CHAPTER IV

RESULTS AND DISCUSSION

1. Isolation and primary screening of actinomycetes

1.1 Isolation of the strains

Ninety eight soil samples were collected from Chiangrai (15 samples), Nan (50 samples), Phatthalung (9 samples), Satun (3 samples), Songkhla (3 samples), Chaiyaphum (10 samples), and Trat (8 samples) provinces, Thailand. A total of 127 actinomycetes were isolated; 24 strains from Chiangrai, 54 strains from Nan, 14 strains from Phatthalung, 3 strains from Satun, 4 strains from Songkhla, 7 strains from Chaiyaphum, and 21 strains from Trat. All strains were cultivated on YMA and kept in cold room at 4 $^{\circ}$C. Sources of samples, pH, date of isolation and strain number were shown in Table 4.1.

Table 4.1 Sources of soil samples, pH, date of isolation and strain number

Sample	Source	pH	Date of isolation	Strain number
Soil	Chiangrai	7.5	10 May 2006	S1-2
Soil	Chiangrai	7.0	10 May 2006	K2-1, K2-2
Soil	Chiangrai	7.0	10 May 2006	S3-1, S3-2, K3-1
Soil	Chiangrai	6.8	10 May 2006	S4-6
Soil	Chiangrai	7.0	10 May 2006	S7-1, K7-1
Soil	Chiangrai	7.0	10 May 2006	S9-1, S9-2, S9-3, S9-4
Soil	Chiangrai	7.5	10 May 2006	S10-1
Soil	Chiangrai	7.0	10 May 2006	S11-1, S11-2, S11-3
Soil	Chiangrai	6.8	10 May 2006	S12-1
Soil	Chiangrai	7.5	10 May 2006	S 13-1, S 13-3, S 13-4, S 13-5, S 13-6, K 13-1
Soil	Nan	7.0	5 July 2006	S 31-1
Soil	Nan	7.5	5 July 2006	S 32-1, S 32-2, S 32-5

Table 4.1 Sources of soil samples, pH, date of isolation and strain number (Continued)

Sample	Source	pH	Date of isolation	Strain number
Soil	Nan	7.0	5 July 2006	S 33-1, S 33-2, S 33-3, S 33-4
Soil	Nan	8.0	5 July 2006	S 35-5
Soil	Nan	6.8	12 July 2006	SB 3-2
Soil	Nan	7.0	12 July 2006	SB 4-1, KB 4-1
Soil	Nan	7.0	12 July 2006	SB 5-2
Soil	Nan	7.5	12 July 2006	SB 7-3
Soil	Nan	7.5	12 July 2006	SB 9-1, SB 9-3
Soil	Nan	7.0	12 July 2006	SB 10-1
Soil	Nan	7.0	12 July 2006	SB 12-1
Soil	Nan	7.0	12 July 2006	SC 13-3, SC 13-4, SC 13-5, SC 13-7, SC 13-9
Soil	Nan	7.5	20 July 2006	SC 18-2
Soil	Nan	7.0	20 July 2006	SC 16-1, SC 16-2
Soil	Nan	6.8	20 July 2006	SC 19-1, KC 19-1, KC 19-2
Soil	Nan	7.0	20 July 2006	KC 20-1
Soil	Nan	7.0	27 July 2006	S 1-5-6
Soil	Nan	7.0	27 July 2006	S 3-1-2, S 3-1-4, S 3-1-5, S 3-1-6
Soil	Nan	7.0	27 July 2006	S 3-2-1, S 3-2-2, S 3-2-5, S 3-2-6
Soil	Nan	7.5	27 July 2006	S 3-3-1, S 3-3-2, S3-3-5, S3-3-6
Soil	Nan	6.8	27 July 2006	S 3-4-4
Soil	Nan	6.8	27 July 2006	S 3-7-4, S 3-7-5
Soil	Nan	7.0	27 July 2006	S 4-1-1
Soil	Nan	6.8	27 July 2006	S 4-2-1
Soil	Nan	7.0	27 July 2006	S 4-2-2
Soil	Nan	7.0	27 July 2006	S 4-5-2, S 4-5-3
Soil	Nan	7.0	27 July 2006	S 4-6-1, S 4-6-2
Soil	Nan	7.5	27 July 2006	S 4-9-1
Soil	Phatthalung	7.0	1 August 2006	S 38-2, K 38-2

Table 4.1 Sources of soil samples, pH, date of isolation and strain number (Continued)

Sample	Source	pH	Date of isolation	Strain number
Soil	Phatthalung	7.0	1 August 2006	S39-7, K 39-4
Soil	Phatthalung	6.8	1 August 2006	S 40-1
Soil	Phatthalung	7.5	1 August 2006	S 42-1, S 42-2
Soil	Phatthalung	7.0	1 August 2006	S 45-4, K 45-2, K 45-3, K 45-5, K 45-6, K 45-9, K 45-10
Soil	Satun	7.5	1 August 2006	S 47-4
Soil	Satun	7.5	1 August 2006	S 48-2, S 48-4
Soil	Songkhla	7.0	1 August 2006	S 49-1, S 49-3, S 49-4, S 49-6
Soil	Chaiyaphum	7.5	1 August 2006	S 55-2, S 55-4
Soil	Chaiyaphum	7.5	1 August 2006	S 57-1, K 57-1, K 57-3
Soil	Chaiyaphum	7.5	1 August 2006	S 65-3
Soil	Chaiyaphum	7.5	1 August 2006	S 68-2
Soil	Trat	7.5	1 December 2006	S 70-2, S 70-4, S 70-5
Soil	Trat	7.0	1 December 2006	S 71-1, S 71-2
Soil	Trat	7.0	1 December 2006	S 72-10, S 72-11, S 72-12, S 72-15
Soil	Trat	7.5	1 December 2006	S 74-1, S 74-5, S 74-6, S74-7
Soil	Trat	7.0	1 December 2006	S 75-1, S 75-3, S 75-4, S 75-5
Soil	Trat	7.5	1 December 2006	S 76-1, S 76-6
Soil	Trat	7.0	1 December 2006	S 77-2, S 77-3

