

CHAPTER IV

RESULTS AND DISCUSSION

1. Isolation and primary screening of actinomycetes

1.1 Isolation of the isolates

Thirty soil and five marine sand samples collected around Samed Island were from Phrao bay (8 samples), Sai Kaew beach (18 samples), Phai bay (1 sample), Phutsa bay (3 samples), Cho bay (1 sample) and Wong Deuan bay (4 samples) in Rayong Province, Thailand. A total of 100 isolates of actinomycetes were isolated on YMA medium and kept as stock culture in a cold room at 4°C. Sources of samples, pH, date of isolation and isolate number were shown in Table 9.

1.2 Primary screening for the antimicrobial activity of the isolates

Antimicrobial activity of all isolates is shown in Table 10. Fifty-five isolates showed an inhibitory activity against *Staphylococcus aureus* ATCC 25923, 56 isolates against *Bacillus subtilis* ATCC 6633, 10 isolates against *Escherichia coli* ATCC 25922, 13 isolates against *Pseudomonas aeruginosa* ATCC 27853 and 18 isolates against *Candida albicans* ATCC 10231. Thirty isolates showing antimicrobial activity was selected for further study. The strain PC 4-3 showing good antimicrobial activity was used to investigation for bioactive metabolites. Characteristics of this strain are shown in Table 17.

Table 9 Sources of soil samples, pH, date of isolation and isolate number

Sample	Source	pH	Date of Isolation	Isolate no.
Soil	Phrao bay	ND	1 July 2000	SC 1-1, SC 1-3 SC 1-4, SC 1-5
Soil	Phrao bay	7.5-8.0	1 July 2000	SC 2-1, SC 2-2 SC 2-4, SC 2-5
Soil	Phrao bay	ND	1 July 2000	SC 4-2, SC 4-3 PC 4-1, PC 4-2 PC 4-3
Marine sand	Phrao bay	7.5-8.0	1 July 2000	PC 5-1
Marine sand	Phrao bay	8.0	1 July 2000	SC 7-1
Soil	Phrao bay	ND	1 July 2000	MS 1-5, MS 1-6
Marine sand	Phrao bay	7.5-8.0	1 July 2000	MS 5-2, MS 5-3 MS 5-6
Marine sand	Phrao bay	8.0	1 July 2000	MS 7-1, MS 7-3
Soil	Sai Kaew beach	7.5-8.0	27 April 2002	SC 11-1, SC 11-2 SC 11-3
Soil	Sai Kaew beach	7.5-8.0	27 April 2002	SC 16-4
Soil	Sai Kaew beach	7.5	27 April 2002	SC 17-1
Soil	Sai Kaew beach	7.5-8.0	27 April 2002	SC 18-1, SC 18-3
Soil	Sai Kaew beach	7.5	27 April 2002	SC 19-1, SC 19-2 SC 19-3, SC 19-4 SC 19-5, SC 19-6
Soil	Sai Kaew beach	7.5	27 April 2002	SC 20-1, SC 20-2
Soil	Sai Kaew beach	7.5-8.0	27 April 2002	SC 21-1, SC 21-2 SC 21-3, SC 21-4
Soil	Sai Kaew beach	7.5	27 April 2002	SC 22-3
Soil	Sai Kaew beach	7.5	27 April 2002	SC 23-1, SC 23-2 SC 23-4, SC 23-5 SC 23-6
Soil	Sai Kaew beach	8.0	27 April 2002	SC 28-3
Marine sand	Sai Kaew beach	8.0	27 April 2002	SC 30-1, SC 30-3
Soil	Phai bay	8.0	27 April 2002	SC 32-1, SC 32-2

Table 9 (continued)

Sample	Source	pH	Date of Isolation	Isolate no.
Soil	Phutsa bay	8.0	27 April 2002	SC 36-1, SC 36-2 SC 36-3
Soil	Phutsa bay	7.5	27 April 2002	SC 37-1, SC 37-2 SC 37-3, SC 37-4
Soil	Phutsa bay	8.0	27 April 2002	SC 38-1, SC 38-2 SC 38-3, SC 38-4 SC 38-5
Soil	Cho bay	8.0	27 April 2002	SC 44-1, SC 44-2
Soil	Wong Deuan bay	8.0	27 April 2002	SC 45-1, SC 45-3 SC 45-4
Soil	Wong Deuan bay	7.5	27 April 2002	SC 46-1, SC 46-2
Soil	Wong Deuan bay	7.5-8.0	27 April 2002	SC 49-1
Soil	Wong Deuan bay	8.0	27 April 2002	SC 50-1, SC 50-2
Soil	Sai Kaew beach	7.5	27 April 2002	SC 54-1, SC 54-2 SC 54-3
Soil	Sai Kaew beach	7.5	27 April 2002	SC 55-1, SC 55-2 SC 55-4, SC 55-5
Soil	Sai Kaew beach	7.5-8.0	27 April 2002	SC 58-1, SC 58-2 SC 58-3, SC 58-4 SC 58-5
Soil	Sai Kaew beach	8.0	27 April 2002	SC 59-1, SC 59-2 SC 59-3, SC 59-4 SC 59-5, SC 59-6 SC 59-8
Soil	Sai Kaew beach	8.0	27 April 2002	SC 60-1, SC 60-3
Soil	Sai Kaew beach	8.0	27 April 2002	SC 61-1, SC 61-2 SC 61-3, SC 61-5
Soil	Sai Kaew beach	8.0	27 April 2002	SC 62-2

ND, not determined

Table 10 Antimicrobial activity of actinomycetes isolates on YMA

Isolate no.	Inhibition zone				
	<i>S. aureus</i> ATCC 25923	<i>B. subtilis</i> ATCC 6633	<i>E. coli</i> ATCC 25922	<i>Ps. aeruginosa</i> ATCC 27853	<i>C. albicans</i> ATCC 10231
SC 1-1	++	+++	-	-	-
SC 1-3	+	++	-	-	-
SC 1-4	-	-	-	+	-
SC 1-5	++	+++	-	-	-
SC 2-1	-	-	-	+	-
SC 2-2	-	-	-	+-	+
SC 2-4	+	+	-	-	-
SC 2-5	+	-	-	+	-
SC 4-2	+-	+-	-	+	-
SC 4-3	+-	+-	-	+	-
PC 4-1	+-	+-	-	-	-
PC 4-2	+-	-	-	-	-
PC 4-3	++++	++++	+	+-	+++
PC 5-1	++	+	-	-	++
SC 7-1	-	+	-	+	-
MS 1-5	+	+	-	-	-
MS 1-6	+	+	-	+-	-
MS 5-2	+-	+-	+-	-	-
MS 5-3	+-	+-	+-	-	-
MS 5-6	+-	+	+	-	-
MS 7-1	++	++	+-	+-	+-
MS 7-3	++	++	+	+-	+
SC 11-1	++++	++++	-	-	++
SC 11-2	+-	-	-	-	-
SC 11-3	++++	++++	-	-	-
SC 16-4	+	+-	-	-	++++
SC 17-1	-	-	-	-	-
SC 18-1	-	-	-	-	-
SC 18-3	-	-	-	-	-

Table 10 (continued)

Isolate no.	Inhibition zone				
	<i>S. aureus</i> ATCC 25923	<i>B. subtilis</i> ATCC 6633	<i>E. coli</i> ATCC 25922	<i>Ps. aeruginosa</i> ATCC 27853	<i>C. albicans</i> ATCC 10231
SC 19-1	-	-	-	-	+/-
SC 19-2	-	-	-	-	-
SC 19-3	-	-	-	-	-
SC 19-4	-	+/-	-	-	-
SC 19-5	-	-	-	-	-
SC 19-6	-	-	-	-	-
SC 20-1	++	+	-	+++	-
SC 20-2	++	-	-	-	+++
SC 21-1	-	-	-	-	-
SC 21-2	++++	++++	-	-	-
SC 21-3	+	++	-	-	-
SC 21-4	++++	++++	-	-	-
SC 22-3	+/-	+/-	-	-	-
SC 23-1	-	+/-	-	-	-
SC 23-2	+++	+/-	-	-	-
SC 23-4	-	-	-	-	-
SC 23-5	++	-	-	-	-
SC 23-6	+	-	-	-	-
SC 28-3	+/-	-	-	-	-
SC 30-1	-	-	-	-	-
SC 30-3	+/-	+/-	-	-	+/-
SC 32-1	-	-	-	-	-
SC 32-2	-	-	-	-	-
SC 36-1	++	+++	-	-	-
SC 36-2	+/-	+/-	-	-	-
SC 36-3	-	++	-	-	-
SC 37-1	-	-	-	-	-
SC 37-2	-	-	-	-	-
SC 37-3	-	-	-	-	-

Table 10 (continued)

Isolate no.	Inhibition zone				
	<i>S. aureus</i> ATCC 25923	<i>B. subtilis</i> ATCC 6633	<i>E. coli</i> ATCC 25922	<i>Ps. aeruginosa</i> ATCC 27853	<i>C. albicans</i> ATCC 10231
SC 37-4	-	-	-	-	-
SC 38-1	+++	++++	-	-	+/-
SC 38-2	-	+	-	-	-
SC 38-3	++	+++	-	-	+/-
SC 38-4	-	-	-	-	-
SC 38-5	++++	++++	-	-	-
SC 44-1	-	-	-	-	-
SC 44-2	-	-	-	-	-
SC 45-1	-	-	-	-	-
SC 45-3	-	+/-	-	-	-
SC 45-4	++++	++	-	-	+/-
SC 46-1	-	-	-	-	-
SC 46-2	-	++++	-	-	++
SC 49-1	++++	+++	-	-	-
SC 50-1	+/-	+	-	+/-	-
SC 50-2	-	++	+	-	-
SC 54-1	-	-	-	-	-
SC 54-2	-	-	+/-	-	-
SC 54-3	++++	++++	+/-	-	-
SC 55-1	-	-	-	-	-
SC 55-2	+/-	+	-	-	-
SC 55-4	-	+/-	-	-	+
SC 55-5	-	-	-	-	-
SC 58-1	+/-	++	-	-	-
SC 58-2	++++	++++	-	-	-
SC 58-3	+/-	+/-	-	-	-
SC 58-4	-	-	-	-	-
SC 58-5	-	-	-	-	-
SC 59-1	++++	++	-	-	-

Table 10 (continued)

Isolate no.	Inhibition zone				
	<i>S. aureus</i> ATCC 25923	<i>B. subtilis</i> ATCC 6633	<i>E. coli</i> ATCC 25922	<i>Ps. aeruginosa</i> ATCC 27853	<i>C. albicans</i> ATCC 10231
SC 59-2	+-	+++	-	-	+-
SC 59-3	+-	++++	-	-	-
SC 59-4	-	-	-	-	-
SC 59-5	-	-	-	-	-
SC 59-6	+-	+-	-	-	-
SC 59-8	-	+	-	-	-
SC 60-1	+	++++	-	-	++++
SC 60-3	-	-	-	-	-
SC 61-1	++++	++++	+-	-	-
SC 61-2	+-	-	-	-	++++
SC 61-3	+	-	-	-	-
SC 61-5	++++	++	-	-	-
SC 62-2	-	-	-	-	-

++++, 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 isolates

2.1 Morphological and cultural characteristics

The morphological and cultural characteristics of 100 isolates on YMA medium at 30°C for 14 days are shown in Tables 11 and 12. Eighty isolates showed powdery colonies. Fifty-nine isolates contained spiral spore chain and 21 isolates were rectiflexibles spore chain. They were identified as *Streptomyces* (Holt, 1989). Twenty isolates showed mucoid colonies with single spore on short sporophore of the vegetative mycelial. They were identified as *Micromonospora* (Holt, 1989). Forty-one isolates can produce soluble pigment including yellow (2 isolates), light brown (17 isolates), brown (11 isolates), dark brown (7 isolates), red (3 isolates), and black (1 isolates). The cultural characteristics of 100 isolates on various media at room temperature for 14 days are shown in Table 13. All isolates grew better on Yeast extract-malt extract agar (YMA) and Oatmeal than Tyrosine, Glycerol-asparagine agar and Inorganic salt-starch agar.

Out of 100 isolates, 24 isolates were white mycelial pigment, 8 yellowish pink, 6 orange, 7 yellowish brown, 9 brown, 7 dark brown, 1 blue, 25 gray, 12 purple and 1 black. The vegetative mycelium of the strain PC 4-3 grown on YMA medium was initially white and turned into dark during the course of incubation (21 days) and temperature (less than 30 °C) as shown in Figure 1.

The scanning electron micrograph of the strain PC 4-3 cultivated on YMA medium at 30°C for 14 days revealed the unbranched aerial hyphae, spiral chains of cylindrical spores with rugose ornamentation as shown in Figure 2.

Table 11 Spore morphology and cultural characteristic of the isolates on YMA incubated for 14 days

Isolate no.	Spore chain	Spore color	Soluble pigment
SC 1-1	Spiral	Pale beige	-
SC 1-3	Spiral	Dark medium gray	Brown
SC 1-4	Spiral	Brownish gold	Light brown
SC 1-5	Spiral	Yellowish white	-
SC 2-1	Spiral	Grayish brown	Dark brown
SC 2-2	Spiral	Brown	-
SC 2-4	Spiral	Light grayish purple	Dark brown
SC 2-5	Spiral	Dark medium gray	Light brown
SC 4-2	Spiral	Light gray	-
SC 4-3	Spiral	Dark yellowish brown	Brown
PC 4-1	Spiral	Pale greenish yellow	Yellow
PC 4-2	Spiral	Pale greenish yellow	Yellow
PC 4-3	Spiral	Black	Light brown
PC 5-1	Spiral	Dark bluish gray	-
SC 7-1	Spiral	Pale pinkish beige	-
MS 1-5	Single spore	Dark brown	-
MS 1-6	Single spore	Yellowish brown	-
MS 5-2	Single spore	Dark brown	-
MS 5-3	Single spore	Dark grayish brown	Light brown
MS 5-6	Single spore	Yellowish brown	-
MS 7-1	Single spore	Vivid orange	-
MS 7-3	Single spore	Dark brown	-
SC 11-1	Spiral	Purplish pink	-
SC 11-2	Spiral	Dark yellowish orange	-
SC 11-3	Spiral	Pinkish white	-
SC 16-4	Rectiflexibles	Pale reddish yellow	-
SC 17-1	Spiral	Dark reddish brown	Dark brown
SC 18-1	Spiral	Pinkish white	-
SC 18-3	Spiral	Pale pinkish beige	-
SC 19-1	Rectiflexibles	Greenish gray	Light brown

Table 11 (continued)

Isolate no.	Spore chain	Spore color	Soluble pigment
SC 19-2	Rectiflexibiles	Light medium gray	Light brown
SC 19-3	Rectiflexibiles	Dull purplish blue	-
SC 19-4	Single spore	Dark grayish brown	Brown
SC 19-5	Spiral	Pale yellowish pink	-
SC 19-6	Single spore	Dark yellowish brown	-
SC 20-1	Spiral	Purplish pink	Light brown
SC 20-2	Spiral	Rose	-
SC 21-1	Spiral	Yellowish pink	Red
SC 21-2	Spiral	Light grayish purple	Dark brown
SC 21-3	Spiral	Rose	Red
SC 21-4	Rectiflexibiles	Pale beige	Brown
SC 22-3	Rectiflexibiles	Greenish gray	Brown
SC 23-1	Single spore	Dark yellowish brown	-
SC 23-2	Spiral	Yellowish white	-
SC 23-4	Single spore	Vivid orange	Light brown
SC 23-5	Spiral	Light greenish yellow	-
SC 23-6	Single spore	Yellowish brown	-
SC 28-3	Spiral	Pale lavender	Brown
SC 30-1	Spiral	Pale yellowish pink	-
SC 30-3	Spiral	Vivid reddish orange	Light brown
SC 32-1	Rectiflexibiles	Light gray	Dark brown
SC 32-2	Rectiflexibiles	Medium gray	Light brown
SC 36-1	Spiral	Yellowish pink	-
SC 36-2	Spiral	Greenish gray	-
SC 36-3	Rectiflexibiles	Light medium gray	Light brown
SC 37-1	Rectiflexibiles	Light medium gray	-
SC 37-2	Rectiflexibiles	Medium gray	-
SC 37-3	Spiral	Pinkish white	-
SC 37-4	Single spore	Yellowish pink	-
SC 38-1	Rectiflexibiles	Yellowish white	-
SC 38-2	Spiral	Grayish leaf	-

Table 11 (continued)

Isolate no.	Spore chain	Spore color	Soluble pigment
SC 38-3	Rectiflexibiles	Pale lavender	-
SC 38-4	Spiral	Yellowish white	Brown
SC 38-5	Rectiflexibiles	Pale reddish yellow	-
SC 44-1	Rectiflexibiles	Bluish gray	-
SC 44-2	Rectiflexibiles	Light medium gray	-
SC 45-1	Single spore	Vivid reddish orange	-
SC 45-3	Spiral	Grayish sky	-
SC 45-4	Spiral	Pale beige	Brown
SC 46-1	Rectiflexibiles	Medium gray	-
SC 46-2	Spiral	Pinkish white	-
SC 49-1	Single spore	Yellowish brown	-
SC 50-1	Spiral	Brownish gray	-
SC 50-2	Spiral	Grayish pink	-
SC 54-1	Spiral	Pale yellowish pink	-
SC 54-2	Spiral	Reddish gray	Brown
SC 54-3	Spiral	Grayish sky	Light brown
SC 55-1	Spiral	Purplish pink	-
SC 55-2	Single spore	Dark red	Red
SC 55-4	Rectiflexibiles	Dark medium gray	Light brown
SC 55-5	Single spore	Yellowish pink	-
SC 58-1	Spiral	Grayish brown	Brown
SC 58-2	Spiral	Pale beige	Light brown
SC 58-3	Single spore	Gold	-
SC 58-4	Single spore	Vivid orange	-
SC 58-5	Spiral	Pinkish white	-
SC 59-1	Spiral	Yellowish pink	-
SC 59-2	Rectiflexibiles	Pale pinkish beige	Light brown
SC 59-3	Spiral	Grayish sky	Brown
SC 59-4	Spiral	Purplish pink	-
SC 59-5	Spiral	Yellowish white	-
SC 59-6	Spiral	Grayish red	Black

Table 11 (continued)

