.5	98.8	96.1	98.8	96.1	98.8	99.2	93.1
14.AB249954	95	95.8	95.9	95.8	95.7	95.8	94.7	95.9	95.9	95.9	95.9	95.9	99.4	100	99.2	99.3	99	99.2	99.1	99.3	94.3	89.3	98.7	96.1	98.7	96.1	98.7	99.2	93.1
15.AY999773	95.4	96.3	96.4	96.3	96.2	96.2	95	96.4	96.4	96.4	96.4	96.4	99.3	99.2	100	99.2	99.1	99.8	98.8	99.7	94.5	89.6	99.1	96.7	99.1	96.5	99.1	99.8	93.1
16.AB249920	94.7	95.7	95.8	95.7	95.5	95.6	94.4	95.7	95.7	95.7	95.7	95.7	99.5	99.3	99.2	100	98.5	99.1	99	99.1	94.5	89.4	98.6	95.8	98.6	95.8	98.6	99.1	93.1

Table 4.10 Similarity percentage of the representative *Streptomyces* strains

Accession no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
17.AF233338	94.7	95.6	95.7	95.6	95.5	95.5	94.3	95.7	95.7	95.7	95.7	95.7	98.9	99	99.1	98.5	100	99	98.9	98.9	94.1	89.1	98.7	96	98.7	96	98.7	99	92.9
18.AY999786	95.4	96.3	96.4	96.3	96.1	96.2	95	96.4	96.4	96.4	96.4	96.4	99.2	99.2	99.8	99.1	99	100	98.8	99.9	94.4	89.6	99.3	96.7	99.3	96.5	99.3	100	93.3
19.AB184504	94.6	95.6	95.7	95.6	95.5	95.5	94.3	95.7	95.7	95.7	95.7	95.7	99.4	99.1	98.8	99	98.9	98.8	100	98.7	94.3	89.6	98.8	95.9	98.7	95.9	98.8	98.8	92.9
20.AF503497	95.5	96.4	96.5	96.4	96.2	96.3	95.1	96.4	96.4	96.4	96.4	96.4	99.1	99.3	99.7	99.1	98.9	99.9	98.7	100	94.3	89.6	99.2	96.7	99.2	96.5	99.2	99.9	93.3
21.D10-1	90.9	91.5	91.4	91.5	91.4	91.4	90.7	91.4	91.4	91.4	91.4	91.4	94.7	94.3	94.5	94.5	94.1	94.4	94.3	94.3	100	84.5	94.2	91.7	94.2	91.4	94.2	94.4	92.7
22.AB037011	87.9	88.6	88.6	88.6	88.6	88.5	87.5	89.1	89.1	89.1	89.1	89.1	89.5	89.3	89.6	89.4	89.1	89.6	89.6	89.6	84.5	100	89.7	88.4	89.6	88	89.7	89.6	84.1
23.AY999917	95.1	96.2	96.2	96.2	96	96.1	94.7	96.1	96.1	96.1	96.1	96.1	98.8	98.7	99.1	98.6	98.7	99.3	98.8	99.2	94.2	89.7	100	96.5	99.6	96.4	100	99.3	93.4
24.AB249970	96.5	98	98	98	97.9	98	96.5	98	98	98	98	98	96.1	96.1	96.7	95.8	96	96.7	95.9	96.7	91.7	88.4	96.5	100	96.6	99.6	96.5	96.7	92.6
25.AB184588	95.1	96.2	96.2	96.2	96	96.1	94.9	96.2	96.2	96.2	96.2	96.2	98.8	98.7	99.1	98.6	98.7	99.3	98.7	99.2	94.2	89.6	99.6	96.6	100	96.5	99.6	99.3	93.5
26.AB184363	96.1	97.6	97.5	97.6	97.4	97.5	96.1	97.6	97.6	97.6	97.6	97.6	96.1	96.1	96.5	95.8	96	96.5	95.9	96.5	91.4	88	96.4	99.6	96.5	100	96.4	96.5	92.6
27.AB184200	95.1	96.2	96.2	96.2	96	96.1	94.7	96.1	96.1	96.1	96.1	96.1	98.8	98.7	99.1	98.6	98.7	99.3	98.8	99.2	94.2	89.7	100	96.5	99.6	96.4	100	99.3	93.4
28.AB184631	95.4	96.3	96.4	96.3	96.1	96.2	95	96.4	96.4	96.4	96.4	96.4	99.2	99.2	99.8	99.1	99	100	98.8	99.9	94.4	89.6	99.3	96.7	99.3	96.5	99.3	100	93.3
29.J17-2	90.9	91.4	91.3	91.4	91.2	91.3	90.8	91.6	91.6	91.6	91.6	91.6	93.1	93.1	93.1	93.1	92.9	93.3	92.9	93.3	92.7	84.1	93.4	92.6	93.5	92.6	93.4	93.3	100