1.2 Primary screening for the antimicrobial activity of the strains.

Forty eight strains showed an inhibitory activity against *Staphylococcus aureus* ATCC 6538, 47 strains against *Bacillus subtilis* ATCC 6633, 16 strains against *Escherichia coli* ATCC 25922, 28 strains against *Micrococcus luteus* ATCC 9341, 21 strains against *Pseudomonas aeruginosa* ATCC 27853, and 18 strains against *Candida albicans* ATCC 10231 as shown in Table 4.2. Eighteen strains that showed antimicrobial activity against many test microorganisms or exhibited an inhibition zone of primary screening at least 20.1 mm (+++) against one test microorganism, were selected from 78 active strains for further study.

Table 4.2 Antimicrobial activity of actinomycetes strains

Strain no.	Inhibition zone					
	S. aureus ATCC 6538	*B. subtilis* ATCC 6633	*E. coli* ATCC 25922	*M. luteus* ATCC 9341	*Ps. aeruginosa* ATCC 27853	*C. albicans* ATCC 10231
S 1-2	++++	++++	+	++++	+	+++
K 2-1	-	-	-	-	-	-
K 2-2	-	-	-	-	-	-
S 3-1	++++	++++	-	++++	+	-
K 3-1	-	-	±	-	-	-
S 3-2	+++	++++	-	+++	±	-
S 4-6	-	-	-	±	-	-
S 7-1	-	-	-	-	-	-
K 7-1	-	-	-	-	-	-
S 9-1	-	±	-	-	-	+
S 9-2	-	-	-	-	-	-
S 9-3	-	++	-	-	-	-
S 9-4	-	++	-	-	±	+
S 10-1	-	-	-	++	-	-
S 11-1	-	-	-	-	-	-

Table 4.2 Antimicrobial activity of actinomycetes strains (Continued)

Strain no.	Inhibition zone					
	S. aureus ATCC 6538	*B. subtilis* ATCC 6633	*E. coli* ATCC 25922	*M. luteus* ATCC 9341	*Ps. aeruginosa* ATCC 27853	*C. albicans* ATCC 10231
S 11-2	-	-	-	-	-	±
S 11-3	+++	-	-	-	-	-
S 12-1	±	-	-	-	-	-
S 13-1	-	-	-	-	-	-
K 13-1	-	+++	-	-	-	-
S 13-3	±	-	-	±	-	-
S 13-4	-	++	-	-	-	-
S 13-5	-	±	-	-	-	-
S 13-6	-	-	-	-	-	-
S 31-1	+++	-	-	-	-	-
S 32-1	-	-	-	-	-	-
S 32-2	-	-	-	-	-	-
S 32-5	-	-	-	++	-	-
S 33-1	++++	-	-	-	-	-
S 33-2	-	-	-	-	-	-
S 33-3	++++	+++	-	-	-	-
S 33-4	-	-	-	-	-	-
S 35-5	-	-	-	-	±	-
SB 3-2	+++	+++	-	++++	+	-
SB 4-1	-	-	-	-	-	-
KB 4-1	±	+++	±	-	-	±
SB 5-2	±	-	-	-	-	-
SB 7-3	+++	++	-	+++	-	-
SB 9-1	-	-	-	-	-	-
SB 9-3	±	-	±	-	-	++
SB 10-1	-	-	-	-	+	-

Table 4.2 Antimicrobial activity of actinomycetes strains (Continued)

Strain no.	Inhibition zone					
	S. *aureus* ATCC 6538	*B. subtilis* ATCC 6633	*E. coli* ATCC 25922	*M. luteus* ATCC 9341	*Ps. aeruginosa* ATCC 27853	*C. albicans* ATCC 10231
SB 12-1	+ +++	++++	-	++++	+	-
SC 13-3	±	±	-	-	-	-
SC 13-4	-	-	-	-	-	-
SC 13-5	-	-	-	-	-	-
SC 13-7	-	-	±	-	±	-
SC 13-9	±	-	-	-	-	±
SC 16-1	+++	-	-	-	±	-
SC 16-2	+++	-	++	-	-	-
SC 18-2	-	-	-	-	-	-
SC 19-1	-	-	-	-	-	-
KC 19-1	+++	-	-	-	-	-
KC 19-2	-	++	-	-	-	-
KC 20-1	++	++	-	++++	+ +	-
S 1-5-6	-	-	±	+	-	±
S 3-1-2	±	++	-	+++	-	-
S 3-1-4	±	-	-	-	-	-
S 3-1-5	-	++	-	-	-	-
S 3-1-6	-	-	-	-	±	-
S 3-2-1	+++	-	+++	-	-	-
S 3-2-2	++++	-	-	-	-	-
S 3-2-5	-	-	-	-	-	-
S 3-2-6	-	-	-	-	-	-
S 3-3-1	+++	+	-	-	-	-
S 3-3-2	-	-	-	-	-	-
S 3-3-5	-	-	-	-	-	-
S 3-3-6	-	-	-	-	-	-

Table 4.2 Antimicrobial activity of actinomycetes strains (Continued)