Isolate no.	Spore chain	Spore color	Soluble pigment
SC 59-8	Spiral	Reddish gray	-
SC 60-1	Rectiflexibiles	Pinkish white	-
SC 60-3	Spiral	Pale beige	Light brown
SC 61-1	Single spore	Dark reddish brown	Dark brown
SC 61-2	Spiral	Purplish pink	-
SC 61-3	Spiral	Grayish yellow	Dark brown
SC 61-5	Rectiflexibiles	Pale lilac	Light brown
SC 62-2	Spiral	Purplish pink	-

Table 12 Cultural characteristics of the isolates on YMA incubated for 14 days

Isolate no.	Colonial appearance	Colony color	
		Upper colony	Lower colony
SC 1-1	Powdery	Dark yellowish brown	Dark yellowish brown
SC 1-3	Powdery	Dark grayish brown	Beige
SC 1-4	Powdery	Yellowish brown	Yellowish brown
SC 1-5	Powdery	Light yellow	Light yellow
SC 2-1	Powdery	Grayish brown	Light grayish brown
SC 2-2	Powdery	Orange yellow	Orange yellow
SC 2-4	Powdery	Dark yellowish brown	Dark brown
SC 2-5	Powdery	Grayish brown	Beige
SC 4-2	Powdery	Bluish white	Bluish white
SC 4-3	Powdery	Dark yellowish brown	Pale beige
PC 4-1	Powdery	Olive yellow	Olive yellow
PC 4-2	Powdery	Olive yellow	Olive yellow
PC 4-3	Powdery	Black	Pale beige
PC 5-1	Powdery	Strong yellowish orange	Strong yellowish orange
SC 7-1	Powdery	Pale beige	Pale beige
MS 1-5	Mucoid	Dark brown	Dark brown
MS 1-6	Mucoid	Yellowish brown	Yellowish brown
MS 5-2	Mucoid	Dark brown	Dark brown
MS 5-3	Mucoid	Grayish brown	Grayish brown
MS 5-6	Mucoid	Yellowish brown	Yellowish brown
MS 7-1	Mucoid	Vivid orange	Vivid orange
MS 7-3	Mucoid	Dark brown	Dark brown
SC 11-1	Powdery	Purplish pink	Dark rose
SC 11-2	Powdery	Dark yellowish orange	Dark yellowish orange
SC 11-3	Powdery	Vivid orange	Vivid orange
SC 16-4	Powdery	Brown	Brown
SC 17-1	Powdery	Brown	Dark brown
SC 18-1	Powdery	Pink	Vivid orange
SC 18-3	Powdery	Vivid yellow orange	Vivid yellow orange
SC 19-1	Powdery	Dark bluish gray	Greenish gray

Table 12 (continued)

Isolate no.	Colonial appearance	Colony color	
		Upper colony	Lower colony
SC 19-2	Powdery	Light yellow	Gold
SC 19-3	Powdery	Pale pinkish beige	Vivid yellow orange
SC 19-4	Mucoid	Dark grayish brown	Dark grayish brown
SC 19-5	Powdery	Yellowish pink	Dark yellowish orange
SC 19-6	Mucoid	Dark yellowish brown	Yellowish brown
SC 20-1	Powdery	Brownish gold	Brownish gold
SC 20-2	Powdery	Deep purplish red	Deep purplish red
SC 21-1	Powdery	Rose	Vivid red
SC 21-2	Powdery	Dark purple	Dark purple
SC 21-3	Powdery	Deep purplish red	Vivid red purple
SC 21-4	Powdery	Brownish olive	Brownish olive
SC 22-3	Powdery	Dark gray	Dark yellowish brown
SC 23-1	Mucoid	Dark yellowish brown	Dark brown
SC 23-2	Powdery	Dark red	Dark red
SC 23-4	Mucoid	Vivid orange	Gold
SC 23-5	Powdery	Vivid greenish yellow	Vivid greenish yellow
SC 23-6	Mucoid	Yellowish brown	Yellowish brown
SC 28-3	Powdery	Light medium gray	Dark yellowish brown
SC 30-1	Powdery	Pale yellowish pink	Vivid orange
SC 30-3	Powdery	Vivid reddish orange	Deep orange
SC 32-1	Powdery	Grayish white	Dark yellowish brown
SC 32-2	Powdery	Light gray	Yellowish gold
SC 36-1	Powdery	Strong reddish orange	Strong reddish orange
SC 36-2	Powdery	Greenish gray	Bright yellow
SC 36-3	Powdery	Light medium gray	Gold
SC 37-1	Powdery	Yellowish white	Yellowish brown
SC 37-2	Powdery	Pale lavender	Dark red
SC 37-3	Powdery	Pinkish white	Pale pinkish beige
SC 37-4	Mucoid	Yellowish pink	Yellowish pink
SC 38-1	Powdery	Light reddish yellow	Light reddish yellow

Table 12 (continued)

Isolate no.	Colonial appearance	Colony color	
		Upper colony	Lower colony
SC 38-2	Powdery	Grayish leaf	Dull reddish yellow
SC 38-3	Powdery	Brownish gold	Brownish gold
SC 38-4	Powdery	Dull reddish yellow	Dull reddish yellow
SC 38-5	Powdery	Strong yellow	Strong yellow
SC 44-1	Powdery	Grayish white	Reddish brown
SC 44-2	Powdery	Reddish gray	Reddish gray
SC 45-1	Mucoid	Vivid reddish orange	Vivid orange
SC 45-3	Powdery	Bluish gray	Dark red
SC 45-4	Powdery	Brownish gold	Dark yellowish brown
SC 46-1	Powdery	Dark gray	Dark red
SC 46-2	Powdery	Orange yellow	Dark yellowish orange
SC 49-1	Mucoid	Yellowish brown	Yellowish brown
SC 50-1	Powdery	Bluish gray	Bluish gray
SC 50-2	Powdery	Brownish gold	Brownish gold
SC 54-1	Powdery	Vivid yellow orange	Vivid yellow orange
SC 54-2	Powdery	Pinkish white	Brownish gold
SC 54-3	Powdery	Dark gray	Strong yellow
SC 55-1	Powdery	Yellowish pink	Vivid orange
SC 55-2	Mucoid	Dark red	Dark red
SC 55-4	Powdery	Pale beige	Dark yellowish orange
SC 55-5	Mucoid	Yellowish pink	Yellowish pink
SC 58-1	Powdery	Yellowish brown	Brown
SC 58-2	Powdery	Dark yellowish brown	Dark yellowish brown
SC 58-3	Mucoid	Gold	Gold
SC 58-4	Mucoid	Vivid orange	Vivid orange
SC 58-5	Powdery	Dull yellow	Dull yellow
SC 59-1	Powdery	Vivid yellow orange	Dark yellowish orange
SC 59-2	Powdery	Light yellowish brown	Brownish gold
SC 59-3	Powdery	Dark red	Dark red
SC 59-4	Powdery	Dark reddish orange	Vivid orange

Table 12 (continued)

Isolate no.	Colonial appearance	Colony color	
		Upper colony	Lower colony
SC 59-5	Powdery	Reddish orange	Vivid yellow orange
SC 59-6	Powdery	Dark reddish brown	Dark brown
SC 59-8	Powdery	Brown	Brown
SC 60-1	Powdery	Yellowish brown	Yellowish brown
SC 60-3	Powdery	Medium gray	Dark brown
SC 61-1	Mucoid	Dark reddish brown	Reddish brown
SC 61-2	Powdery	Rose	Deep yellowish red
SC 61-3	Powdery	Dark brown	Dark brown
SC 61-5	Powdery	Brown	Brown
SC 62-2	Powdery	Yellowish pink	Vivid orange

Table 13 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
SC 1-1	YM	+++	Pale beige	Dark yellowish brown	Dark yellowish brown
	Tyrosine	+++	Pale yellow	Yellowish brown	Pale yellow
	Oatmeal	+++	Light yellow	Bright yellow	Bright yellow
	Asparagine	+++	Grayish yellow	Light reddish yellow	Light reddish yellow
	Inorg. salt	+++	Pale reddish yellow	Pale reddish yellow	Pale reddish yellow
SC 1-3	YM	+++	Dark medium gray	Dark grayish brown	Beige
	Tyrosine	+++	Light yellowish brown	Brownish olive	Dull reddish yellow
	Oatmeal	+++	Dull yellow	Dull yellow	Olive yellow
	Asparagine	+++	Pale beige	Brownish gold	Brownish gold
	Inorg. salt	+++	Pale beige	Brownish gold	Brownish gold
SC 1-4	YM	+++	Brownish gold	Yellowish brown	Yellowish brown
	Tyrosine	+++	Brownish olive	Brownish olive	Brownish olive
	Oatmeal	+++	Beige	Beige	Beige
	Asparagine	+++	Grayish yellow	Grayish yellow	Grayish yellow
	Inorg. salt	+++	Brownish gold	Yellowish brown	Yellowish brown
SC 1-5	YM	+++	Yellowish white	Light yellow	Light yellow
	Tyrosine	++	Pale greenish yellow	Light yellowish brown	Light yellowish brown
	Oatmeal	+++	Beige	Grayish yellow	Grayish yellow
	Asparagine	+++	Yellowish white	Light yellow	Light yellow
	Inorg. salt	+++	Yellowish white	Pale reddish yellow	Pale yellow
SC 2-1	YM	+++	Grayish brown	Grayish brown	Light grayish brown
	Tyrosine	+++	Grayish red	Grayish brown	Dark yellowish brown
	Oatmeal	+++	Brownish olive	Brownish olive	Brownish olive
	Asparagine	+++	Pale lavender	Pale reddish yellow	Light yellowish brown
	Inorg. salt	+++	Brown	Brown	Brown
SC 2-2	YM	+++	Brown	Orange yellow	Orange yellow
	Tyrosine	+	Gold	Strong yellow	Brown
	Oatmeal	+++	Brownish gold	Brownish gold	Brownish gold
	Asparagine	+	Pale beige	Pale beige	Yellowish white
	Inorg. salt	++	Dull reddish yellow	Dull reddish yellow	Brownish gold

Table 13 (continued)

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
SC 2-4	YM	+++	Light grayish purple	Dark yellowish brown	Dark brown
	Tyrosine	+++	Grayish pink	Grayish brown	Dark brown
	Oatmeal	+++	Yellowish brown	Yellowish brown	Yellowish brown
	Asparagine	+++	Light bluish gray	Light yellowish brown	Dark yellowish brown
	Inorg. salt	+++	Light reddish brown	Dull red purple	Yellowish brown
SC 2-5	YM	+++	Dark medium gray	Grayish brown	Beige
	Tyrosine	+++	Yellowish white	Brownish gold	Dark yellowish brown
	Oatmeal	+++	Storm yellow	Storm yellow	Dark yellowish brown
	Asparagine	+++	Pale yellow	Brownish gold	Brownish gold
	Inorg. salt	+++	Pale beige	Light reddish yellow	Light reddish yellow
SC 4-2	YM	+++	Light gray	Bluish white	Bluish white
	Tyrosine	+++	Dull reddish yellow	Grayish yellow	Pale reddish yellow
	Oatmeal	+++	Grayish yellow	Grayish yellow	Pale yellow
	Asparagine	+++	Grayish white	Pale yellow	Pale yellow
	Inorg. salt	+++	Yellowish white	Brownish gold	Yellowish brown
SC 4-3	YM	+++	Dark yellowish brown	Dark yellowish brown	Pale beige
	Tyrosine	+++	Grayish pink	Grayish brown	Dark brown
	Oatmeal	+++	Dark yellowish brown	Dark yellowish brown	Pale beige
	Asparagine	+++	Pinkish white	Yellowish brown	Brownish gold
	Inorg. salt	+++	Light reddish brown	Dull red purple	Dark yellowish brown
PC 4-1	YM	+++	Pale greenish yellow	Olive yellow	Olive yellow
	Tyrosine	+	Light greenish yellow	Olive yellow	Olive yellow
	Oatmeal	++	Pale greenish yellow	Olive yellow	Olive yellow
	Asparagine	+	Light greenish yellow	Olive yellow	Olive yellow
	Inorg. salt	+	Pale reddish white	Gold	Dark brown
PC 4-2	YM	+++	Pale greenish yellow	Olive yellow	Olive yellow
	Tyrosine	+	Light greenish yellow	Olive yellow	Olive yellow
	Oatmeal	++	Light greenish yellow	Olive yellow	Olive yellow
	Asparagine	+	Light greenish yellow	Olive yellow	Olive yellow
	Inorg. salt	+	Pale reddish white	Gold	Dark brown

Table 13 (continued)

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
PC 4-3	YM	+++	Black	pale beige	Pale beige
	Tyrosine	+++	Black	Pale yellow	Dark brown
	Oatmeal	+++	Dark medium gray	Pale yellow	Pale yellow
	Asparagine	+++	Dark medium gray	Medium gray	Medium gray
	Inorg. salt	+++	Dark gray	Pale beige	Dark gray
PC 5-1	YM	+++	Dark bluish gray	Dark yellowish orange	Dark yellowish orange
	Tyrosine	++	Yellowish white	Brown	Yellowish brown
	Oatmeal	++	Bright yellow	Bright yellow	Dull yellow
	Asparagine	++	Yellowish white	Pale yellow	Pale reddish yellow
	Inorg. salt	++	Brownish gray	Brownish gray	Dark yellowish brown
SC 7-1	YM	+++	Pale pinkish beige	Pale beige	Pale beige
	Tyrosine	++	Yellowish pink	Yellowish pink	Yellowish pink
	Oatmeal	+++	Pale pinkish white	Yellowish pink	Yellowish pink
	Asparagine	++	Yellowish pink	Yellowish pink	Yellowish pink
	Inorg. salt	++	Yellowish pink	Yellowish pink	Pale beige
MS 1-5	YM	+++	Dark brown	Dark brown	Dark brown
	Tyrosine	++	Vivid yellow orange	Vivid yellow orange	Dark reddish orange
	Oatmeal	+++	Vivid orange	Vivid orange	Vivid orange
	Asparagine	+++	Vivid yellow orange	Vivid yellow orange	Vivid yellow orange
	Inorg. salt	++	Vivid orange	Vivid orange	Vivid orange
MS 1-6	YM	+++	Yellowish brown	Yellowish brown	Yellowish brown
	Tyrosine	+++	Yellowish brown	Dark yellowish brown	Dark yellowish brown
	Oatmeal	+++	Yellowish brown	Yellowish brown	Yellowish brown
	Asparagine	+++	Dark yellowish brown	Dark yellowish brown	Dark yellowish brown
	Inorg. salt	+++	Yellowish brown	Yellowish brown	Yellowish brown
MS 5-2	YM	+++	Dark brown	Dark brown	Dark brown
	Tyrosine	+++	Dark yellowish brown	Brown	Dark brown
	Oatmeal	+++	Dark yellowish brown	Dark yellowish brown	Dark yellowish brown
	Asparagine	+++	Dark brown	Dark brown	Dark brown
	Inorg. salt	+++	Dark brown	Dark brown	Dark brown

Table 13 (continued)

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
MS 5-3	YM	+++	Dark grayish brown	Grayish brown	Grayish brown
	Tyrosine	+++	Yellowish brown	Yellowish brown	Yellowish pink
	Oatmeal	+++	Yellowish brown	Yellowish brown	Yellowish brown
	Asparagine	+++	Vivid orange	Vivid orange	Vivid orange
	Inorg. salt	+++	Yellowish brown	Yellowish brown	Dark yellowish brown
MS 5-6	YM	+++	Yellowish brown	Yellowish brown	Yellowish brown
	Tyrosine	+++	Yellowish brown	Yellowish brown	Yellowish brown
	Oatmeal	+++	Yellowish brown	Yellowish brown	Yellowish brown
	Asparagine	+++	Yellowish brown	Yellowish brown	Yellowish brown
	Inorg. salt	+++	Yellowish brown	Yellowish brown	Yellowish brown
MS 7-1	YM	+++	Vivid orange	Vivid orange	Vivid orange
	Tyrosine	++	Vivid orange	Vivid orange	Vivid orange
	Oatmeal	++	Vivid yellow orange	Vivid yellow orange	Vivid yellow orange
	Asparagine	++	Vivid orange	Vivid orange	Vivid orange
	Inorg. salt	+	Vivid orange	Vivid orange	Vivid orange
MS 7-3	YM	+++	Dark brown	Dark brown	Dark brown
	Tyrosine	+++	Dark brown	Dark brown	Dark brown
	Oatmeal	+++	Dark yellowish brown	Dark yellowish brown	Dark yellowish brown
	Asparagine	+++	Dark brown	Dark brown	Dark brown
	Inorg. salt	++	Dark yellowish brown	Dark yellowish brown	Dark yellowish brown
SC 11-1	YM	+++	Purplish pink	Purplish pink	Dark rose
	Tyrosine	+++	Rose	Rose	Dark rose
	Oatmeal	+++	Purplish rose	Rose	Rose
	Asparagine	+++	Purplish pink	Purplish rose	Dark rose
	Inorg. salt	+++	Purplish rose	Rose	Rose
SC 11-2	YM	+++	Dark yellowish orange	Dark yellowish orange	Dark yellowish orange
	Tyrosine	+++	Vivid yellow orange	Vivid yellow orange	Vivid yellow orange
	Oatmeal	+++	Vivid yellow orange	Vivid yellow orange	Vivid yellow orange
	Asparagine	+++	Orange yellow	Orange yellow	Orange yellow
	Inorg. salt	+++	Vivid yellow	Vivid yellow	Vivid yellow

Table 13 (continued)