Figure 4.13 Unroot neighbor-joining tree base on nearly complete 16S rDNA sequences, showing the position of the representation *Streptomyces* strains in the *Streptomyces* tree.

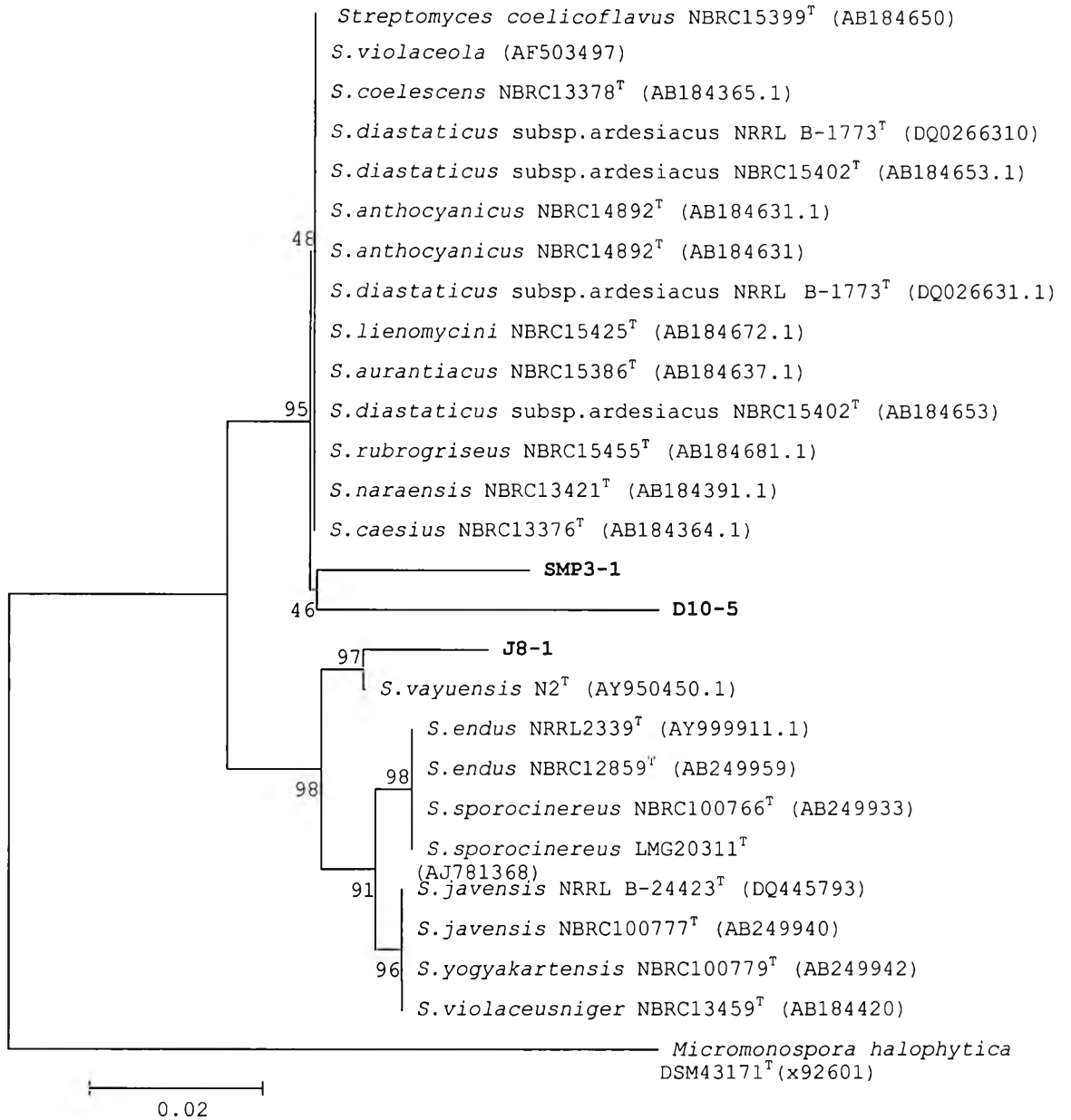


Table 4.11 Similarity percentage of the representative *Streptomyces* strains

Accession no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28		
1.J8-1	100	98.5	97.2	96.8	96.8	96.8	97.2	97.2	96.8	97.2	95.9	95.9	95.9	95.9	95.9	93.3	95.9	95.9	95.9	95.9	95.9	95.9	95.9	95.9	95.9	95.9	95.9	93.3	86.8	
2.AY950450	98.5	100	98.7	98.3	98.3	98.3	98.7	98.7	98.3	98.7	97.4	97.4	97.4	97.4	97.4	94.7	97.4	97.4	97.4	97.4	97.4	97.4	97.4	97.4	97.4	97.4	97.4	93.3	88.5	
3.AY999911	97.2	98.7	100	99.3	99.3	99.3	100	100	99.3	100	96.8	96.8	96.8	96.8	96.8	94.1	96.8	96.8	96.8	96.8	96.8	96.8	96.8	96.8	96.8	96.8	96.8	92.7	87.8	
4.DQ445793	96.8	98.3	99.3	100	100	100	99.3	99.3	100	99.3	97	97	97	97	97	94.3	97	97	97	97	97	97	97	97	97	97	97	92.9	87.6	
5.AB249942	96.8	98.3	99.3	100	100	100	99.3	99.3	100	99.3	97	97	97	97	97	94.3	97	97	97	97	97	97	97	97	97	97	97	92.9	87.6	
6.AB249940	96.8	98.3	99.3	100	100	100	99.3	99.3	100	99.3	97	97	97	97	97	94.3	97	97	97	97	97	97	97	97	97	97	97	92.9	87.6	
7.AB249933	97.2	98.7	100	99.3	99.3	99.3	100	100	99.3	100	96.8	96.8	96.8	96.8	96.8	94.1	96.8	96.8	96.8	96.8	96.8	96.8	96.8	96.8	96.8	96.8	96.8	92.7	87.8	
8.AJ781368	97.2	98.7	100	99.3	99.3	99.3	100	100	99.3	100	96.8	96.8	96.8	96.8	96.8	94.1	96.8	96.8	96.8	96.8	96.8	96.8	96.8	96.8	96.8	96.8	96.8	92.7	87.8	
9.AB184420	96.8	98.3	99.3	100	100	100	99.3	99.3	100	99.3	97	97	97	97	97	94.3	97	97	97	97	97	97	97	97	97	97	97	92.9	87.6	
10.AB249959	97.2	98.7	100	99.3	99.3	99.3	100	100	99.3	100	96.8	96.8	96.8	96.8	96.8	94.1	96.8	96.8	96.8	96.8	96.8	96.8	96.8	96.8	96.8	96.8	96.8	92.7	87.8	