Strain no.	Inhibition zone					
	S. *aureus* ATCC 6538	*B. subtilis* ATCC 6633	*E. coli* ATCC 25922	*M. luteus* ATCC 9341	*Ps. aeruginosa* ATCC 27853	*C. albicans* ATCC 10231
S 3-4-4	-	-	-	-	-	-
S 3-7-4	+++	-	-	-	+	++
S 3-7-5	±	-	-	-	-	-
S 4-1-1	-	++	±	-	-	-
S 4-2-1	+ ++	±	-	+	-	-
S 4-2-2	-	-	-	-	-	-
S 4-5-2	-	-	-	-	-	-
S 4-5-3	±	++++	-	+++	-	++
S 4-6-1	+ +	-	-	-	-	-
S 4-6-2	-	-	-	-	-	-
S 4-9-1	-	-	-	-	-	-
S 38-2	±	+++	-	++++	+	-
K 38-2	-	-	-	-	-	-
K 39-4	+	++	-	+++	±	-
S 39-7	++++	++++	++++	++++	+	-
S 40-1	-	-	-	-	-	-
S 42-1	-	-	-	-	-	-
S 42-2	-	+++	-	-	-	-
K 45-3	-	+++	-	+++	-	+
K 45-2	-	-	-	-	-	-
S 45-4	-	-	-	-	-	-
K 45-5	+	-	±	+++	-	-
K 45-6	-	++	-	-	-	-
K 45-9	-	-	-	-	-	-
K 45-10	-	++	-	++	-	-
S 47-4	-	-	-	-	-	-

Table 4.2 Antimicrobial activity of actinomycetes strains (Continued)

Strain no.	Inhibition zone					
	S. *aureus* ATCC 6538	*B. subtilis* ATCC 6633	*E. coli* ATCC 25922	*M. luteus* ATCC 9341	*Ps. aeruginosa* ATCC 27853	*C. albicans* ATCC 10231
S 48-2	-	+++	-	-	-	-
S 48-4	+ ++	+++	-	++++	-	-
S 49-1	+++	++++	+	++++	+	-
S 49-3	-	-	-	-	-	-
S 49-4	-	+++	-	-	-	-
S 49-6	-	-	-	-	-	-
S 55-2	-	-	-	-	-	-
S 55-4	++++	+++	-	-	-	+
S 57-1	-	-	-	-	-	-
K 57-1	-	-	++	+++	-	-
K 57-3	-	-	-	-	-	-
S 65-3	-	-	-	-	-	-
S 68-2	-	-	-	-	-	-
S 70-2	-	-	-	-	-	-
S 70-4	++	+++	-	-	-	-
S 70-5	-	-	-	-	-	-
S 71-1	++++	-	-	++	-	+++
S 71-2	-	-	-	-	-	-
S 72-10	++++	++++	-	-	+	-
S 72-11	-	-	-	-	-	-
S 72-12	-	-	-	-	-	-
S 72-15	++	++++	-	-	-	-
S 74-1	-	-	-	-	-	-
S 74-5	-	-	-	-	-	-
S 74-6	-	+++	-	-	+	-
S 74-7	+++	+++	-	-	-	±

Table 4.2 Antimicrobial activity of actinomycetes strains (Continued)

Strain no.	Inhibition zone					
	S. *aureus* ATCC 6538	*B. subtilis* ATCC 6633	*E. coli* ATCC 25922	*M. luteus* ATCC 9341	*Ps. aeruginosa* ATCC 27853	*C. albicans* ATCC 10231
S 75-1	++	+++	-	-	-	±
S 75-3	-	+++	+	-	-	++
S 75-4	±	-	-	-	-	-
S 75-5	++++	++++	+	++++	+	+++
S 76-1	++++	++++	-	-	+	-
S 76-6	-	+++	-	-	-	-
S 77-2	-	+++	±	+	-	-
S 77-3	++	++	-	++	-	++

++++, 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 of strains

2.1 Morphological and cultural characteristics

The morphological and cultural characteristics of 127 strains on YMA plate at 30°C for 14 days were shown in Table 4.3 and 4.4. Spore morphological study showed that the spore chains from 21 strains were spiral, 103 strains were rectiflexibiles and 3 strains were rectinaculiaperti. Spore color of 127 strains were black (2 strains), gray (29 strains), red (5 strains), white (59 strains), yellow (5 strains), pale beige (11 strains), orange (3 strains), brown (5 strains), gold (2 strains) and purplish pink (6 strains). The spore color of strain S1-2 and S75-5 grew on YMA medium were initially white and turned into dark during incubation for 14 days.Twenty one strains could produce soluble pigment including light brown (6 strains), brown (9 strains), orange yellow (3 strains), bright yellow (2 strain), and dark red (1 strains). Colonies on agar media showed the presence of powdery colonies in 90 strains, granular colonies in 31 strains, and velvety colonies in 6 strains. The cultural characteristics of 127 strains on various media after incubation at 30°C for 14 days showed that all strains grew better on Yeast Extract- Malt Extract Agar, and Oatmeal agar than Tyrosine, Glycerol-Asparagine agar, and Inorganic salt-Starch agar.

Colonial appearance and scanning electron micrograph of the strains S3-1, S38-2, S72-10, S75-5, SB7-3, S39-7, KC19-1, KC20-1, K57-1, SB3-2 cultivated on YMA medium after incubation at 30°C for 14 days were shown in Figures 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 4.10, respectively.