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
SC 11-3	YM	+++	Pinkish white	Vivid orange	Vivid orange
	Tyrosine	+++	Pinkish white	Vivid orange	Vivid orange
	Oatmeal	+++	Pinkish white	Vivid orange	Vivid orange
	Asparagine	+++	Pinkish white	Vivid orange	Vivid orange
	Inorg. salt	+++	Pinkish white	Vivid orange	Vivid orange
SC 16-4	YM	+++	Pale reddish yellow	Brown	Brown
	Tyrosine	+++	Dull yellow	Brownish gold	Brownish gold
	Oatmeal	++	Pale reddish yellow	Brown	Brown
	Asparagine	+++	Pale reddish yellow	Brown	Brown
	Inorg. salt	+++	Dull yellow	Brownish gold	Brownish gold
SC 17-1	YM	+++	Dark reddish brown	Brown	Dark brown
	Tyrosine	+++	Dull orange	Brown	Brown
	Oatmeal	+++	Bluish white	Greenish gray	Greenish gray
	Asparagine	+++	Dull orange	Brown	Brown
	Inorg. salt	++	Yellowish brown	Yellowish brown	Yellowish brown
SC 18-1	YM	+++	Pinkish white	Pink	Vivid orange
	Tyrosine	+++	Brownish white	Dull reddish yellow	Dull yellow
	Oatmeal	+++	Pinkish white	Pink	Orange yellow
	Asparagine	+++	Pale yellow	Light yellow	Light yellow
	Inorg. salt	++	Pinkish white	Pink	Vivid orange
SC 18-3	YM	+++	Pale pinkish beige	Vivid yellow orange	Vivid yellow orange
	Tyrosine	+++	Pale beige	Vivid yellow orange	Vivid yellow orange
	Oatmeal	+++	Yellowish white	Orange yellow	Orange yellow
	Asparagine	+++	Pale beige	Orange yellow	Orange yellow
	Inorg. salt	+++	Yellowish white	Pale reddish yellow	Pale reddish yellow
SC 19-1	YM	+++	Greenish gray	Dark bluish gray	Dark bluish gray
	Tyrosine	+++	Grayish yellow	Brownish olive	Yellowish brown
	Oatmeal	+++	Light medium gray	Grayish yellow	Brownish gold
	Asparagine	+++	Light gray	Brown	Brown
	Inorg. salt	+++	Grayish red	Grayish red	Light yellowish brown

Table 13 (continued)

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
SC 19-2	YM	+++	Light medium gray	Light yellow	Gold
	Tyrosine	+++	Light reddish yellow	Dull reddish yellow	Dark brown
	Oatmeal	+++	Light yellowish brown	Light yellowish brown	Yellowish brown
	Asparagine	+++	Bluish gray	Brownish gold	Brownish gold
	Inorg. salt	+++	Bluish gray	Brownish gold	Brownish gold
SC 19-3	YM	+++	Dull purplish blue	Pale pinkish beige	Vivid yellow orange
	Tyrosine	+++	Pale lilac	Yellowish brown	Dark yellowish brown
	Oatmeal	++	Pinkish white	Beige	Beige
	Asparagine	+++	Grayish sky	Grayish yellow	Light yellowish brown
	Inorg. salt	++	Light grayish green	Yellowish brown	Yellowish brown
SC 19-4	YM	+++	Grayish brown	Grayish brown	Dark grayish brown
	Tyrosine	+++	Light greenish gray	Grayish leaf	Light yellowish brown
	Oatmeal	+++	Greenish gray	Greenish white	Light grayish brown
	Asparagine	+++	Dark reddish brown	Dark reddish brown	Dark reddish brown
	Inorg. salt	++	Greenish gray	Dark grayish brown	Dark grayish brown
SC 19-5	YM	+++	Pale yellowish pink	Yellowish pink	Dark yellowish orange
	Tyrosine	+++	Pale yellowish pink	Yellowish pink	Yellowish pink
	Oatmeal	+++	Yellowish pink	Yellowish pink	Yellowish pink
	Asparagine	+++	Yellowish pink	Yellowish pink	Yellowish pink
	Inorg. salt	+++	Pale yellowish pink	Yellowish pink	Yellowish pink
SC 19-6	YM	+++	Dark yellowish brown	Dark yellowish brown	Yellowish brown
	Tyrosine	+++	Pale yellow	Yellowish brown	Yellowish brown
	Oatmeal	+++	Dull reddish yellow	Yellowish brown	Yellowish brown
	Asparagine	++	Pale yellow	Light reddish yellow	Dull reddish yellow
	Inorg. salt	+	Dark yellowish brown	Yellowish brown	Yellowish brown
SC 20-1	YM	+++	Purplish pink	Brownish gold	Brownish gold
	Tyrosine	+++	Purplish pink	Brownish gold	Brownish gold
	Oatmeal	+++	Purplish rose	Gold	Gold
	Asparagine	++	Purplish rose	Gold	Gold
	Inorg. salt	+++	Purplish rose	Gold	Brownish gold

Table 13 (continued)

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
SC 20-2	YM	+++	Rose	Deep purplish red	Deep purplish red
	Tyrosine	+++	Deep purplish red	Deep purplish red	Dull red purple
	Oatmeal	+++	Purplish rose	Purplish rose	Deep purplish red
	Asparagine	+++	Purplish pink	Purplish rose	Purplish rose
	Inorg. salt	+++	Purplish rose	Purplish rose	Vivid purple
SC 21-1	YM	+++	Yellowish pink	Rose	Vivid red
	Tyrosine	+++	Pinkish white	Purplish pink	Purplish rose
	Oatmeal	+++	Pinkish white	Purplish pink	Purplish pink
	Asparagine	+++	Pinkish white	Purplish pink	Purplish pink
	Inorg. salt	+++	Purplish pink	Purplish pink	Rose
SC 21-2	YM	+++	Light grayish purple	Dark purple	Dark purple
	Tyrosine	+++	Light grayish purple	Dark purple	Dark purple
	Oatmeal	+++	Light grayish purple	Dark purple	Dark purple
	Asparagine	+++	Light grayish purple	Dark purple	Dark purple
	Inorg. salt	+++	Light grayish purple	Dark purple	Dark purple
SC 21-3	YM	+++	Rose	Deep purplish red	Vivid red purple
	Tyrosine	+++	Rose	Dull red purple	Dull red purple
	Oatmeal	+++	Rose	Dull red purple	Dull red purple
	Asparagine	+++	Rose	Deep purplish red	Vivid red purple
	Inorg. salt	+++	Rose	Dull red purple	Dull red purple
SC 21-4	YM	+++	Pale beige	Brownish olive	Brownish olive
	Tyrosine	+++	Pale reddish yellow	Yellowish brown	Yellowish brown
	Oatmeal	+++	Pale yellow	Beige	Beige
	Asparagine	+++	Pale yellow	Beige	Beige
	Inorg. salt	+++	Pale yellow	Grayish yellow	Grayish yellow
SC 22-3	YM	+++	Greenish gray	Medium gray	Dark yellowish brown
	Tyrosine	+++	Dull yellow	Brownish olive	Dark brown
	Oatmeal	++	Pale greenish yellow	Yellowish brown	Dark yellowish brown
	Asparagine	+++	Light greenish gray	Dark yellowish brown	Dark brown
	Inorg. salt	+++	Grayish yellow	Dark brown	Dark brown

Table 13 (continued)

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
SC 23-1	YM	+++	Dark yellowish brown	Dark yellowish brown	Dark brown
	Tyrosine	+++	Dark gray	Dark gray	Dark grayish brown
	Oatmeal	+++	Yellowish brown	Yellowish brown	Dark yellowish brown
	Asparagine	+++	Grayish red	Brown	Grayish red
	Inorg. salt	++	Dark yellowish brown	Dark yellowish brown	Dark brown
SC 23-2	YM	+++	Yellowish white	Dark red	Dark red
	Tyrosine	+++	Pale yellow	Brownish olive	Yellowish brown
	Oatmeal	+++	Yellowish white	Grayish yellow	Yellowish brown
	Asparagine	+++	Light yellow	Strong yellow	Strong yellow
	Inorg. salt	+++	Yellowish white	Pale greenish yellow	Pale greenish yellow
SC 23-4	YM	+++	Vivid orange	Vivid orange	Gold
	Tyrosine	+++	Orange yellow	Orange yellow	Orange yellow
	Oatmeal	+++	Vivid yellow	Vivid yellow	Vivid yellow
	Asparagine	++	Vivid yellow orange	Vivid yellow orange	Pale reddish yellow
	Inorg. salt	++	Vivid yellow orange	Vivid yellow orange	Brownish gold
SC 23-5	YM	+++	Light greenish yellow	Vivid greenish yellow	Vivid greenish yellow
	Tyrosine	+++	Light greenish yellow	Greenish yellow	Vivid greenish yellow
	Oatmeal	+++	Light greenish yellow	Greenish yellow	Greenish yellow
	Asparagine	++	Light greenish yellow	Vivid greenish yellow	Vivid greenish yellow
	Inorg. salt	++	Light greenish yellow	Greenish yellow	Greenish yellow
SC 23-6	YM	+++	Yellowish brown	Yellowish brown	Yellowish brown
	Tyrosine	+++	Yellowish brown	Yellowish brown	Yellowish brown
	Oatmeal	+++	Yellowish brown	Yellowish brown	Dark yellowish brown
	Asparagine	+++	Yellowish brown	Yellowish brown	Dark yellowish brown
	Inorg. salt	++	Yellowish brown	Yellowish brown	Dark yellowish brown
SC 28-3	YM	+++	Pale lavender	Light medium gray	Dark yellowish brown
	Tyrosine	+++	Grayish red	Brown	Dark brown
	Oatmeal	++	Grayish white	Dark yellowish brown	Dark yellowish brown
	Asparagine	+++	Grayish yellow	Brown	Brownish gold
	Inorg. salt	+++	Dark medium gray	Yellowish brown	Light reddish yellow

Table 13 (continued)

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
SC 30-1	YM	+++	Pale yellowish pink	Pale yellowish pink	Vivid orange
	Tyrosine	+++	Light reddish yellow	Yellowish orange	Dark yellowish orange
	Oatmeal	++	Pinkish white	Pale yellowish pink	Yellowish brown
	Asparagine	+++	Grayish red	Light yellowish brown	Brown
	Inorg. salt	+++	Grayish white	Grayish yellow	Grayish yellow
SC 30-3	YM	+++	Vivid reddish orange	Vivid reddish orange	Deep orange
	Tyrosine	++	Vivid orange	Vivid orange	Orange yellow
	Oatmeal	+++	Vivid yellow orange	Vivid yellow orange	Beige
	Asparagine	++	Vivid reddish orange	Vivid orange	Vivid orange
	Inorg. salt	++	Vivid orange	Brownish gold	Brownish gold
SC 32-1	YM	+++	Light gray	Yellowish brown	Dark yellowish brown
	Tyrosine	+++	Light yellowish brown	Yellowish brown	Brown
	Oatmeal	+++	Yellowish Gray	Brown	Brown
	Asparagine	+++	Yellowish white	Light reddish yellow	Dull reddish yellow
	Inorg. salt	+	Yellowish white	Brown	Brown
SC 32-2	YM	+++	Medium gray	Light gray	Yellowish gold
	Tyrosine	+++	Dark brown	Dark brown	Dark brown
	Oatmeal	+++	Pale beige	Brown	Brownish gold
	Asparagine	+++	Dark medium gray	Dark medium gray	Leaf
	Inorg. salt	+++	Pale reddish yellow	Medium gray	Dull reddish yellow
SC 36-1	YM	+++	Yellowish pink	Strong reddish orange	Strong reddish orange
	Tyrosine	+++	Yellowish pink	Strong reddish orange	Strong reddish orange
	Oatmeal	+++	Yellowish pink	Vivid orange	Vivid orange
	Asparagine	+++	Yellowish pink	Strong reddish orange	Strong reddish orange
	Inorg. salt	+++	Yellowish pink	Vivid orange	Vivid orange
SC 36-2	YM	+++	Greenish gray	Greenish gray	Bright yellow
	Tyrosine	+++	Medium gray	Medium gray	Dark gray
	Oatmeal	+++	Pale beige	Dull yellow	Beige
	Asparagine	+++	Dark medium gray	Dark medium gray	Dull green
	Inorg. salt	+++	Dark gray	Dark gray	Dark medium gray

Table 13 (continued)

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
SC 36-3	YM	+++	Light medium gray	Light medium gray	Gold
	Tyrosine	+++	Pale beige	Strong yellow	Brown
	Oatmeal	+++	Pale yellow	Brownish gold	Gold
	Asparagine	+++	Yellowish white	Light greenish yellow	Light greenish yellow
	Inorg. salt	+++	Pale yellow	Medium gray	Light reddish yellow
SC 37-1	YM	+++	Light medium gray	Yellowish white	Yellowish brown
	Tyrosine	+++	Reddish gray	Brown	Dark brown
	Oatmeal	+++	Pale reddish yellow	Dull reddish yellow	Dull yellow
	Asparagine	+++	Pinkish beige	Medium gray	Pale yellow
	Inorg. salt	+++	Reddish gray	Grayish red	Pale reddish yellow
SC 37-2	YM	+++	Medium gray	Pale lavender	Dark red
	Tyrosine	+++	Brownish gray	Brownish olive	Grayish brown
	Oatmeal	+++	Light bluish gray	Grayish brown	Grayish brown
	Asparagine	+++	Yellowish white	Dull red purple	Light reddish yellow
	Inorg. salt	+++	Grayish red	Pale lilac	Grayish brown
SC 37-3	YM	+++	Pinkish white	Beige	Pale pinkish beige
	Tyrosine	++	Yellowish white	Pale beige	Pale beige
	Oatmeal	+++	Grayish yellow	Beige	Beige
	Asparagine	+	Grayish yellow	Pale beige	Pale pinkish beige
	Inorg. salt	++	Pinkish white	Beige	Pale beige
SC 37-4	YM	+++	Yellowish pink	Yellowish pink	Yellowish pink
	Tyrosine	+++	Brown	Brown	Light grayish brown
	Oatmeal	+++	Yellowish pink	Yellowish pink	Brownish gold
	Asparagine	+++	Vivid yellow orange	Vivid yellow orange	Vivid yellow orange
	Inorg. salt	+++	Light yellowish brown	Light yellowish brown	Brownish gold
SC 38-1	YM	+++	Yellowish white	Light reddish yellow	Light reddish yellow
	Tyrosine	+++	Yellowish white	Light reddish yellow	Light reddish yellow
	Oatmeal	++	Pale lilac	Dull reddish yellow	Dull reddish yellow
	Asparagine	+++	Pale lilac	Dull reddish yellow	Dull reddish yellow
	Inorg. salt	+++	Yellowish white	Dull reddish yellow	Dull reddish yellow

Table 13 (continued)

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
SC 38-2	YM	+++	Grayish leaf	Grayish leaf	Dull reddish yellow
	Tyrosine	++	Pale beige	Light reddish yellow	Dull reddish yellow
	Oatmeal	+++	Pale yellow	Grayish yellow	Dull reddish yellow
	Asparagine	+++	Grayish yellow	Grayish yellow	Vivid reddish yellow
	Inorg. salt	+++	Grayish red	Grayish leaf	Pale yellow
SC 38-3	YM	+++	Pale lavehder	Brownish gold	Brownish gold
	Tyrosine	+++	Pale lavehder	Brownish gold	Brownish gold
	Oatmeal	+++	Pale lavehder	Brownish gold	Brownish gold
	Asparagine	+++	Pale lavehder	Brownish gold	Brownish gold
	Inorg. salt	+++	Pale lavehder	Brownish gold	Brownish gold
SC 38-4	YM	+++	Yellowish white	Dull reddish yellow	Dull reddish yellow
	Tyrosine	+++	Pale pinkish beige	Yellowish pink	Light yellowish brown
	Oatmeal	++	Beige	Beige	Dull reddish yellow
	Asparagine	+++	Beige	Beige	Grayish yellow
	Inorg. salt	+++	Yellowish white	Yellowish pink	Light yellowish brown
SC 38-5	YM	+++	Pale reddish yellow	Brownish gold	Brownish gold
	Tyrosine	+++	Pale reddish yellow	Gold	Gold
	Oatmeal	+++	Light reddish yellow	Dull reddish yellow	Dull reddish yellow
	Asparagine	+++	Bright yellow	Brownish gold	Brownish gold
	Inorg. salt	+++	Pale reddish yellow	Brownish gold	Brownish gold
SC 44-1	YM	+++	Bluish gray	Grayish white	Reddish brown
	Tyrosine	+++	Pinkish white	Medium gray	Dark brown
	Oatmeal	+++	Grayish leaf	Grayish leaf	Dark brown
	Asparagine	+++	Pale purplish pink	Pale purplish pink	Deep purplish red
	Inorg. salt	+++	Grayish red	Reddish gray	Dark wine
SC 44-2	YM	+++	Light medium gray	Reddish gray	Reddish gray
	Tyrosine	+++	Medium gray	Reddish gray	Reddish gray
	Oatmeal	++	Light medium gray	Reddish gray	Reddish gray
	Asparagine	+++	Light medium gray	Reddish gray	Reddish gray
	Inorg. salt	++	Medium gray	Reddish gray	Reddish gray

Table 13 (continued)

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
SC 45-1	YM	+++	Vivid reddish orange	Vivid reddish orange	Vivid orange
	Tyrosine	+++	Yellowish pink	Yellowish pink	Yellowish pink
	Oatmeal	++	Vivid yellow orange	Vivid yellow orange	Vivid yellow orange
	Asparagine	+++	Vivid yellow orange	Vivid yellow orange	Vivid yellow orange
	Inorg. salt	+++	Vivid orange	Brownish gold	Brownish gold
SC 45-3	YM	+++	Grayish sky	Bluish gray	Dark red
	Tyrosine	+++	Dark medium gray	Dull yellow	Brown
	Oatmeal	+++	Brownish white	Light medium gray	Dark brown
	Asparagine	+++	Dark medium gray	Brown	Brown
	Inorg. salt	+++	Brownish gray	Brownish gray	Grayish brown
SC 45-4	YM	+++	Pale beige	Brownish gold	Dark yellowish brown
	Tyrosine	+++	Light reddish yellow	Brownish olive	Dark yellowish brown
	Oatmeal	+++	Pink white	Brownish gold	Brownish gold
	Asparagine	+++	Pale yellow	Light yellow	Light reddish yellow
	Inorg. salt	+++	Yellowish white	Yellowish white	Pale beige
SC 46-1	YM	+++	Medium gray	Dark gray	Dark red
	Tyrosine	+++	Medium gray	Dark gray	Dark brown
	Oatmeal	+++	Grayish yellow	Brown	Brown
	Asparagine	+++	Dark medium gray	Brownish gray	Brownish gray
	Inorg. salt	+++	Reddish gray	Pinkish white	Brown
SC 46-2	YM	+++	Pinkish white	Orange yellow	Dark yellowish orange
	Tyrosine	+++	Pale beige	Dull yellow	Brownish olive
	Oatmeal	+++	Beige	Beige	Dull reddish yellow
	Asparagine	+++	Pale beige	Pale yellow	Pale yellow
	Inorg. salt	+++	Light yellowish brown	Pale beige	Light yellowish brown
SC 49-1	YM	+++	Vivid yellow orange	Yellowish brown	Yellowish brown
	Tyrosine	+++	Vivid yellow orange	Yellowish brown	Yellowish brown
	Oatmeal	+++	Vivid yellow orange	Yellowish brown	Yellowish brown
	Asparagine	+++	Vivid yellow orange	Yellowish brown	Yellowish brown
	Inorg. salt	++	Vivid yellow orange	Yellowish brown	Yellowish brown