11.AB184653	95.9	97.4	96.8	97	97	97	96.8	96.8	97	96.8	100	100	100	100	100	97.4	100	100	100	100	100	100	100	100	100	100	100	95.9	88.9	
12.DQ026631	95.9	97.4	96.8	97	97	97	96.8	96.8	97	96.8	100	100	100	100	100	97.4	100	100	100	100	100	100	100	100	100	100	100	100	95.9	88.9
13.AB184650	95.9	97.4	96.8	97	97	97	96.8	96.8	97	96.8	100	100	100	100	100	97.4	100	100	100	100	100	100	100	100	100	100	100	100	95.9	88.9
14.AF503497	95.9	97.4	96.8	97	97	97	96.8	96.8	97	96.8	100	100	100	100	100	97.4	100	100	100	100	100	100	100	100	100	100	100	100	95.9	88.9
15.AB184631	95.9	97.4	96.8	97	97	97	96.8	96.8	97	96.8	100	100	100	100	100	97.4	100	100	100	100	100	100	100	100	100	100	100	100	95.9	88.9
16.SMP3-1	93.3	94.7	94.1	94.3	94.3	94.3	94.1	94.1	94.3	94.1	97.4	97.4	97.4	97.4	97.4	100	97.4	97.4	97.4	97.4	97.4	97.4	97.4	97.4	97.4	97.4	97.4	93.5	86.3	
17.AB184653	95.9	97.4	96.8	97	97	97	96.8	96.8	97	96.8	100	100	100	100	100	97.4	100	100	100	100	100	100	100	100	100	100	100	100	95.9	88.9
18.DQ026631.1	95.9	97.4	96.8	97	97	97	96.8	96.8	97	96.8	100	100	100	100	100	97.4	100	100	100	100	100	100	100	100	100	100	100	100	95.9	88.9
19.AB184672.1	95.9	97.4	96.8	97	97	97	96.8	96.8	97	96.8	100	100	100	100	100	97.4	100	100	100	100	100	100	100	100	100	100	100	100	95.9	88.9
20.AB184650.1	95.9	97.4	96.8	97	97	97	96.8	96.8	97	96.8	100	100	100	100	100	97.4	100	100	100	100	100	100	100	100	100	100	100	100	95.9	88.9
21.AB184637.1	95.9	97.4	96.8	97	97	97	96.8	96.8	97	96.8	100	100	100	100	100	97.4	100	100	100	100	100	100	100	100	100	100	100	100	95.9	88.9
22.AB184631.1	95.9	97.4	96.8	97	97	97	96.8	96.8	97	96.8	100	100	100	100	100	97.4	100	100	100	100	100	100	100	100	100	100	100	100	95.9	88.9