Table 4.3 Morphological and cultural characteristics of the strains on YMA after 14 days incubation

Strain no.	Spore chain	Spore color	Soluble pigment	Colonial appearance	Colony color	
					Upper colony	Lower colony
S 1-2	S	Black	Light brown	Powdery	Pale beige	Pale beige
K 2-1	R	Graynish white	-	Granular	Graynish white	Dark brown
K 2-2	R	Graynish yellow	-	Granular	Graynish yellow	Dark brown
S 3-1	R	Vivid red	-	Velvety	Vivid red	Vivid red
K 3-1	R	Yellowish gray	-	Granular	Yellowish gray	Dark brown
S 3-2	R	Vivid red	-	Velvety	Vivid red	Vivid red
S 4-6	S	Strong yellow	-	Granular	Strong yellow	Pale yellow
S 7-1	R	Pinkish white	-	Granular	Pinkish white	Dark brown
K 7-1	R	Pale beige	Orange yellow	Granular	Pale beige	Orange yellow
S 9-1	R	Yellowish white	-	Granular	Yellowish white	Gold
S 9-2	R	Brownish white	-	Powdery	Brownish white	Brown
S 9-3	R	Pinkish white	-	Granular	Pinkish white	Gold
S 9-4	R	Brownish white	-	Granular	Brownish white	Brownish gold
S 10-1	S	Brownish white	-	Granular	Brownish white	Pale beige
S 11-1	R	Yellowish white	-	Granular	Yellowish white	Pale yellow
S 11-2	R	Pale beige	-	Velvety	Pale beige	Pale beige
S 11-3	R	Brownish white	-	Granular	Brownish white	Gold
S 12-1	R	Yellowish white	-	Powdery	Yellowish white	Gold
S 13-1	S	Yellowish white	-	Powdery	Yellowish white	Pale beige
K 13-1	R	Grayish white	-	Powdery	Grayish white	Pale beige
S 13-3	R	Grayish white	-	Velvety	Grayish white	Pale beige
S 13-4	R	Yellowish white	-	Granular	Yellowish white	Pale beige
S 13-5	R	Brownish white	-	Powdery	Brownish white	Pale beige
S 13-6	R	Grayish white	-	Powdery	Grayish white	Gold
S 31-1	S	Grayish white	-	Powdery	Grayish white	Pale beige
S 32-1	R	Yellowish white	-	Powdery	Yellowish white	Brown

Table 4.3 Morphological and cultural characteristics of the strains on YMA after 14 days incubation (continued)

Strain no.	Spore chain	Spore color	Soluble pigment	Colonial appearance	Colony color	
					Upper colony	Lower colony
S 32-2	R	Light gray	-	Powdery	Light gray	Pale beige
S 32-5	R	Yellowish gray	Brown	Powdery	Yellowish gray	Dark reddish brown
S 33-1	R	Light medium gray	-	Powdery	Light medium gray	Yellowish brown
S 33-2	R	Yellowish gray	Brown	Granular	Yellowish gray	Brownish gold
S 33-3	R	Grayish white	-	Powdery	Grayish white	Pale beige
S 33-4	R	Grayish white	Light brown	Powdery	Grayish white	Brown
S 35-5	R	Medium gray	-	Powdery	Medium gray	Light brown
SB 3-2	R	Medium gray	Brown	Powdery	Medium gray	Grayish brown
SB 4-1	S	Grayish white	-	Powdery	Grayish white	Gold
KB 4-1	R	Medium gray	-	Granular	Medium gray	Grayish brown
SB 5-2	S	Pale beige	-	Powdery	Pale beige	Pale beige
SB 7-3	R	Pinkish white	Light brown	Powdery	Pinkish white	Dark red
SB 9-1	R	Pale beige	-	Powdery	Pale beige	Pale beige
SB 9-3	R	Yellowish white	-	Granular	Yellowish white	Gold
SB 10-1	R	Pale beige	-	Powdery	Pale beige	Gold
SB 12-1	R	Vivid red	-	Velvety	Vivid red	Vivid red
SC 13-3	RC	Pale beige	-	Granular	Pale beige	Pale beige
SC 13-4	S	Yellowish white	-	Powdery	Yellowish white	Pale beige
SC 13-5	R	Pale beige	-	Powdery	Yellowish white	Pale beige
SC 13-7	R	Gold	-	Powdery	Gold	Light brown
SC 13-9	RC	Deep orange	-	Powdery	Deep orange	Pale beige
SC 16-1	R	Deep orange	-	Granular	Deep orange	Gold
SC 16-2	R	Strong yellow	-	Granular	Strong yellow	Gold
SC 18-2	S	Medium gray	Brown	Powdery	Medium gray	Reddish brown
SC 19-1	R	Bright yellow	Orange yellow	Powdery	Bright yellow	Gold
KC19-1	R	Brownish white	-	Powdery	Brownish white	Pale beige

Table 4.3 Morphological and cultural characteristics of the strains on YMA after 14 days incubation (continued)