Table 13 (continued)

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
SC 50-1	YM	+++	Brownish gray	Bluish gray	Bluish gray
	Tyrosine	+++	Brownish gray	Bluish gray	Light medium gray
	Oatmeal	+++	Brownish gray	Bluish gray	Bluish gray
	Asparagine	+++	Brownish gray	Bluish gray	Bluish gray
	Inorg. salt	+	Grayish yellow	Bluish gray	Light medium gray
SC 50-2	YM	+++	Grayish pink	Brownish gold	Brownish gold Brownish
	Tyrosine	+++	Grayish pink	Brownish gold	gold
	Oatmeal	+++	Grayish pink	Brownish gold	Brownish gold
	Asparagine	+++	Grayish pink	Brownish gold	Brownish gold
	Inorg. salt	++	Grayish pink	Yellowish brown	Yellowish brown
SC 54-1	YM	+++	Pale yellowish pink	Vivid yellow orange	Vivid yellow orange
	Tyrosine	+++	Yellowish white	Dull reddish yellow	Dull reddish yellow
	Oatmeal	+++	Grayish white	Dull reddish yellow	Dull reddish yellow
	Asparagine	+++	Pale yellow	Vivid yellow	Vivid yellow
	Inorg. salt	+++	Pale beige	Vivid yellow orange	Vivid yellow orange
SC 54-2	YM	+++	Reddish gray	Pinkish white	Brownish gold
	Tyrosine	+++	Grayish yellow	Medium gray	Grayish yellow
	Oatmeal	+++	Pale yellow	Light yellowish brown	Light yellowish brown
	Asparagine	+++	Olive yellow	Olive yellow	Olive yellow
	Inorg. salt	+++	Light gray	Yellowish white	Brownish olive
SC 54-3	YM	+++	Grayish sky	Dark gray	Strong yellow
	Tyrosine	+++	Grayish sky	Yellowish brown	Gold
	Oatmeal	+++	Light grayish green	Brown	Gold
	Asparagine	+++	Light grayish green	Dull reddish yellow	Dull reddish yellow
	Inorg. salt	+++	Grayish sky	Greenish gray	Dull reddish yellow
SC 55-1	YM	+++	Yellowish pink	Yellowish pink	Vivid orange
	Tyrosine	+++	Pale reddish yellow	Dull orange	Light yellowish brown
	Oatmeal	+++	Pinkish white	Dull orange	Vivid orange
	Asparagine	+++	Pinkish white	Dark yellowish brown	Dark yellowish brown
	Inorg. salt	+++	Grayish white	Grayish white	Grayish brown

Table 13 (continued)

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
SC 55-2	YM	+++	Purplish rose	Dark red	Dark red
	Tyrosine	+++	Purplish pink	Rose	Dull red purple
	Oatmeal	+++	Light reddish yellow	Rose	Dull red purple
	Asparagine	++	Purplish pink	Purplish rose	Purplish rose
	Inorg. salt	+++	Pinkish white	Vivid red purple	Vivid red purple
SC 55-4	YM	+++	Dark medium gray	Pale beige	Dark Yellowish orange
	Tyrosine	+++	Grayish yellow	Brownish gold	Brownish gold
	Oatmeal	+++	Bluish white	Brown	Brown
	Asparagine	+++	Pale reddish yellow	Brown	Brown
	Inorg. salt	+++	Light medium gray	Medium gray	Yellowish brown
SC 55-5	YM	+++	Yellowish pink	Yellowish pink	Yellowish pink
	Tyrosine	+++	Yellowish pink	Yellowish pink	Yellowish pink
	Oatmeal	+++	Dull reddish yellow	Dull reddish yellow	Yellowish pink
	Asparagine	+++	Yellowish pink	Yellowish pink	Yellowish pink
	Inorg. salt	++	Pale beige	Dull reddish yellow	Dull reddish yellow
SC 58-1	YM	+++	Grayish brown	Yellowish brown	Brown
	Tyrosine	++	Yellowish brown	Yellowish brown	Brown
	Oatmeal	++	Pale yellow	Light yellow	Pale yellow
	Asparagine	+++	Pale yellow	Light yellow	Vivid yellow
	Inorg. salt	+++	Pinkish white	Pinkish white	Pale yellow
SC 58-2	YM	+++	Pale beige	Dark yellowish brown	Dark yellowish brown
	Tyrosine	+++	Pale beige	Dark yellowish brown	Dark yellowish brown
	Oatmeal	+++	Pale beige	Dark yellowish brown	Dark yellowish brown
	Asparagine	+++	Pale beige	Dark yellowish brown	Dark yellowish brown
	Inorg. salt	+++	Pale beige	Dark yellowish brown	Dark yellowish brown
SC 58-3	YM	+++	Gold	Gold	Gold
	Tyrosine	+++	Yellowish brown	Yellowish brown	Yellowish brown
	Oatmeal	+++	Vivid yellow orange	Gold	Gold
	Asparagine	+++	Vivid yellow orange	Vivid yellow orange	Vivid yellow orange
	Inorg. salt	+++	Vivid orange	Vivid orange	Gold

Table 13 (continued)

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
SC 58-4	YM	+++	Vivid orange	Vivid orange	Vivid orange
	Tyrosine	+++	Vivid orange	Vivid orange	Vivid orange
	Oatmeal	+++	Brownish gold	Brownish gold	Brownish gold
	Asparagine	+++	Brownish gold	Brownish gold	Brownish gold
	Inorg. salt	+++	Vivid orange	Vivid orange	Vivid orange
SC 58-5	YM	+++	Pinkish white	Dull yellow	Dull yellow
	Tyrosine	+++	Pale yellow	Pale yellow	Dull yellow
	Oatmeal	+++	Light gray	Dull yellow	Dull yellow
	Asparagine	+++	Light gray	Brown	Dark brown
	Inorg. salt	++	Pinkish white	Pale yellow	Dull yellow
SC 59-1	YM	+++	Yellowish pink	Vivid yellow orange	Dark yellowish orange
	Tyrosine	+++	Yellowish pink	Vivid yellow orange	Vivid yellow orange
	Oatmeal	+++	Yellowish pink	Yellowish pink	Vivid orange
	Asparagine	++	Yellowish pink	Yellowish pink	Vivid orange
	Inorg. salt	++	Grayish yellow	Yellowish pink	Beige
SC 59-2	YM	+++	Pale pinkish beige	Light yellowish brown	Brownish gold
	Tyrosine	+++	Pale pinkish beige	Light yellowish brown	Brownish gold
	Oatmeal	+++	Pale pinkish beige	Light yellowish brown	Brownish gold
	Asparagine	+++	Pale pinkish beige	Light yellowish brown	Brownish gold
	Inorg. salt	+++	Pale pinkish beige	Light yellowish brown	Brownish gold
SC 59-3	YM	+++	Grayish sky	Dark red	Dark red
	Tyrosine	+++	Grayish yellow	Grayish red	Reddish brown
	Oatmeal	+++	Purplish pink	Purplish rose	Purplish rose
	Asparagine	+++	Dark reddish orange	Dark reddish orange	Dark yellowish red
	Inorg. salt	+++	Light greenish gray	Light reddish brown	Light reddish brown
SC 59-4	YM	+++	Purplish pink	Dark reddish orange	Vivid orange
	Tyrosine	+++	Pale pinkish beige	Pale pinkish beige	Grayish yellow
	Oatmeal	+++	Yellowish pink	Pale reddish yellow	Pale reddish yellow
	Asparagine	+++	Yellowish pink	Yellowish pink	Yellowish pink
	Inorg. salt	+	Purplish pink	Dark reddish orange	Vivid orange

Table 13 (continued)

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
SC 59-5	YM	+++	Yellowish white	Reddish orange	Vivid yellow orange
	Tyrosine	+++	Pale beige	Pale beige	Beige
	Oatmeal	+++	Pinkish white	Deep orange	Dull reddish yellow
	Asparagine	+++	Pinkish white	Dull reddish yellow	Dull reddish yellow
	Inorg. salt	+++	Yellowish white	Reddish orange	Vivid yellow orange
SC 59-6	YM	+++	Grayish red	Dark reddish brown	Dark brown
	Tyrosine	+++	Yellowish brown	Dark yellowish brown	Dark brown
	Oatmeal	++	Light yellow	Dark yellowish brown	Dark brown
	Asparagine	+++	Light yellow	Yellowish brown	Yellowish brown
	Inorg. salt	+++	Pale lilac	Yellowish brown	Dark yellowish brown
SC 59-8	YM	+++	Reddish gray	Brown	Brown
	Tyrosine	+++	Gray	Medium gray	Dark purple
	Oatmeal	+++	Pale beige	Dark gray	Dark gray
	Asparagine	+++	Pale beige	Brownish gold	Brownish gold
	Inorg. salt	+++	Grayish yellow	Pinkish gray	Grayish brown
SC 60-1	YM	+++	Pinkish white	Yellowish brown	Yellowish brown
	Tyrosine	+++	Pinkish white	Yellowish brown	Yellowish brown
	Oatmeal	++	Pinkish white	Yellowish brown	Yellowish brown
	Asparagine	+++	Pinkish white	Yellowish brown	Yellowish brown
	Inorg. salt	+	Pinkish white	Yellowish brown	Yellowish brown
SC 60-3	YM	+++	Pale beige	Medium gray	Dark brown
	Tyrosine	+++	Grayish red	Grayish red	Grayish brown
	Oatmeal	+++	Bluish gray	Pale yellow	Pale yellow
	Asparagine	+++	Bluish gray	Dark gray	Dark gray
	Inorg. salt	+++	Dark gray	Yellowish white	Dark gray
SC 61-1	YM	+++	Dark reddish brown	Dark reddish brown	Reddish brown
	Tyrosine	++	Dark yellowish brown	Dark yellowish brown	Dull reddish yellow
	Oatmeal	+++	Dark yellowish brown	Dark yellowish brown	Dull reddish yellow
	Asparagine	+++	Dark reddish brown	Dark reddish brown	Reddish brown
	Inorg. salt	++	Brownish gold	Brownish gold	Brownish gold

Table 13 (continued)

Isolate no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
SC 61-2	YM	+++	Purplish pink	Rose	Deep Yellowish red
	Tyrosine	+++	Purplish pink	Purplish rose	Dull red purple
	Oatmeal	++	Pale yellow	Pale yellow	Brown
	Asparagine	+++	Purplish pink	Purplish rose	Purplish rose
	Inorg. salt	+++	Grayish pink	Purplish pink	Purplish rose
SC 61-3	YM	+++	Grayish yellow	Dark brown	Dark brown
	Tyrosine	+++	Pale beige	Grayish brown	Dark brown
	Oatmeal	+++	Medium gray	Dark medium gray	Dark medium gray
	Asparagine	+++	Medium gray	Dark yellowish brown	Dark yellowish brown
	Inorg. salt	+++	Light medium gray	Light medium gray	Brown
SC 61-5	YM	+++	Pale lilac	Brown	Brown
	Tyrosine	+++	Pale lilac	Dull reddish yellow	Dark yellowish brown
	Oatmeal	++	Pale lilac	Dull reddish yellow	Dark yellowish brown
	Asparagine	+++	Pale lilac	Yellowish brown	Yellowish brown
	Inorg. salt	+++	Pale lilac	Brown	Dull reddish yellow
SC 62-2	YM	+++	Purplish pink	Yellowish pink	Vivid orange
	Tyrosine	+++	Yellowish pink	Bluish white	Brownish white
	Oatmeal	+++	Pinkish white	Pinkish white	Brownish white
	Asparagine	+++	Pinkish white	Yellowish pink	Yellowish pink
	Inorg. salt	+++	Grayish yellow	Yellowish pink	Grayish yellow

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.

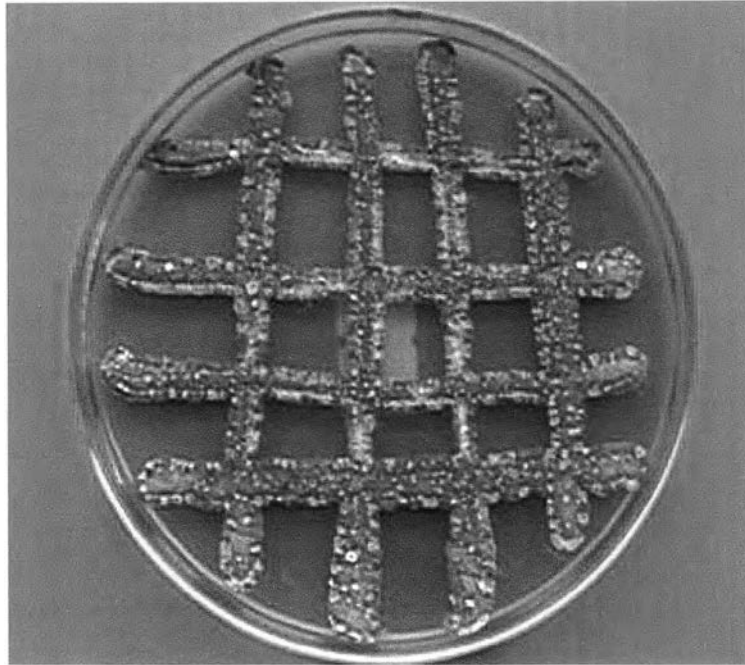


Figure 1 The colonial appearance of the strain PC 4-3 on YMA medium (14 days)

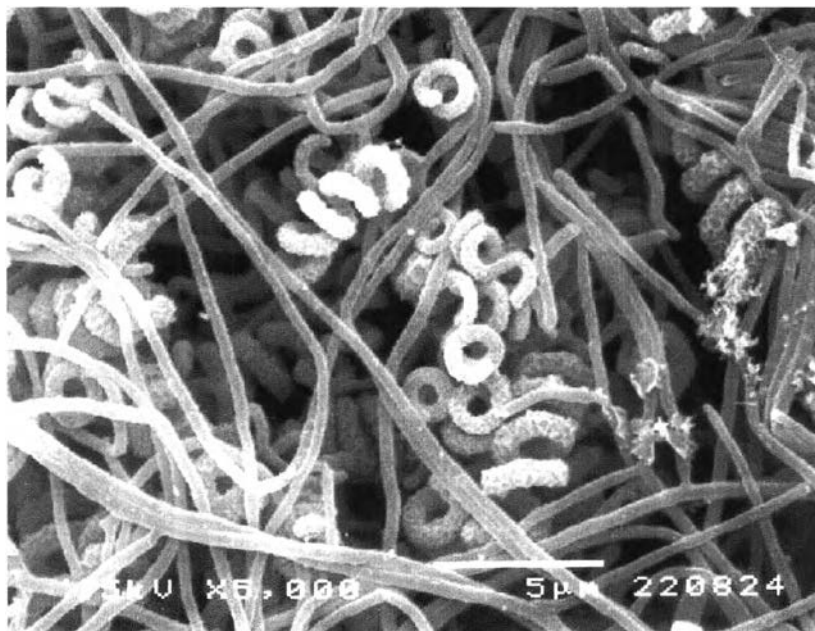


Figure 2 Scanning electron micrograph of spore-bearing substrate mycelium of the strain PC 4-3 on YMA medium (14 days)

2.2 Physiological and biochemical characteristics

The physiological characteristics of 30 selected isolates are shown in Table 14. Most strains grew in YMA with 2% and 4% NaCl, at pH 5.0, 7.0, and 9.0, and at 10°C and 28°C, whereas fewer strains grew in 6% NaCl, at pH 4.0 and 10.0, and at 45°C.

All strains could not hydrolyse chitin and decompose cellulose. Most strains formed melanin, coagulated skim milk and liquefied gelatin, whereas fewer strains reduced nitrate, hydrolysed starch and produced H₂S (Table 15). Most strains used glucose, glycerol, L-arabinose, D-xylose, mannitol, fructose, sucrose, melibiose, rhamnose, and raffinose, whereas fewer strains did not use any carbon sources (Table 16).

2.3 Cell wall analysis

The cell wall chemical analysis revealed that the strain PC 4-3 contained L-isomer of diaminopimelic acid (DAP), indicating the type I cell wall of *Streptomyces* (Holt, 1989).

2.4 16S rDNA sequence analysis and phylogenetic tree construction

2.4.1 16S rDNA amplification by PCR

The chromosomal DNA from the strain PC 4-3 was used as the DNA templates for 16S rDNA amplification by PCR with forward and reverse primer (27F 5' AGAGTTTGATCCTGGCTCAG 3' and 1492R 5' TACGGCTACCTTGTTACGACTT 3'). The PCR amplified 16S rDNA was analyzed in 1.0% agarose gel electrophoresis. 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.

Table 14 Physiological characteristics of 30 isolates showing antimicrobial activity

Isolate no.	NaCl			pH					Temperature		
	2%	4%	6%	4	5	7	9	10	10°C	28°C	45°C
SC 1-1	+	+	+	+	+	+	+	+	-	+	+
SC 1-5	+	+	+	-	+-	+	+	-	+-	+	-
SC 2-4	+	+	+	-	+-	+	+	+	+	+	+
PC 4-3	+	+	+	+	+	+	+	+	+	+	-
PC 5-1	+	+	+	-	+-	+	+	+	+-	+	-
MS 1-5	+-	-	-	-	-	+	+	-	-	+	-
MS 1-6	+	-	-	-	-	+	+	+	+-	+	-
MS 5-6	+-	-	-	-	-	+	+-	-	-	+	-
MS 7-1	+-	-	-	-	-	+	+-	-	+	+	-
MS 7-3	+-	-	-	-	-	+	+	-	-	+	-
SC 11-1	+	+	-	-	+	+	+	+	+	+	+
SC 11-3	+	+	-	-	+	+	+	+	+	+	-
SC 16-4	+	-	-	-	+	+	+	-	+	+	-
SC 20-1	+	+	-	-	+	+	+	+	+-	+	-
SC 21-2	+	+	-	-	+	+	+	-	-	+	+
SC 21-3	+-	-	-	-	+-	+	-	-	-	+	+
SC 21-4	+	+	-	-	+	+	+	+	-	+	+
SC 36-1	+	+	+	-	+	+	+	+	+-	+	-
SC 38-1	+	+	-	-	+	+	+	-	+	+	-
SC 38-3	+	+	+	-	+-	+	+	+	-	+	+
SC 38-5	+	+	+	-	+	+	+	-	+	+	-
SC 45-4	+	-	-	-	+	+	+	-	-	+	+
SC 49-1	+-	-	-	-	+-	+	+	-	-	+	+
SC 54-3	+	+	+	-	+	+	+	+	+-	+	-
SC 58-2	+	+	+	+	+	+	+	+	-	+	+
SC 59-1	+	+	+	-	+	+	+	+	+-	+	-
SC 59-2	+	+	-	-	+	+	+	+	-	+	+
SC 60-1	+	+	+	-	+	+	+	-	+	+	-
SC 61-1	+-	-	-	-	+-	+	+	-	-	+	+
SC 61-5	+	+	-	-	+	+	+	-	+	+	-

Table 15 (continued)

Isolate no.	NO ₃ reduction	H ₂ S production	Melanin formation	Starch hydrolysis	Chitin hydrolysis	Cellulose decomposition	Gelatin liquefaction	Skim milk	
								Coagulation	Peptonization
SC 59-2	-	-	-	-	-	-	+	-	-
SC 60-1	-	-	-	-	-	-	+	+	-
SC 61-1	-	-	-	-	-	-	-	-	-
SC 61-5	+	-	+	-	-	-	+	+	-

+, positive; ±, weakly; -, negative.