Table 4.11 Similarity percentage of the representative *Streptomyces* strains

Accession no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
23.AB184391.1	95.9	97.4	96.8	97	97	97	96.8	96.8	97	96.8	100	100	100	100	100	97.4	100	100	100	100	100	100	100	100	100	100	100	95.9	88.9
24.AB184365.1	95.9	97.4	96.8	97	97	97	96.8	96.8	97	96.8	100	100	100	100	100	97.4	100	100	100	100	100	100	100	100	100	100	100	95.9	88.9
25.AB184364.1	95.9	97.4	96.8	97	97	97	96.8	96.8	97	96.8	100	100	100	100	100	97.4	100	100	100	100	100	100	100	100	100	100	100	95.9	88.9
26.D10-5	93.3	93.3	92.7	92.9	92.9	92.9	92.7	92.7	92.9	92.7	95.9	95.9	95.9	95.9	95.9	93.5	95.9	95.9	95.9	95.9	95.9	95.9	95.9	95.9	95.9	95.9	95.9	100	84.8
27.X92601	86.8	88.5	87.8	87.6	87.6	87.6	87.8	87.8	87.6	87.8	88.9	88.9	88.9	88.9	88.9	86.3	88.9	88.9	88.9	88.9	88.9	88.9	88.9	88.9	88.9	88.9	88.9	84.8	100