Strain no.	Spore chain	Spore color	Soluble pigment	Colonial appearance	Colony color	
					Upper colony	Lower colony
KC19-2	R	Pinkish white	Bright yellow	Powdery	Pinkish white	Yellowish pink
KC20-1	R	Yellowish white	-	Powdery	Yellowish white	Vivid orange
S 1-5-6	R	Yellowish gray	-	Powdery	Yellowish gray	Pale beige
S 3-1-2	S	Strong yellow	Bright yellow	Powdery	Strong yellow	Vivid yellow
S 3-1-4	S	Grayish white	-	Powdery	Grayish white	Brownish gold
S 3-1-5	S	Pale beige	-	Powdery	Pale beige	Bright yellow
S 3-1-6	S	Medium gray	Orange yellow	Powdery	Medium gray	Gold
S 3-2-1	R	Vivid orange	-	Granular	Pale beige	Pale beige
S 3-2-2	R	Yellowish brown	-	Granular	Yellowish brown	Brown
S 3-2-5	R	Grayish yellow	-	Powdery	Grayish yellow	Light brown
S 3-2-6	R	Light gray	-	Powdery	Light gray	Pale beige
S 3-3-1	RC	Strong yellow	-	Powdery	Strong yellow	Strong yellowish pink
S 3-3-2	R	Yellowish white	-	Powdery	Yellowish white	Pale beige
S 3-3-5	R	Deep purplish red	-	Granular	Deep purplish red	Deep red purple
S 3-3-6	R	Medium gray	-	Powdery	Medium gray	Grayish brown
S 3-4-4	R	Medium gray	Brown	Powdery	Medium gray	Yellowish brown
S 3-7-4	S	Brownish white	-	Granular	Brownish white	Pale beige
S 3-7-5	S	Brown	-	Powdery	Brown	Brown
S 4-1-1	R	Grayish white	-	Powdery	Grayish white	Pale beige
S 4-2-1	R	Medium gray	-	Powdery	Medium gray	Brown
S 4-2-2	R	Dark yellowish brown	Brown	Powdery	Dark yellowish brown	Dark brown
S 4-5-2	R	Light gray	-	Powdery	Light gray	Pale yellow
S 4-5-3	R	Medium gray	-	Powdery	Medium gray	Pale beige
S 4-6-1	R	Bluish gray	-	Powdery	Bluish gray	Gold
S 4-6-2	R	Light medium gray	-	Powdery	Light medium gray	Pale beige

Table 4.3 Morphological and cultural characteristics of the strains on YMA after 14 days incubation (continued)

Strain no.	Spore chain	Spore color	Soluble pigment	Colonial appearance	Colony color	
					Upper colony	Lower colony
S 4-9-1	R	Pale beige	-	Powdery	Pale beige	Gold
S 38-2	R	Light medium gray	-	Powdery	Light medium gray	Yellowish brown
K 38-2	R	Pinkish white	-	Powdery	Pinkish white	Brown
K 39-4	R	Grayish white	-	Powdery	Grayish white	Medium brown
S 39-7	R	Pinkish white	Dark red	Powdery	Pinkish white	Vivid purplish red
S 40-1	S	Light gray	Brown	Powdery	Light gray	Brown
S 42-1	S	Yellowish white	-	Powdery	Yellowish white	Pale beige
S 42-2	R	Grayish white	-	Powdery	Grayish white	Pale beige
K 45-3	R	Grayish white	-	Powdery	Grayish white	Pale beige
K 45-2	R	Brownish white	-	Powdery	Brownish white	Yellowish brown
S 45-4	R	Light bluish gray	-	Powdery	Light bluish gray	Brown
K 45-5	R	Pale beige	-	Powdery	Pale beige	Pale beige
K 45-6	R	Brownish white	-	Powdery	Brownish white	Light brown
K 45-9	R	Yellowish white	-	Powdery	Yellowish white	Pale beige
K 45-10	R	Pale beige	-	Powdery	Pale beige	Light brown
S 47-4	S	Grayish white	-	Powdery	Grayish white	Pale beige
S 48-2	R	Purplish pink	-	Powdery	Purplish pink	Pale beige
S 48-4	R	Pinkish white	-	Powdery	Pinkish white	Brownish gold
S 49-1	R	Purplish pink	-	Granular	Purplish pink	Vivid purplish red
S 49-3	R	Brownish white	-	Powdery	Brownish white	Pale beige
S 49-4	R	Yellowish white	-	Powdery	Yellowish white	Brown
S 49-6	R	Grayish white	-	Powdery	Grayish white	Pale beige
S 55-2	R	Grayish white	-	Powdery	Grayish white	Pale beige
S 55-4	R	Brownish white	-	Powdery	Brownish white	Light brown
S 57-1	R	Grayish pink	-	Powdery	Grayish pink	Pale beige

Table 4.3 Morphological and cultural characteristics of the strains on YMA after 14 days incubation (continued)

Strain no.	Spore chain	Spore color	Soluble pigment	Colonial appearance	Colony color	
					Upper colony	Lower colony
K 57-1	R	Yellowish white	-	Powdery	Yellowish white	Pale beige
K 57-3	R	Brownish white	-	Powdery	Brownish white	Light brown
S 65-3	R	Gold	-	Granular	Gold	Pale beige
S 68-2	S	Yellowish white	-	Powdery	Yellowish white	Pale beige
S 70-2	R	Yellowish gray	-	Powdery	Yellowish gray	Pale beige
S 70-4	R	Brownish white	-	Granular	Brownish white	Brownish gold
S 70-5	R	Light gray	-	Powdery	Light gray	Yellowish brown
S 71-1	R	Yellowish white	-	Granular	Yellowish white	Pale beige
S 71-2	R	Light medium gray	-	Powdery	Light medium gray	Pale beige
S 72-10	R	Purplish pink	Brown	Powdery	Purplish pink	Light brown
S 72-11	R	Yellowish brown	Light brown	Powdery	Yellowish brown	Brown
S 72-12	R	Brownish white	-	Powdery	Brownish white	Pale beige
S 72-15	R	Purplish pink	-	Granular	Purplish pink	Pale beige
S 74-1	R	Light brown	-	Granular	Light brown	Pale beige
S 74-5	R	Purplish pink	-	Powdery	Purplish pink	Pale beige
S 74-6	R	Light medium gray	-	Powdery	Light medium gray	Brownish gold
S 74-7	R	Yellowish white	-	Powdery	Yellowish white	Pale beige
S 75-1	R	Yellowish white	-	Granular	Yellowish white	Pale beige
S 75-3	R	Brownish white	-	Granular	Brownish white	Pale beige
S 75-4	R	Vivid red	-	Velvety	Vivid red	Vivid red
S 75-5	S	Black	Light brown	Powdery	Pale beige	Pale beige
S 76-1	R	Purplish pink	Brown	Powdery	Purplish pink	Light brown
S 76-6	R	Yellowish white	-	Powdery	Yellowish white	Pale beige
S 77-2	S	Yellowish white	-	Powdery	Yellowish white	Pale yellow
S 77-3	R	Brownish white	Light brown	Powdery	Brownish white	Brownish gold