Table 16 Utilization of various carbon sources by 30 isolates showing antimicrobial activity

Isolate no.	None	Glucose	Glycerol	L-Arabinose	D-Xylose	Mannitol	Fructose	Sucrose	Melibiose	Rhamnose	Raffinose
SC 1-1	-	+	+	+	+	+	+	+	+	+	-
SC 1-5	-	+	-	-	-	+	+	+	-	-	-
SC 2-4	-	+	+	+	+	+	+	+	+	+	+
PC 4-3	+	+	+	+	+	+	+	+	+	+	+
PC 5-1	-	+	+	-	+	+	-	+	-	+	+
MS 1-5	-	+	-	-	-	-	-	-	-	-	-
MS 1-6	-	+	+	-	+	+	-	+	+	-	-
MS 5-6	-	+	-	+	+	-	-	-	-	+	-
MS 7-1	-	+	-	-	-	-	-	-	-	-	-
MS 7-3	-	+	+	+	-	+	-	-	-	+	-
SC 11-1	-	+	+	+	-	+	+	-	+	+	-
SC 11-3	-	+	-	+	+	+	+	+	+	+	-
SC 16-4	-	+	-	-	+	+	+	+	-	+	+
SC 20-1	-	+	+	+	+	+	+	+	+	+	+
SC 21-2	-	+	-	+	+	+	-	+	-	+	+
SC 21-3	-	+	-	-	+	-	-	-	-	-	-
SC 21-4	-	+	+	+	+	+	+	-	+	+	-
SC 36-1	+	+	+	+	+	+	+	+	+	+	+
SC 38-1	-	+	+	+	-	+	-	+	+	+	+
SC 38-3	-	+	+	+	+	-	+	+	-	+	-
SC 38-5	-	+	+	+	-	+	-	+	+	+	+
SC 45-4	+	+	+	+	+	+	+	+	+	+	+
SC 49-1	-	+	-	-	+	-	+	+	-	+	+
SC 54-3	+	+	+	+	+	+	+	+	+	+	+
SC 58-2	-	+	+	+	+	+	+	+	-	-	+
SC 59-1	-	+	+	+	+	+	+	+	-	+	-
SC 59-2	-	+	+	-	+	+	+	-	+	+	+
SC 60-1	-	+	-	-	-	+	-	-	-	-	+

Table 16 (continued)

Isolate no.	None	Glucose	Glycerol	L-Arabinose	D-Xylose	Mannitol	Fructose	Sucrose	Melibiose	Rhamnose	Raffinose
SC 61-1	-	+	-	-	+	+	-	+	-	+	+
SC 61-5	-	+	+	+	+	+	+	+	+	+	+

+, positive; \pm , weakly; -, negative.

Table 17 Characteristics of *Streptomyces* sp. PC 4-3

Characteristics	Result
Spore chain	Spiral
Spore surface	Rugose
Vegetative mycelium diameter	0.5-0.6 μm
Aerial spore-mass color	White --> Black
Soluble pigment	Light brown
Growth on agar medium:	
Yeast extract-malt extract, Tyrosine, Oatmeal, Asparagine and Inorganic salt-starch	+
Growth at:	
10°C-30°C	+
45°C	-
pH 4-10	+
Growth in:	
NaCl (2-6%)	+
Decomposition of:	
Starch	-
Chitin	-
Cellulose	-
Gelatin	+
Production of:	
Hydrogen sulphide	-
Melanin	-
Reduction of:	
Nitrate	-
Coagulation of:	
Skim milk	+
Growth on sole carbon source (1%w/v)	
D-Xylose, Fructose, Glucose, Glycerol, L-Arabinose, Mannitol, Melibiose, Raffinose, Rhamnose and Sucrose	+
Cell wall	L-diaminopimelic acid

+, positive; -, negative.

2.4.2 16S rDNA sequencing

The selected PCR amplified 16S rDNA from the strain PC 4-3 and *Streptomyces* sp. NRRL B-1865 were determined for their nucleotide sequences. The obtained nucleotide sequences were illustrated in Figure 3.

2.4.3 16S rDNA sequence analysis and phylogenetic tree construction

Based on 16S rDNA sequences from the strain PC 4-3, the phylogenetic tree (Figure 4) was constructed from evolutionary distances by using the neighbor-joining method. It was located in the lineage of the genus *Streptomyces*. The genus *Streptomyces* showed a monophyletic lineage with a high bootstrap value of 56% when high bootstrap value (1,000) was used.

The percentage of 16S rDNA sequence similarity of the strain PC 4-3 to another strains was shown in Table 18. An almost complete 16S rDNA sequence was determined for the strain PC 4-3 (1534 nucleotides). This sequence served to distinguish it from other Streptomycetes for which full or partial nucleotide sequences are available. The alignment of 16S rDNA sequences of the strain PC 4-3 to 16S rDNA genes of *Streptomyces* sp. NRRL B-1865 showed the highest similarity percentage of 99.52%. Hence, it is conclude that the strain PC 4-3 is *Streptomyces*.

```

PC 4-3      GCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCGGTTTCG
NRRL B-1865 GCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCGGTTTCG
*****

PC 4-3      GCCGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGA
NRRL B-1865 GCCGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGA
*****

PC 4-3      CAAGCCCTGGAACGGGGTCTAATACCGGATACGACGCGTCCCGCATGGGATACGCGTG
NRRL B-1865 CAAGCCCTGGAACGGGGTCTAATACCGGATACGACGCGTCCCGCATGGGATACGCGTG
*****

PC 4-3      GAAAGCTCCAGCGGTGCAGGATGAGCCCGCGCCTATCAGCTTGTGGTGGGGTGATGGC
NRRL B-1865 GAAAGCTCCAGCGGTGCAGGATGAGCCCGCGCCTATCAGCTTGTGGTGGGGTGATGGC
*****

PC 4-3      CTACCAAGCGCAGCAGGGTAGCCGGCTGAGAGGGCGACCGGCCACTGGGACTGAGA
NRRL B-1865 CTACCAAGCGCAGCAGGGTAGCCGGCTGAGAGGGCGACCGGCCACTGGGACTGAGA
*****

PC 4-3      CACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGCGAAAGCCT
NRRL B-1865 CACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGCGAAAGCCT
*****

PC 4-3      GATGCAGCGACCCCGTGAGGGATGACGGCCTTCGGGTTGTAACCTCTTTCAGCAGGG
NRRL B-1865 GATGCAGCGACCCCGTGAGGGATGACGGCCTTCGGGTTGTAACCTCTTTCAGCAGGG
* *****

PC 4-3      AAGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCG
NRRL B-1865 AAGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCG
*****

PC 4-3      CGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGGC
NRRL B-1865 CGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGGC
*****

PC 4-3      GCTTGTGCGCGTGGATGTGAAAGCCCGGGCTTAACCCGGGTCTGCATTTCGATACGGGC
NRRL B-1865 GCTTGTGCGCGTGGATGTGAAAGCCCGGGCTTAACCCGGGTCTGCATTTCGATACGGGC
*****

PC 4-3      AGGCTAGAGTTCGGTAGGGGAGATCGGAATCCTGGTGTAGCGGTGAAATGCGCAGATAT
NRRL B-1865 AGGCTAGAGTTCGGTAGGGGAGATCGGAATCCTGGTGTAGCGGTGAAATGCGCAGATAT
*****

PC 4-3      CAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAA
NRRL B-1865 CAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAA
*****

PC 4-3      AGCGTGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACCGTTGGGAACT
NRRL B-1865 AGCGTGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACCGTTGGGAACT
*****

PC 4-3      AGGTGTGGGCGACATTCACGTTGTCCGTGCCGCAGCTAACGCATTAAGTCCCGCCTG
NRRL B-1865 AGGTGTGGGCGACATTCACGTTGTCCGTGCCGCAGCTAACGCATTAAGTCCCGCCTG
*****

PC 4-3      GAGAGTACGGCCGAAGGCTAAAACCTCAAAGGAATTGACGGGGCCCGCACAAAGTGGCGG
NRRL B-1865 GAGAGTACGGCCGAAGGCTAAAACCTCAAAGGAATTGACGGGGCCCGCACAAAGTGGCGG
* *****

PC 4-3      AGCATGTGGCTTAATTCGACGCAACCGGAAGAACCTTACCAAGGCTTGACATACCCGGA
NRRL B-1865 AGCATGTGGCTTAATTCGACGCAACCGGAAGAACCTTACCAAGGCTTGACATACCCGGA
*****

PC 4-3      AAACCCGAGAGCAGGGTCCCCCTTGTGGTGGTGTACAGGTGGTGCATGGCTGTGCTCA
NRRL B-1865 AAACCCGAGAGCAGGGTCCCCCTTGTGGTGGTGTACAGGTGGTGCATGGCTGTGCTCA
*****

```

Figure 3 Comparison of 16S rDNA nucleotide sequences between the strain PC 4-3 and NRRL B-1865

PC 4-3 GCTGTCGTGAGATGTTGGGTAAAGTCCCGCAACGAGCGCAACCCTTGTTCTGTGTTG
 NRRL B-1865 GCTGTCGTGAGATGTTGGGTAAAGTCCCGCAACGAGCGCAACCCTTGTTCTGTGTTG

PC 4-3 CCAGCATGCCTTTCGGGGTGATGGGGACTCACAGGAGACTGCCGGGGTCAACTCGGAGGA
 NRRL B-1865 CCAGCATGCCTTTCGGGGTGATGGGGACTCACAGGAGACTGCCGGGGTCAACTCGGAGGA

PC 4-3 AGGTGGGGACGACGTCAAGTCATCATGCCCTTATGTCTTGGGCTGCACACGTGCTACAA
 NRRL B-1865 AGGTGGGGACGACGTCAAGTCATCATGCCCTTATGTCTTGGGCTGCACACGTGCTACAA

PC 4-3 TGGCCGGTACAATGAGCTGCGAAGCCGTGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCA
 NRRL B-1865 TGGCCGGTACAATGAGCTGCGAAGCCGTGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCA

PC 4-3 GTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATC
 NRRL B-1865 GTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATC

PC 4-3 AGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGACACACCGCCCGTCACGTACGAAA
 NRRL B-1865 AGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGACACACCGCCCGTCACGTACGAAA

PC 4-3 GTCGGTAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCGAAGGTGGGAC
 NRRL B-1865 GTCGGTAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCGAAGGTGGGAC

PC 4-3 TGGCGATTGGGACGAAGTCGTAACAAGGTACGTA
 NRRL B-1865 TGGCGATTGGGACGAAGTCGTAACAAGGTACGTA

Figure 3 (continued)

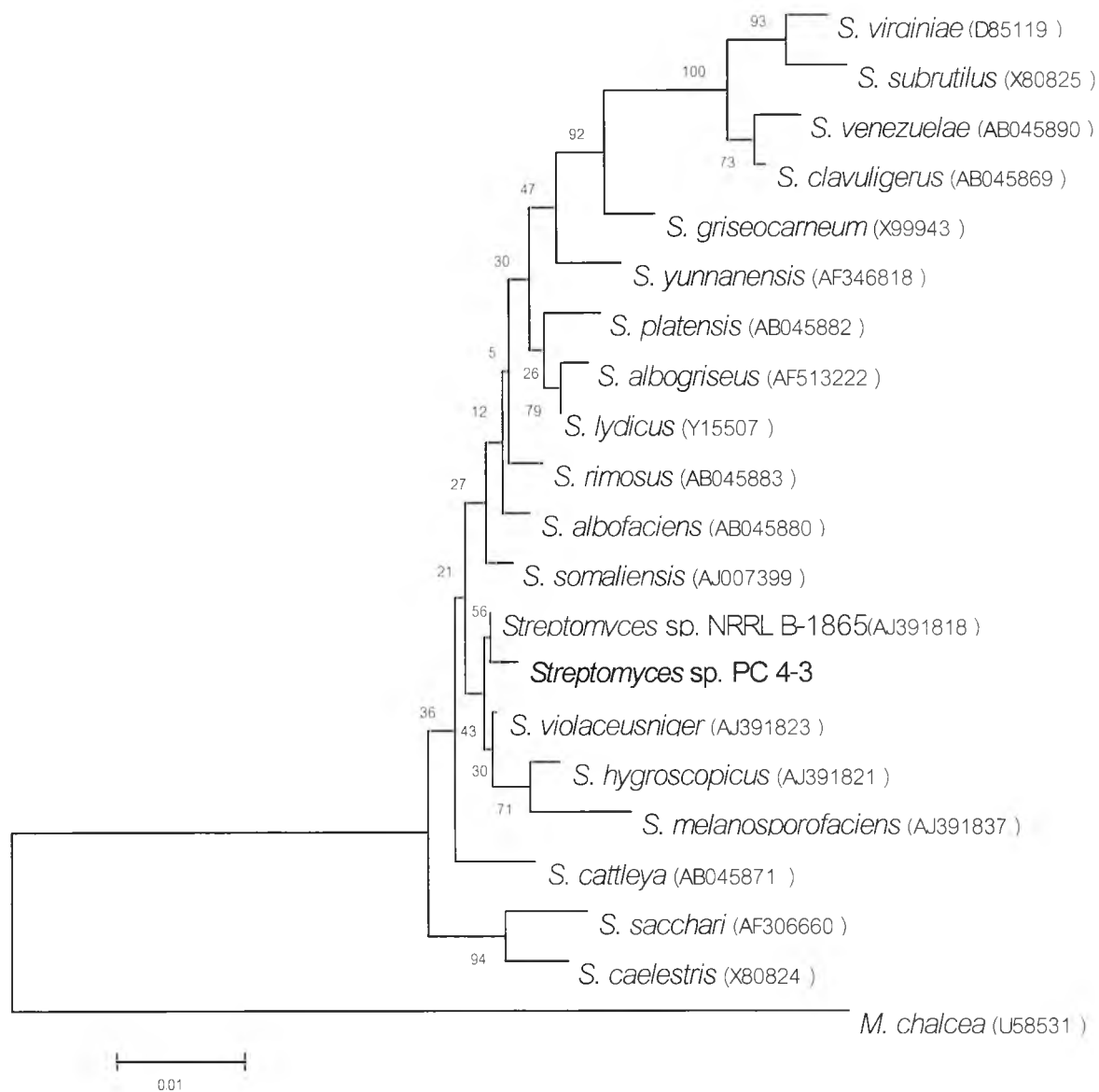


Figure 4 Phylogenetic relationships of the strain PC 4-3 based on the 16S rDNA sequences

Table 18 16S rDNA sequence similarity of the strain PC 4-3 to closely related species

Species	% 16S rDNA sequence similarity to strain PC 4-3
<i>Streptomyces sp. NRRL B-1865</i> (AJ391818)	99.52
<i>S. violaceusniger</i> (AJ391823)	99.26
<i>S. hygrosopicus</i> (AJ391821)	99.15
<i>S. melanosporofaciens</i> (AJ391837)	98.41
<i>S. cattleyar</i> (AB045871)	98.22
<i>S. somaliensis</i> (AJ007399)	97.82
<i>S. albofaciens</i> (AB045880)	97.72
<i>S. rimosus</i> (AB045883)	97.63
<i>S. lydicus</i> (Y15507)	97.54
<i>S. albogriseuss</i> (AF513222)	97.35
<i>S. platensis</i> (AB045882)	97.24
<i>S. yunnanensis</i> (AF346818)	97.13
<i>S. griseocarneum</i> (X99943)	97.00
<i>S. sacchari</i> (AF306660)	96.73
<i>S. caelestris</i> (X80824)	96.56
<i>S. clavuligerus</i> (AB045869)	96.54
<i>S. venezuelae</i> (AB045890)	96.00
<i>S. subutilus</i> (X80825)	95.83
<i>S. virginiae</i> (D85119)	95.76
<i>Micromonospora chalcea</i> (U58531)	93.66

3. Fermentation and antimicrobial activity test

3.1 Antimicrobial production of 30 isolates showing antimicrobial activity in YMB medium

Thirty isolates showing antimicrobial activity were fermented in YMB medium at 30°C for 10 days. Antimicrobial activities of these strains in YMB medium are shown in Table 19. Eighteen isolates exhibited antimicrobial activity against *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633 and 12 isolates against all tested organisms (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231). The strain PC 4-3 showing good antimicrobial activity was selected for secondary metabolite fermentation study.

3.2 Antimicrobial production of the strain PC 4-3 in various media and effect of pH

The strain PC 4-3 was fermented in 4 kinds of production media including GMP, PY, YMB and GBY. Antimicrobial activities of this strain on various media are shown in Table 20. The results of fermentation in YMB medium of this strain displayed good antimicrobial activity against all tested organisms. Effects of pH on antimicrobial activity of the strain PC 4-3 is shown in Table 21. This strain exhibited good antimicrobial activity with the high potency of inhibition when cultivated at all selected pH especially pH 6.0.