3. Antimicrobial activity (agar disc diffusion)

Two new isolate numbers SAM2-1 and SMP3-1 and 6 high antimicrobial activity *Streptomyces* sp. isolate number B10-3-1, B10-3-2, B10-3-4, C10-2, C10-6 and J15-1 were selected for antimicrobial activity test by agar disc diffusion method. They were culture in YMB medium (seed medium) on rotary shaker (200 rpm) for 4 days. Then each cultures was transferred into production medium (YMB containing calcium carbonate), and incubated at the same above conditions for 10 days. The culture filtrate was extracted by ethyl acetate, rotary evaporator dried, dissolved in methanol. Vacuum dried and redissolved in methanol were applied on a paper disc (0.6 cm diameter) at 1 mg/disc. The applied disc placed on agar media spreaded over by the tested microorganisms revealed inhibition zone as shown in Table 4.12. Streptomycin (20 μ g/disc) was used as positive control. All culture extracts inhibited Gram positive bacteria; *Staphylococcus aureus* ATCC 6538P, *Bacillus subtilis* ATCC 16633, *Micrococcus luteus* ATCC 9341. Culture extracts of isolates SMP3-1, B10-3-1, C10-2, C10-6 inhibited *Escherichia coli* ATCC 25922, and those of isolate SAM2-1, SMP3-1 inhibited *Pseudomonas aeruginosa* ATCC 27853. None of them inhibited *Candida albicans* ATCC 10231. All ethyl acetate extracts of isolates were analyzed by magnetic resonance (NMR). Only, the culture filtrate of strain C10-6 revealed interesting signals on the NMR spectrum as shown in Fig 4.14. Chloroform signal is 7.2 whereas the signals between 6 and 9 are the interesting signals to be occurred the compound ring structure of the secondary metabolites. The other NMR signals other than 6 to 9, could be primary metabolites that were the direct chains belonged to fat and sugars. Therefore, the strain C10-6 was selected for chemical study.

Table 4.12 Antimicrobial activity of the selected isolates in YMB medium

Isolation no.	Inhibitory activity (cm)					
	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 6538P	<i>C. albicans</i> ATCC 10231	<i>B. subtilis</i> ATCC 6633	<i>Ps.aeruginosa</i> ATCC 27853	<i>M.luteus</i> ATCC 9341
Negative control	-	-	-	-	-	-
SAM2-1	-	0.8	-	0.8	0.8	1.1
SMP3-1	1.6	0.8	-	0.8	0.8	0.8
B10-3-1	0.8	1	-	1	-	0.8
B10-3-2	-	1	-	0.9	-	0.8
B10-3-4	-	0.8	-	1.3	-	1
C10-2	1.7	1	-	1.2	-	0.9
C10-6	0.8	0.8	-	0.8	-	0.8
J15-1	-	1.2	-	0.7	-	0.8
Positive control	1	1.8	-	1.1	1	2.1