Table 19 Antimicrobial activity of 30 isolates showing antimicrobial activity in YMB

Isolate no.	Inhibition zone (mm.)				
	<i>S. aureus</i> ATCC 25923	<i>B. subtilis</i> ATCC 6633	<i>E. coli</i> ATCC 25922	<i>Ps. aeruginosa</i> ATCC 27853	<i>C. albicans</i> ATCC 10231
SC 1-1	15.0	21.4	-	-	-
SC 1-5	16.4	20.8	-	-	-
SC 2-4	8.6	14.1	10.0	-	8.4
PC 4-3	28.2	22.0	12.0	8.0	22.0
PC 5-1	15.5	10.7	-	-	-
MS 1-5	11.2	10.1	-	-	-
MS 1-6	10.4	10.9	-	9.1	-
MS 5-6	9.3	10.3	11.7	-	-
MS 7-1	12.0	11.2	-	-	8.1
MS 7-3	15.8	16.3	10.5	9.2	10.8
SC 11-1	21.0	30.1	11.8	-	15.5
SC 11-3	19.1	18.0	7.2	-	-
SC 16-4	13.0	7.1	-	-	10.4
SC 20-1	18.8	10.3	-	-	-
SC 21-2	17.2	19.6	-	-	-
SC 21-3	12.5	18.7	-	-	-
SC 21-4	16.6	18.0	-	-	-
SC 36-1	11.7	13.4	-	-	-
SC 38-1	12.0	17.2	-	-	-
SC 38-3	8.1	11.0	12.0	-	12.4
SC 38-5	10.4	19.2	-	-	-
SC 45-4	17.0	19.0	-	-	-
SC 49-1	13.1	7.3	-	-	-
SC 54-3	11.2	14.7	-	-	-
SC 58-2	19.5	21.0	-	-	-
SC 59-1	15.2	10.1	-	-	-
SC 59-2	6.7	15.5	-	-	-
SC 60-1	7.3	19.0	-	-	11.6

Table 19 (continued)

Isolate no.	Inhibition zone (mm.)				
	<i>S. aureus</i> ATCC 25923	<i>B. subtilis</i> ATCC 6633	<i>E. coli</i> ATCC 25922	<i>Ps. aeruginosa</i> ATCC 27853	<i>C. albicans</i> ATCC 10231
SC 61-1	20.0	21.4	-	-	-
SC 61-5	22.3	13.0	10.4	-	8.0

0.5 mg/disc.

Table 20 Antimicrobial activity of the strain PC 4-3 in various media

Medium	Inhibition zone (mm.)				
	<i>S. aureus</i> ATCC 25923	<i>B. subtilis</i> ATCC 6633	<i>E. coli</i> ATCC 25922	<i>Ps. aeruginosa</i> ATCC 27853	<i>C. albicans</i> ATCC 10231
GMP	6.3	6.8	-	-	-
PY	-	8.1	-	7.5	-
YMB	28.3	36.0	11.0	8.8	16.3
GBY	24.8	20.0	8.6	-	11.2

GMP, Glucose-Malt extract-Peptone medium; PY, Peptone-Yeast extract medium; YMB, Yeast extract-Malt extract medium; GBY, Glucose-Beef extract-Yeast extract-Tryptone medium.

Table 21 Effect of pH on antimicrobial activity of the strain PC 4-3

pH before sterilization	pH after sterilization	Inhibition zone (mm.)				
		<i>S. aureus</i> ATCC 25923	<i>B. subtilis</i> ATCC 6633	<i>E. coli</i> ATCC 25922	<i>Ps. eruginosa</i> ATCC 27853	<i>C. albicans</i> ATCC10231
6.01	5.89	30.0	30.0	9.0	9.0	31.0
7.01	6.88	31.0	27.0	12.0	-	25.0
8.00	7.95	29.0	30.0	14.0	-	28.0

1 mg/disc.

4. Structure elucidation and antimicrobial activity of the isolated compounds

Two compounds were isolated from the ethyl acetate extract of YM fermentation broth of the strain PC 4-3 after several chromatographic techniques. The extracts gave two known compounds, geldanamycin (FPC43001a, 130 mg, 0.89% of ethyl acetate extract, and 2.28×10^{-4} % w/v of YM fermentation broth) and 17-*O*-demethylgeldanamycin (SPC43004a, 14 mg, 0.09% of ethyl acetate extract, and 2.46×10^{-5} % w/v of YM fermentation broth). The yield percentage of these two compounds is based on the ethyl acetate extract (5.79 g of ethyl acetate extract of YM fermentation broth). The chemical structures of these isolated compounds were determined by analyses of their spectroscopic data including UV, IR, MS, and NMR spectral data.

4.1 Structure elucidation of geldanamycin (FPC43001a)

Compound FPC43001a (130 mg) was obtained as a yellow amorphous powder showing optical rotation $[\alpha]_D^{25} +11.32^\circ$ (c, 0.100, CHCl₃). The UV spectrum in MeOH of compound FPC43001a (Figure 6) exhibited $\lambda_{max} (\epsilon)$ at 260 (10,360), 310 (12,104) and a

broad weak shoulder at 410 (574) nm. The IR absorption spectrum (Figure 7) displayed characteristic band at $3,441\text{ cm}^{-1}$ (O-H stretching), $1,704\text{ cm}^{-1}$ (C=O stretching) and $1,655\text{ cm}^{-1}$ (C=O stretching, amide I band). The ESI-TOF MS of this compound exhibited the pseudomolecular ion peak $[M+Na]^+$ (Figure 8) at m/z 583.29, therefore the molecular formula of this compound should be $C_{29}H_{40}N_2O_9$ with the MW 560. The 300 MHz $^1\text{H-NMR}$ spectrum of compound FPC43001a in CDCl_3 (Figure 9) revealed that this compound contained four methyl proton signals at δ 0.93, 0.94, 1.75 and 1.97 ppm; three methoxy proton signals at δ 3.25, 3.32 and 4.10 ppm; two methylene proton signals at δ 1.75 and 2.44 ppm; eleven methine proton signals at δ 1.61, 2.76, 3.38, 3.51, 4.29, 5.17, 5.77, 5.83, 6.55, 6.92 and 7.26 ppm; and three exchangeable proton signals at δ 3.01, 4.76 and 8.69 ppm. The 75 MHz $^{13}\text{C-NMR}$ spectrum of compound FPC43001a in CDCl_3 (Figure 10) gave twenty-nine carbon signals, consistent with the molecular formula. Analysis of the $^{13}\text{C-NMR}$ spectrum indicated the presence of two quinone carbonyl carbons at δ 183.0 and 184.73 ppm, one amide carbonyl carbon at δ 168.01 ppm and one carbamate carbon at δ 155.76 ppm.

Comparison the spectral data of compound FPC43001a to those reported in the literatures (Rinehart, Jr. and Shield, 1976; Omura *et al.*, 1979) confirmed that it was the known compound, geldanamycin (Table 22, Figure 5). Geldanamycin was previously obtained from the culture broth of *Streptomyces hygroscopicus* var. *geldanus* in 1970 (DeBoer *et al.*, 1970) and from mangrove *Streptomyces* sp. TRA 9875-2 (Tadtong, 2000).

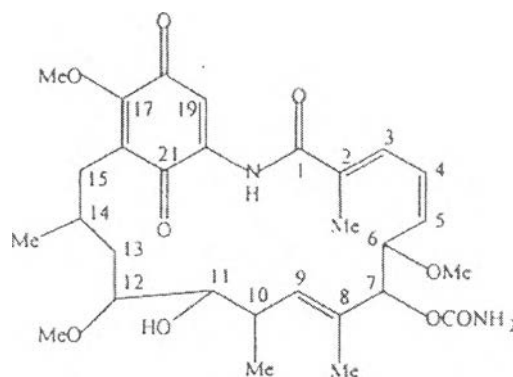


Figure 5 Structure of compound FPC43001a

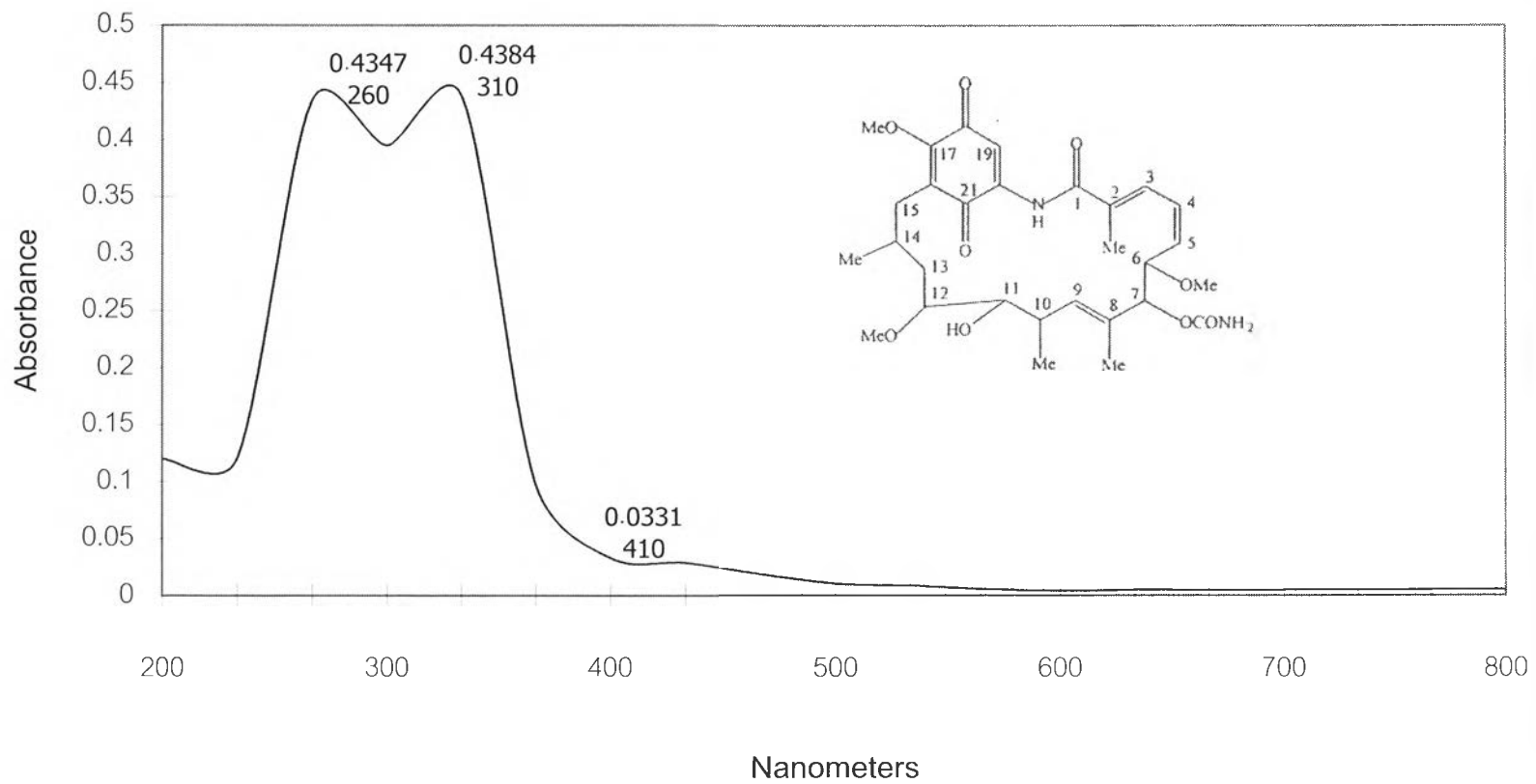


Figure 6 The UV spectrum of geldanamycin (FPC43001a)

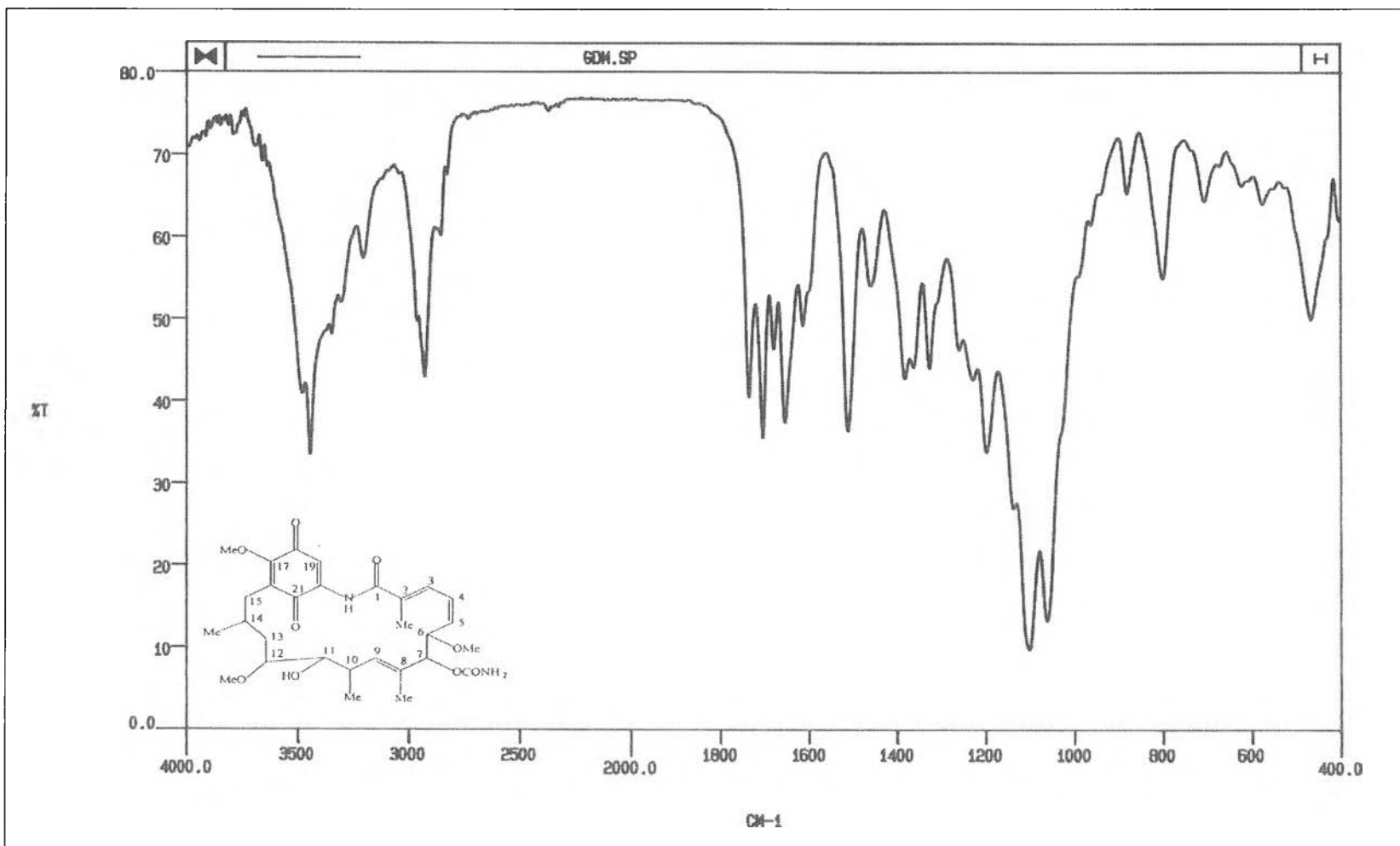


Figure 7 The IR spectrum of geldanamycin (FPC43001a)

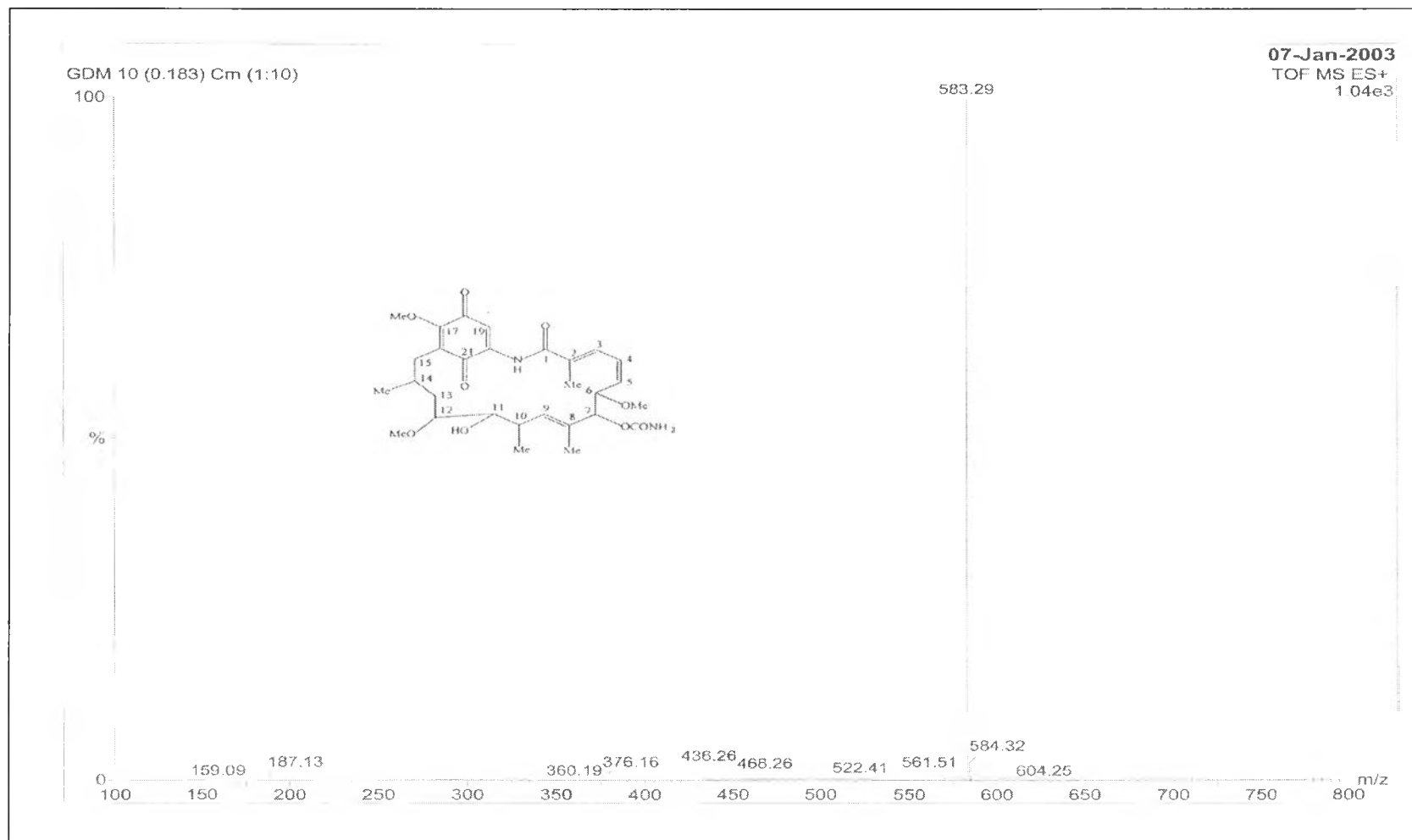


Figure 8 The ESI-TOF mass spectrum of geldanamycin (FPC43001a)

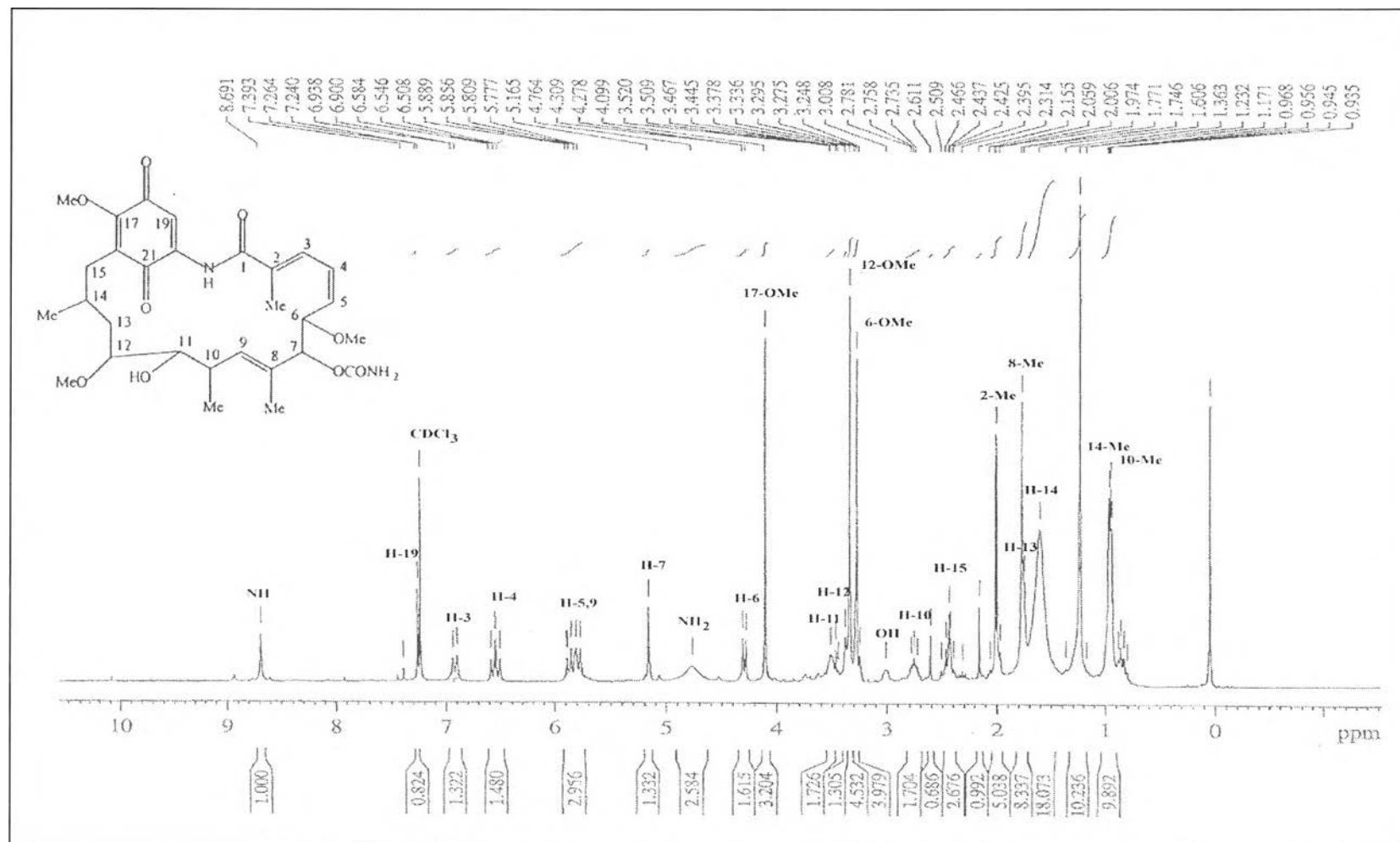


Figure 9 ¹H NMR spectrum (300 MHz) of geldanamycin (FPC43001a) in CDCl₃

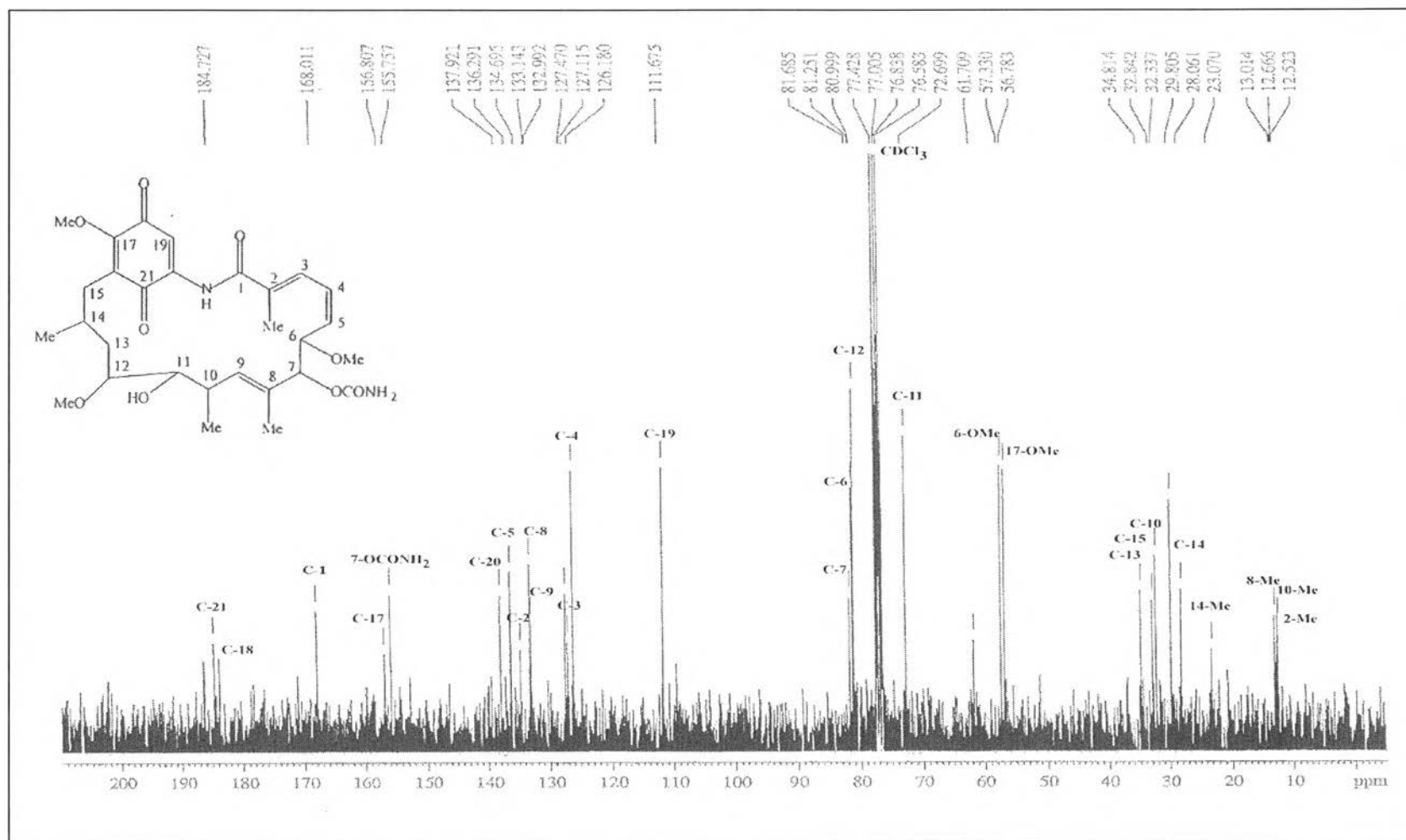


Figure 10 ^{13}C NMR spectrum (75 MHz) of geldanamycin (FPC43001a) in CDCl_3

Table 22 Comparison of ^1H and ^{13}C -NMR spectral data of FPC43001a with that of geldanamycin

Position	FPC43001a		Geldanamycin ^a	
	δ_c (ppm)	δ_H (ppm), mult, (J in Hz)	δ_c (ppm)	δ_H (ppm), mult, (J in Hz)
1	168.01	-	167.99	-
2	134.70	-	134.66	-
2-Me	12.52	1.97, 3H, s	12.50	1.98, 3H, s
3	127.12	6.92, d, (12)	127.07	6.90, d, (12)
4	126.18	6.55, t, (12, 11)	126.18	6.53, t, (12, 11)
5	136.29	5.83, t, (11, 10)	136.25	5.83, t, (11, 10)
6	81.25	4.29, d, (10)	81.23	4.29, d, (10)
6-OMe	57.33	3.25, 3H, s	57.31	3.25, 3H, s
7	81.69	5.17, brs	81.64	5.14, brs
7-OCONH ₂	155.76	4.76, NH ₂ , brs	155.77	4.80, NH ₂ , brs
8	133.14	-	133.11	-
8-Me	13.01	1.75, 3H, s	13.01	1.75, 3H, s
9	132.99	5.77, d, (10)	132.97	5.77, d, (10)
10	32.34	2.76, m	32.28	2.73, m
10-Me	12.67	0.94, 3H, d, (7)	12.66	0.93, 3H, d, (7)
11	72.70	3.51, m	72.62	3.48, m
11-OH	-	3.01, OH, brs	-	3.01, OH, brs
12	80.99	3.38, m	80.93	3.35, m
12-OMe	56.78	3.32, 3H, s	56.77	3.31, 3H, s
13	34.81	1.75, 2H, m	34.76	1.73, 2H, m
14	28.06	1.61, brs	27.99	1.63, brs
14-Me	23.07	0.93, 3H, d, (6)	23.06	0.92, 3H, d, (6)
15	32.84	2.44, 2H, m	32.85	2.41, 2H, m
16	127.47	-	127.42	-
17	156.81	-	156.75	-
17-OMe	61.71	4.10, 3H, s	61.71	4.08, 3H, s
18	183.0	-	183.88	-
19	111.66	7.26, s	111.63	7.22, s
20	137.92	-	137.86	-
21	184.73	-	184.70	-
NH	-	8.69, NH, brs	-	8.67, NH, brs

^aobserved in CDCl₃ (Tadtong, 2000).

4.2 Structure elucidation of 17-O-demethylgeldanamycin (SPC43004a)

Compound SPC43004a was obtained as orange needles with optical rotation $[\alpha]_D^{25} +5.34^\circ$ (c, 0.100, MeOH). The UV absorption spectrum in MeOH of compound FPC43001a (Figure 12) showed maximal absorption (ϵ) at 235 (20,219) and 315 (23,615) nm. The IR absorption spectrum (Figure 13) displayed characteristic band at $3,441\text{ cm}^{-1}$ (O-H stretching), $1,697\text{ cm}^{-1}$ (C=O stretching) and $1,651\text{ cm}^{-1}$ (C=O stretching, amide I band). The ESI-TOF MS of this compound exhibited the pseudomolecular ion peak $[M+Na]^+$ (Figure 14) at m/z 569.29, thus the molecular formula of this compound should be $C_{28}H_{38}N_2O_9$ with the MW 546. The 300 MHz $^1\text{H-NMR}$ spectrum of compound SPC43004a in $\text{CDCl}_3 + \text{DMSO-}d_6$ (Figure 15) presented four methyl proton signals at δ 0.77, 0.92, 1.65 and 1.91 ppm; two methoxy proton signals at δ 3.21 and 3.23 ppm; two methylene proton signals at δ 1.54 and 2.28 ppm; eleven methine proton signals at δ 1.86, 2.62, 3.32, 3.55, 4.24, 4.97, 5.65, 5.80, 6.45, 6.92 and 7.11 ppm; and two exchangeable proton signals at δ 5.40 and 8.92 ppm. The 75 MHz $^{13}\text{C-NMR}$ spectrum of compound SPC43004a in $\text{CDCl}_3 + \text{DMSO-}d_6$ (Figure 16) gave twenty-eight carbon signals.

The ^1H and $^{13}\text{C-NMR}$ spectral data of SPC43004a as summarized in Table 23 indicated that this compound is structurally similar to geldanamycin. The absence of 17-OMe of this compound was identical to 17-O-demethylgeldanamycin (Figure 11) which was previously reported as a demethylation product of geldanamycin by the reaction with barium hydroxide ($\text{Ba}(\text{OH})_2$) (Rinehart, Jr. and Shield, 1976), and found for the first time as a naturally occurring substance produced by the mangrove *Streptomyces* sp. TRA 9875-2 (Tadtong, 2000).

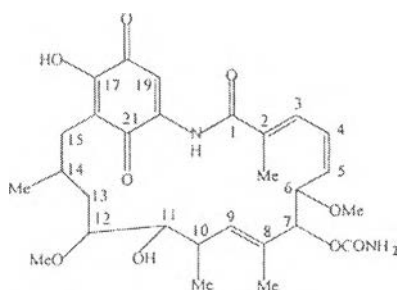


Figure 11 Structure of compound SPC43004a

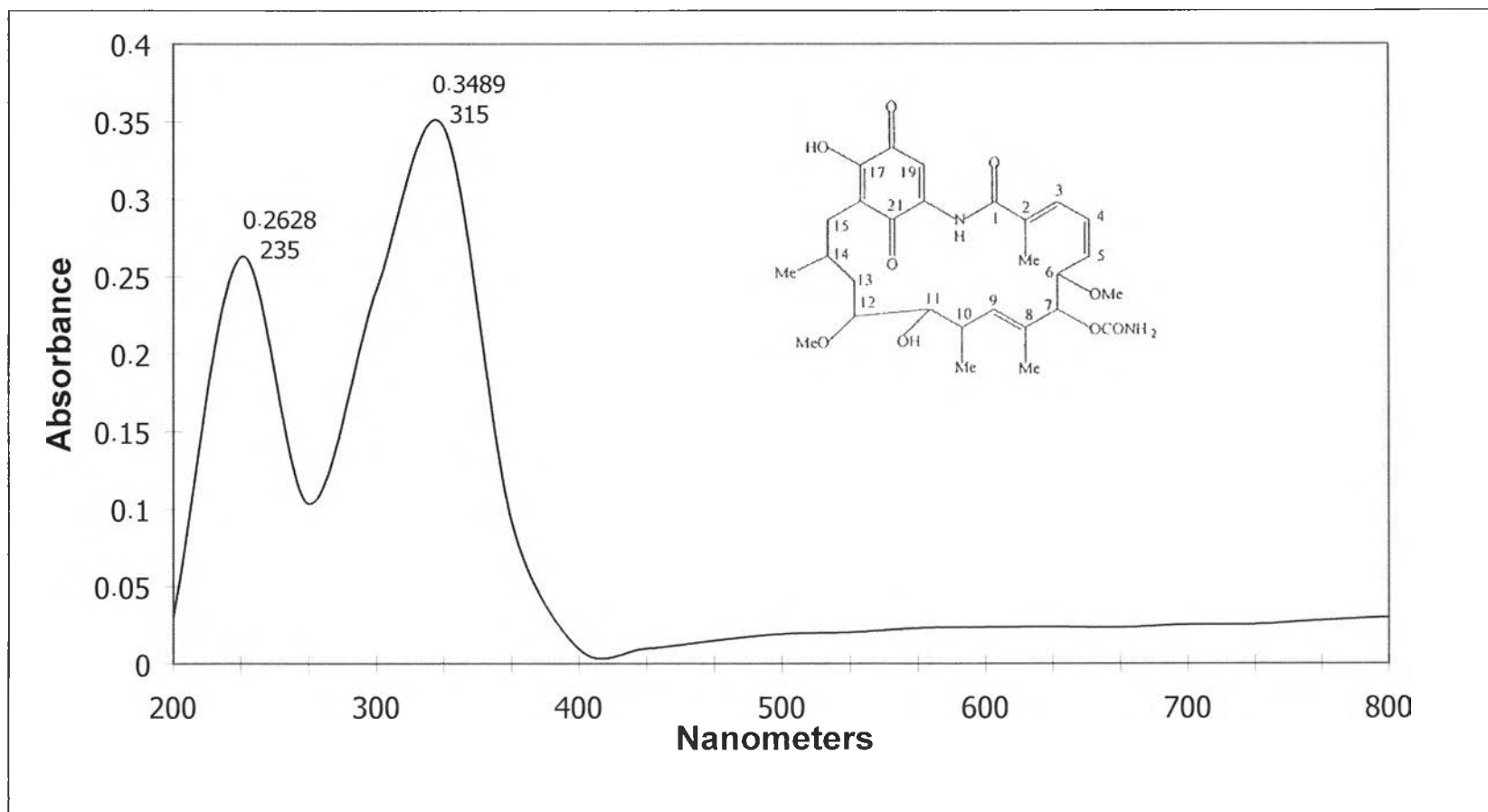


Figure 12 The UV spectrum of 17-O-demethylgeldanamycin (SPC43004a)