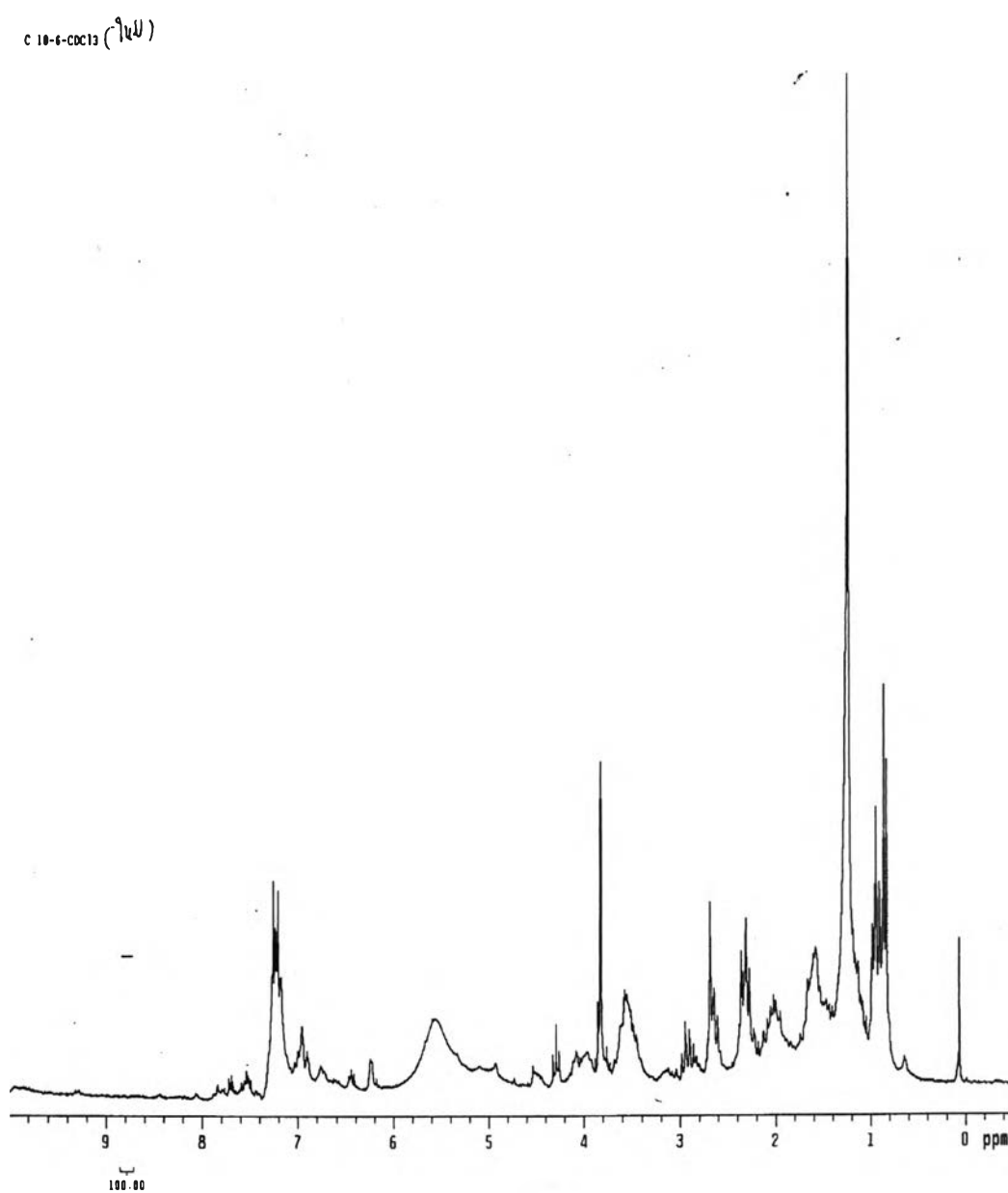


Figure 4.14 NMR spectrum of strain C10-6

2. Extraction and isolation of antimicrobial products from *Streptomyces sp.* C10-6

Yield of strain C10-6 was 438.2 mg of the ethyl acetate extract (dark brown oily liquid). Eight fractions were dissolved in chloroform-*d* and 2 fractions were dissolved in acetone-*d*₆ and subjected to nuclear magnetic resonance (NMR) analysis. NMR spectra of fractions 4, 5, 6, 7, 8 and 9 revealed interesting signals. Fraction 4, 5, 6, 7, 8 and 9 were tested for antimicrobial activity by agar disc diffusion method (Table 4.13). The 8 fraction exhibited antibacterial activity against Gram positive bacteria, *M. luteus* ATCC 9341 and Gram negative bacteria, *Ps. aeruginosa* ATCC 27853.

Table 4.13 Antimicrobial activity of chromatographic fractions of the extract of the strain C10-6

Fraction no. of C10-6	Inhibitory activity (cm)					
	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 6538P	<i>C. albicans</i> ATCC 10231	<i>B. subtilis</i> ATCC 6633	<i>Ps.aeruginosa</i> ATCC 27853	<i>M.luteus</i> ATCC 9341
4	-	0.9	-	1	-	-
5	-	0.9	-	0.8	-	-
6	-	0.8	1	-	-	-
7	-	0.8	1.1	-	-	-
8	1	0.9	-	0.7	1.1	1
9	-	0.9	-	0.7	-	0.7