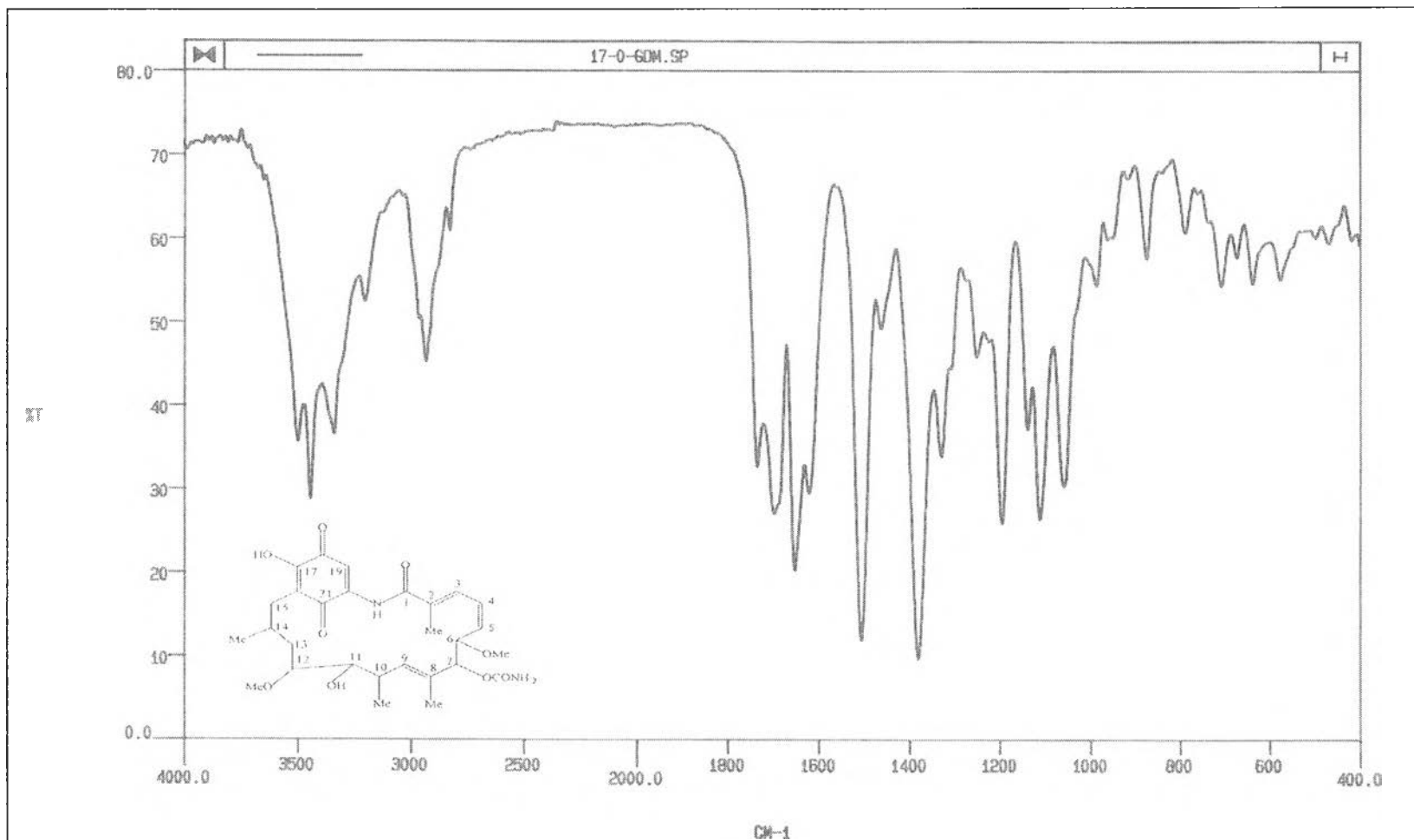


Figure 13 The IR spectrum of 17-O-demethylgeldanamycin (SPC43004a)

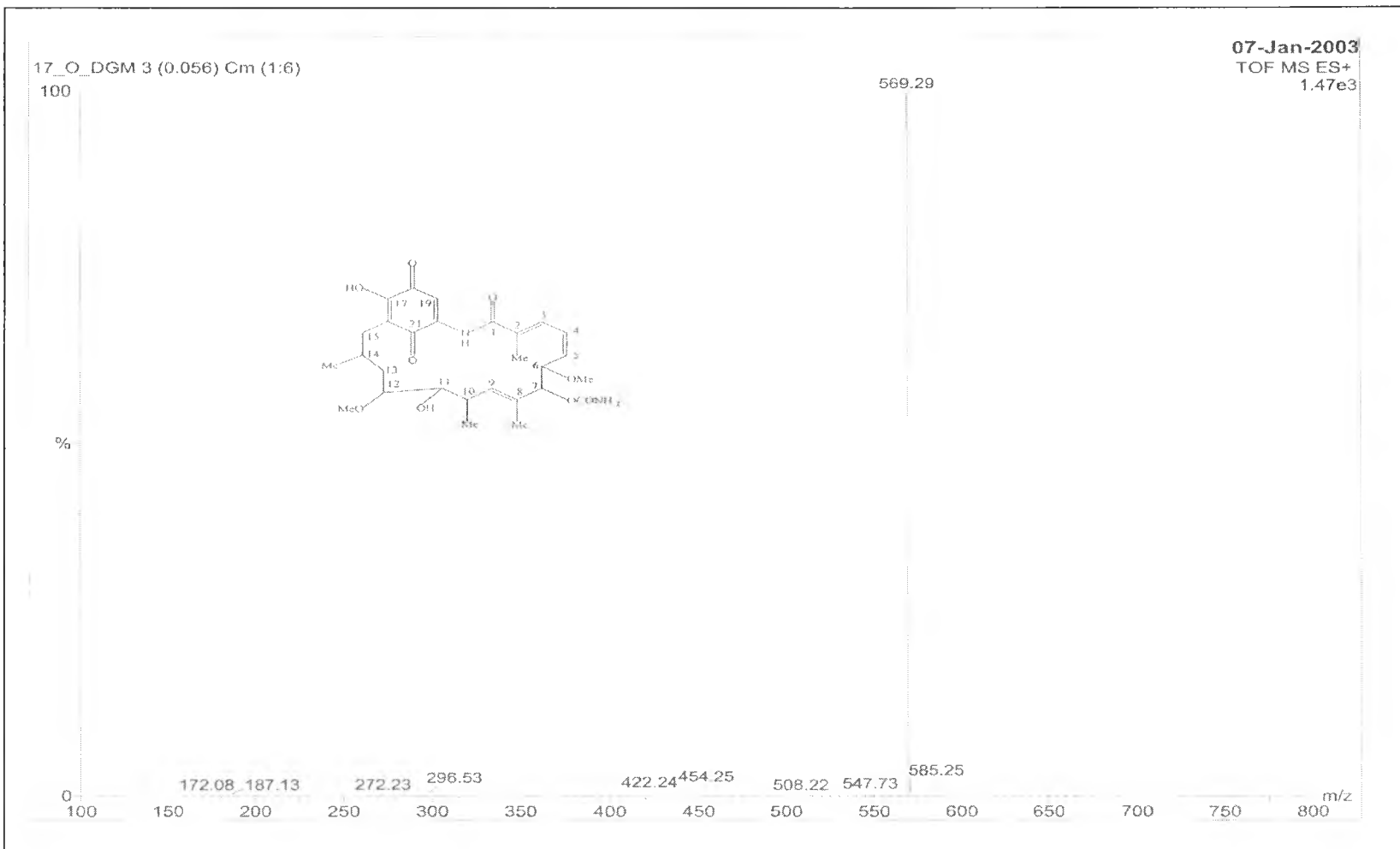


Figure 14 The ESI-TOF mass spectrum of 17-O-demethylgeldanamycin (SPC43004a)

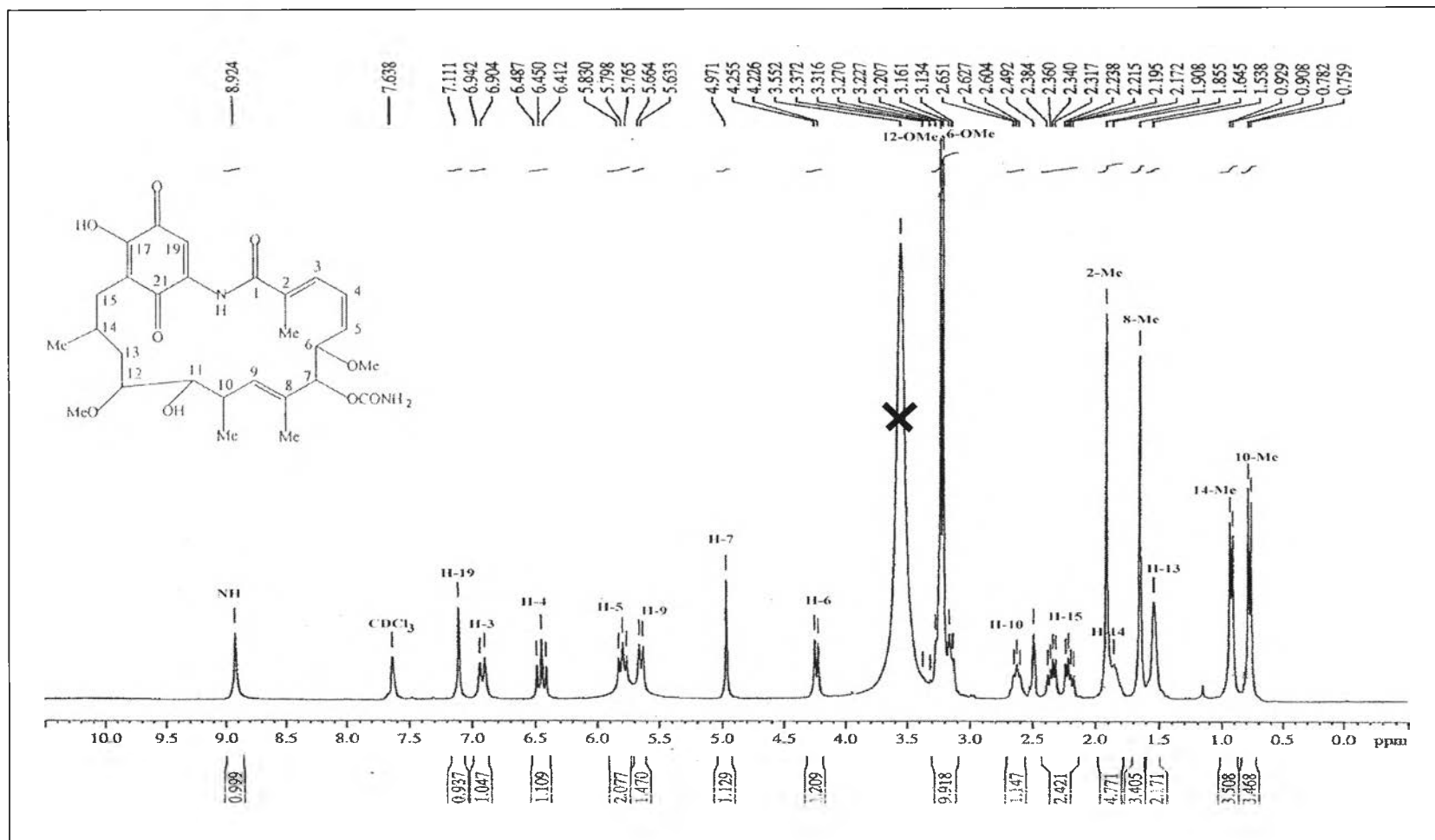


Figure 15 ¹H NMR spectrum (300 MHz) of 17-O-demethylgeldanamycin (SPC43004a) in CDCl₃ + DMSO-d₆

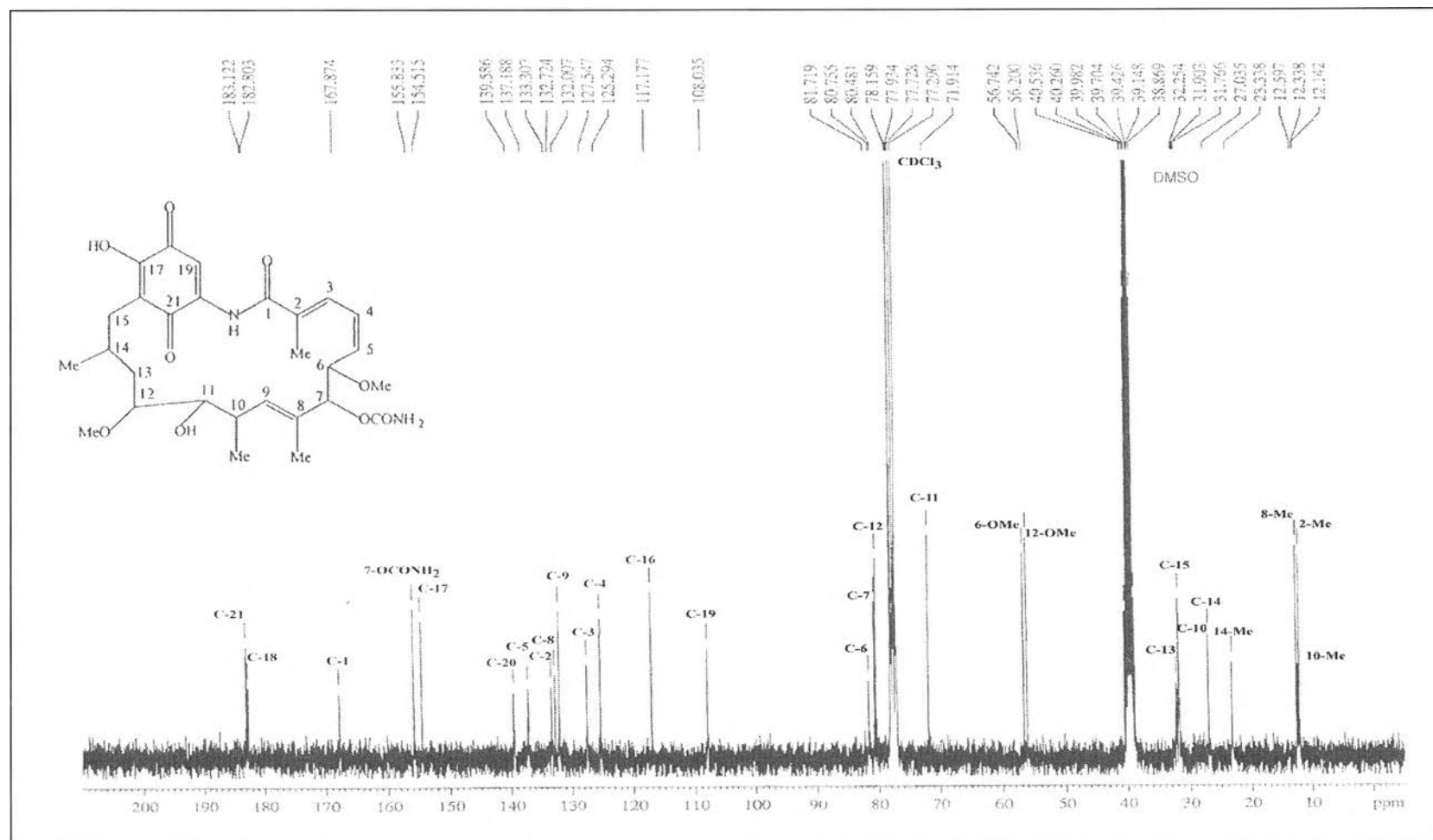


Figure 16 ¹³C NMR spectrum (75 MHz) of 17-O-demethylgeldanamycin (SPC43004a) in CDCl₃ + DMSO-d₆

Table 23 Comparison of ^1H and ^{13}C -NMR spectral data of SPC43004a with that of 17-O-demethylgeldanamycin

Position	SPC43004a		17-O-demethylgeldanamycin ^a	
	δ_c (ppm)	δ_H (ppm), mult, (J in Hz)	δ_c (ppm)	δ_H (ppm), mult, (J in Hz)
1	167.87	-	167.82	-
2	133.30	-	133.62	-
2-Me	12.14	1.91, 3H, s	12.19	1.92, 3H, s
3	127.55	6.92, d, (12)	127.28	6.91, d, (12)
4	125.29	6.45, t, (12, 11)	125.48	6.46, t, (12, 11)
5	137.18	5.80, t, (11, 9)	136.78	5.80, t, (11, 9)
6	81.71	4.24, d, (9)	81.48	4.23, d, (9)
6-OMe	56.74	3.21, 3H, s	56.85	3.20, 3H, s
7	80.75	4.97, brs	80.97	5.01, brs
7-OCONH ₂	155.83	5.40, NH ₂ , brs	155.77	5.43, NH ₂ , brs
8	132.72	-	132.74	-
8-Me	12.60	1.65, 3H, s	12.60	1.67, 3H, s
9	132.00	5.65, d, (9)	132.28	5.69, d, (9)
10	31.76	2.62, m	31.87	2.69, m
10-Me	12.33	0.77, 3H, d, (7)	12.19	0.81, 3H, d, (7)
11	71.91	3.55, m	71.99	3.49, m
12	80.48	3.32, m	80.53	3.33, m
12-OMe	56.20	3.23, 3H, s	56.29	3.24, 3H, s
13	32.25	1.54, 2H, brs	33.09	1.60, 2H, brs
14	27.03	1.86, m	27.31	1.77, m
14-Me	23.34	0.92, 3H, d, (6)	23.22	0.91, 3H, d, (6)
15	31.90	2.20, 2.35, 2H, m	32.06	2.31, 2H, m
16	117.18	-	117.26	-
17	154.52	-	155.77	-
18	182.80	-	183.16	-
19	108.04	7.11, s	108.18	7.17, s
20	139.59	-	139.50	-
21	183.12	-	183.16	-
NH	-	8.92, NH, brs	-	8.90, NH, brs

^a Data were observed in CDCl₃+ DMSO-*d*₆ (Tadtong, 2000).

4.3 Antimicrobial activity of the isolated compounds

The results of antimicrobial activity of the liquor (YMPC 4-3) and two isolated compounds, geldanamycin (FPC43001a) and 17-O-demethylgeldanamycin (SPC43004a), which obtained from the strain PC 4-3 are shown in Table 24

Table 24 Antimicrobial activity from the mother liquor, isolated geldanamycin and 17-O-demethylgeldanamycin of the strain PC 4-3

Sample	Inhibition zone (mm.)		
	<i>S. aureus</i> ATCC 25923	<i>B. subtilis</i> ATCC 6633	<i>C. albicans</i> ATCC10231
YMPC 4-3	37.0	29.0	30.0
FPC43001a	12.2	-	20.0
SPC43004a	-	-	-

0.5 mg/disc.

The mother liquor (YMPC 4-3) displayed strong antimicrobial activity against all tested microorganisms. Geldanamycin (FPC43001a) showed mild antimicrobial activity against *S. aureus* ATCC 25923, and *C. albicans* ATCC 10231, while 17-O-demethylgeldanamycin (SPC43004a) exhibited no antimicrobial activity.

Higher potency of the mother liquor than the isolated geldanamycin suggested that the mother liquor might contain other interesting compounds having stronger antimicrobial activity than the isolated geldanamycin. Therefore, further investigation of the mother liquor should be studied.

Geldanamycin has been previously isolated from the terrestrial *Streptomyces hygroscopicus* var. *geldanus* in 1970 displaying moderately activity against protozoa, bacteria and fungi (DeBoer *et al.*, 1970).