R, Rectiflexibiles; RC, Rectinaculiaperti; S, Spiral

Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
S1-2	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
K 2-1	YM	+++	Grayish white	Grayish white	Dark brown
	Tyrosine	++	Yellowish white	Yellowish white	Dark brown
	Oatmeal	+++	Grayish white	Grayish white	Light brown
	Asparagine	++	Yellowish white	Yellowish white	Light brown
	Inorg. salt	++	Yellowish white	Yellowish white	Light brown
K 2-2	YM	+++	Grayish yellow	Grayish yellow	Dark brown
	Tyrosine	++	Grayish brown	Grayish yellow	Dark brown
	Oatmeal	+++	Medium gray	Pale lavender	Light brown
	Asparagine	+++	Medium gray	Light medium gray	Light brown
	Inorg. salt	+++	Medium gray	Light medium gray	Light brown
S 3-1	YM	+++	Vivid red	Vivid red	Vivid red
	Tyrosine	+++	Vivid red	Vivid red	Vivid red
	Oatmeal	+++	Vivid red	Vivid red	Vivid red
	Asparagine	+++	Strong yellowish red	Strong yellowish red	Deep yellowish red
	Inorg. salt	+++	Vivid red	Vivid purplish red	Strong yellowish red
K 3-1	YM	+++	Yellowish gray	Yellowish gray	Dark brown
	Tyrosine	+++	Yellowish gray	Yellowish gray	Dark brown
	Oatmeal	+++	Yellowish gray	Grayish yellow	Brownish gold
	Asparagine	+++	Yellowish gray	Grayish yellow	Dark brown
	Inorg. salt	+++	Yellowish gray	Yellowish gray	Brownish gold
S 3-2	YM	+++	Vivid red	Vivid red	Vivid red
	Tyrosine	+++	Vivid red	Vivid red	Vivid red
	Oatmeal	+++	Vivid red	Vivid red	Vivid red
	Asparagine	+++	Strong yellowish red	Strong yellowish red	Deep yellowish red
	Inorg. salt	+++	Vivid red	Vivid purplish red	Strong yellowish red

Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

(Continued)

Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
S 4-6	YM	+++	Strong yellow	Strong yellow	Pale yellow
	Tyrosine	+++	Strong yellow	Vivid reddish yellow	Pale beige
	Oatmeal	+++	Strong yellow	Strong yellow	Pale beige
	Asparagine	+++	Strong yellow	Vivid reddish yellow	Pale yellow
	Inorg. salt	+++	Strong yellow	Vivid reddish yellow	Pale yellow
S 7-1	YM	+++	Pinkish white	Pinkish white	Dark brown
	Tyrosine	+++	Brownish white	Pinkish white	Dark red
	Oatmeal	+++	Pinkish white	Pinkish white	Dark brown
	Asparagine	+++	Pinkish white	Pinkish white	Dark red
	Inorg. salt	+++	Brownish white	Brownish white	Dark red
K 7-1	YM	+++	Pale beige	Pale beige	Orange yellow
	Tyrosine	+++	Light yellow	Yellowish white	Orange yellow
	Oatmeal	+++	Yellowish white	Yellowish white	Orange yellow
	Asparagine	++	Light yellow	Pale beige	Pale yellow
	Inorg. salt	++	Light yellow	Yellowish white	Pale yellow
S 9-1	YM	+++	Yellowish white	Yellowish white	Gold
	Tyrosine	+++	Yellowish white	Yellowish white	Light brown
	Oatmeal	+++	Yellowish white	Yellowish white	Gold
	Asparagine	+++	Yellowish white	Yellowish white	Brown
	Inorg. salt	+++	Yellowish white	Yellowish white	Gold
S 9-2	YM	+++	Brownish white	Brownish white	Brown
	Tyrosine	++	Brownish white	Brownish white	Light brown
	Oatmeal	+++	Brownish white	Brownish white	Pale beige
	Asparagine	++	Brownish white	Yellowish white	Pale beige
	Inorg. salt	+	Brownish white	Yellowish white	Pale beige
S 9-3	YM	+++	Pinkish white	Pinkish white	Gold
	Tyrosine	+++	Brownish white	Grayish white	Brown
	Oatmeal	+++	Brownish white	Pinkish white	Brownish gold
	Asparagine	+++	Brownish white	Pinkish white	Gold
	Inorg. salt	+++	Pinkish white	Pinkish white	Gold

Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

(Continued)

Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
S 9-4	YM	+++	Brownish white	Brownish white	Brownish gold
	Tyrosine	+++	Grayish white	Yellowish white	Gold
	Oatmeal	+++	Brownish white	Yellowish white	Gold
	Asparagine	+++	Grayish white	Brownish white	Gold
	Inorg. salt	+++	Grayish white	Brownish white	Gold
S 10-1	YM	+++	Brownish white	Brownish white	Pale beige
	Tyrosine	++	Brownish white	Brownish white	Pale beige
	Oatmeal	+++	Brownish white	Brownish white	Dull yellow
	Asparagine	++	Brownish white	Yellowish white	Dull yellow
	Inorg. salt	++	Brownish white	Yellowish white	Pale beige
S 11-1	YM	+++	Yellowish white	Yellowish white	Pale yellow
	Tyrosine	+++	Brownish white	Brownish white	Pale yellow
	Oatmeal	+++	Brownish white	Brownish white	Pale yellow
	Asparagine	+++	Yellowish white	Yellowish white	Pale yellow
	Inorg. salt	+++	Yellowish white	Yellowish white	Pale yellow
S 11-2	YM	+++	Pale beige	Pale beige	Pale beige
	Tyrosine	+++	Pale yellow	Pale beige	Pale yellow
	Oatmeal	+++	Pale reddish yellow	Pale beige	Pale yellow
	Asparagine	+++	Pale beige	Pale beige	Pale beige
	Inorg. salt	+++	Pale beige	Pale beige	Pale beige
S 11-3	YM	+++	Brownish white	Brownish white	Gold
	Tyrosine	+++	Brownish white	Brownish white	Brown
	Oatmeal	+++	Brownish white	Brownish white	Brownish gold
	Asparagine	+++	Yellowish white	Yellowish white	Gold
	Inorg. salt	+++	Yellowish white	Yellowish white	Gold
S 12-1	YM	+++	Yellowish white	Yellowish white	Gold
	Tyrosine	+++	Brownish white	Yellowish white	Light brown
	Oatmeal	+++	Yellowish white	Yellowish white	Gold
	Asparagine	+++	Yellowish white	Yellowish white	Dull reddish yellow
	Inorg. salt	+++	Yellowish white	Yellowish white	Dull reddish yellow

Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

(Continued)

Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
S 13-1	YM	+++	Yellowish white	Yellowish white	Pale beige
	Tyrosine	+++	Yellowish white	Yellowish white	Pale beige
	Oatmeal	+++	Yellowish white	Yellowish white	Pale beige
	Asparagine	+++	Pale beige	Yellowish white	Pale beige
	Inorg. salt	+++	Yellowish white	Yellowish white	Pale beige
K 13-1	YM	+++	Grayish white	Grayish white	Pale beige
	Tyrosine	+++	Grayish white	Grayish white	Pale beige
	Oatmeal	+++	Grayish white	Grayish white	Gold
	Asparagine	+++	Grayish white	Grayish white	Light brown
	Inorg. salt	+++	Grayish white	Grayish white	Pale beige
S 13-3	YM	+++	Grayish white	Grayish white	Pale beige
	Tyrosine	+++	Grayish white	Grayish white	Pale yellow
	Oatmeal	+++	Grayish white	Grayish white	Pale beige
	Asparagine	+++	Grayish white	Grayish white	Pale yellow
	Inorg. salt	+++	Grayish white	Grayish white	Pale beige
S 13-4	YM	+++	Yellowish white	Yellowish white	Pale beige
	Tyrosine	+++	Yellowish white	Yellowish white	Pale beige
	Oatmeal	+++	Yellowish white	Yellowish white	Pale beige
	Asparagine	+++	Brownish white	Brownish white	Pale beige
	Inorg. salt	+++	Brownish white	Brownish white	Pale beige
S 13-5	YM	+++	Brownish white	Brownish white	Pale beige
	Tyrosine	+++	Brownish white	Brownish white	Pale beige
	Oatmeal	+++	Brownish white	Brownish white	Pale beige
	Asparagine	+++	Brownish white	Brownish white	Pale beige
	Inorg. salt	+++	Brownish white	Brownish white	Pale beige
S 13-6	YM	+++	Grayish white	Grayish white	Gold
	Tyrosine	+++	Grayish white	Grayish white	Gold
	Oatmeal	+++	Grayish white	Grayish white	Gold
	Asparagine	+++	Grayish white	Grayish white	Light brown
	Inorg. salt	+++	Grayish white	Grayish white	Light brown

Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

(Continued)

Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
S 31-1	YM	+++	Grayish white	Grayish white	Pale beige
	Tyrosine	+++	Grayish white	Grayish white	Pale yellow
	Oatmeal	+++	Grayish white	Grayish white	Pale beige
	Asparagine	+++	Grayish white	Grayish white	Dull yellow
	Inorg. salt	+++	Grayish white	Grayish white	Dull yellow
S 32-1	YM	+++	Yellowish white	Yellowish white	Brown
	Tyrosine	+++	Yellowish white	Yellowish white	Brownish gold
	Oatmeal	+++	Yellowish white	Yellowish white	Brownish gold
	Asparagine	+++	Brownish white	Yellowish white	Brownish gold
	Inorg. salt	+++	Brownish white	Yellowish white	Gold
S 32-2	YM	+++	Light gray	Light gray	Pale beige
	Tyrosine	+++	Light medium gray	Light gray	Dull yellow
	Oatmeal	+++	Yellowish gray	Yellowish gray	Dull yellow
	Asparagine	+++	Light gray	Light gray	Pale beige
	Inorg. salt	+++	Light gray	Light gray	Pale beige
S 32-5	YM	+++	Yellowish gray	Yellowish gray	Dark reddish brown
	Tyrosine	+++	Brownish gray	Brownish gray	Dark brown
	Oatmeal	+++	Grayish yellow	Grayish yellow	Brown
	Asparagine	+++	Dark gray	Brownish gray	Brownish olive
	Inorg. salt	+++	Grayish brown	Grayish brown	Dark brown
S 33-1	YM	+++	Light medium gray	Light medium gray	Yellowish brown
	Tyrosine	+++	Medium gray	Light medium gray	Dark gray
	Oatmeal	+++	Medium gray	Light medium gray	Dark gray
	Asparagine	+++	Light gray	Light medium gray	Yellowish brown
	Inorg. salt	+++	Light gray	Light gray	Yellowish brown
S 33-2	YM	+++	Yellowish gray	Yellowish gray	Brownish gold
	Tyrosine	+++	Brownish gray	Brownish gray	Dark brown
	Oatmeal	+++	Grayish yellow	Grayish yellow	Brown
	Asparagine	+++	Dark gray	Brownish gray	Brownish olive
	Inorg. salt	+++	Grayish brown	Grayish brown	Brownish gold

Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

(Continued)

Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
S 33-3	YM	+++	Grayish white	Grayish white	Pale beige
	Tyrosine	+++	Grayish white	Grayish white	Pale beige
	Oatmeal	+++	Grayish white	Grayish white	Pale beige
	Asparagine	+++	Grayish white	Yellowish white	Pale beige
	Inorg. salt	+++	Grayish white	Yellowish white	Pale beige
S 33-4	YM	+++	Grayish white	Grayish white	Brown
	Tyrosine	+++	Light gray	Light gray	Light brown
	Oatmeal	+++	Grayish white	Grayish white	Brownish gold
	Asparagine	+++	Grayish white	Grayish white	Brownish gold
	Inorg. salt	+++	Grayish white	Grayish white	Brownish gold
S 35-5	YM	+++	Medium gray	Medium gray	Light brown
	Tyrosine	+++	Yellowish gray	Yellowish gray	Light brown
	Oatmeal	+++	Medium gray	Light medium gray	Light brown
	Asparagine	+++	Grayish leaf	Grayish leaf	Pale yellow
	Inorg. salt	+++	Grayish leaf	Pale yellowish green	Pale yellow
SB 3-2	YM	+++	Medium gray	Medium gray	Grayish brown
	Tyrosine	++	Medium gray	Medium gray	Grayish brown
	Oatmeal	+++	Gray	Medium gray	Dark medium gray
	Asparagine	++	Light medium gray	Light gray	Dark bluish gray
	Inorg. salt	++	Medium gray	Medium gray	Grayish brown
SB 4-1	YM	+++	Grayish white	Grayish white	Gold
	Tyrosine	+++	Grayish white	Grayish white	Gold
	Oatmeal	+++	Grayish white	Grayish white	Gold
	Asparagine	+++	Grayish white	Grayish white	Light brown
	Inorg. salt	+++	Grayish white	Grayish white	Light brown
KB 4-1	YM	+++	Medium gray	Medium gray	Grayish brown
	Tyrosine	+++	Light medium gray	Light medium gray	Grayish brown
	Oatmeal	+++	Medium gray	Medium gray	Brown
	Asparagine	+++	Light medium gray	Light medium gray	Grayish brown
	Inorg. salt	+++	Light medium gray	Light medium gray	Grayish brown

Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

(Continued)

Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
SB 5-2	YM	+++	Pale beige	Pale beige	Pale beige
	Tyrosine	+++	Pale yellow	Pale beige	Pale yellow
	Oatmeal	+++	Pale reddish yellow	Pale beige	Pale yellow
	Asparagine	+++	Pale beige	Pale beige	Pale beige
	Inorg. salt	+++	Pale beige	Pale beige	Pale beige
SB 7-3	YM	+++	Pinkish white	Pinkish white	Dark red
	Tyrosine	+++	Pinkish white	Pinkish white	Brown
	Oatmeal	+++	Pinkish white	Pinkish white	Light brown
	Asparagine	+++	Pinkish white	Pale purplish pink	Pale purplish pink
	Inorg. salt	+++	Pinkish white	Pinkish white	Pale pinkish beige
SB 9-1	YM	+++	Pale beige	Pale beige	Pale beige
	Tyrosine	+++	Pale yellow	Pale beige	Pale yellow
	Oatmeal	+++	Pale reddish yellow	Pale beige	Pale yellow
	Asparagine	++	Pale beige	Pale beige	Pale beige
	Inorg. salt	++	Pale beige	Pale beige	Pale beige
SB 9-3	YM	+++	Yellowish white	Yellowish white	Gold
	Tyrosine	+++	Yellowish white	Yellowish white	Brownish gold
	Oatmeal	+++	Yellowish white	Yellowish white	Brownish gold
	Asparagine	+++	Brownish white	Yellowish white	Brownish gold
	Inorg. salt	+++	Brownish white	Yellowish white	Gold
SB 10-1	YM	+++	Pale beige	Pale beige	Gold
	Tyrosine	++	Pale beige	Pale beige	Light brown
	Oatmeal	+++	Pale beige	Pale beige	Gold
	Asparagine	++	Pale reddish yellow	Pale beige	Brownish gold
	Inorg. salt	+++	Pale beige	Pale beige	Gold
SB 12-1	YM	+++	Vivid red	Vivid red	Vivid red
	Tyrosine	+++	Vivid red	Vivid red	Vivid red
	Oatmeal	+++	Vivid red	Vivid red	Vivid red
	Asparagine	+++	Strong yellowish red	Strong yellowish red	Deep yellowish red
	Inorg. salt	+++	Vivid red	Vivid purplish red	Strong yellowish red

Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

(Continued)

Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
SC 13-3	YM	+++	Pale beige	Pale beige	Pale beige
	Tyrosine	++	Pale yellow	Pale beige	Pale yellow
	Oatmeal	+++	Pale reddish yellow	Pale beige	Pale yellow
	Asparagine	++	Pale beige	Pale beige	Pale beige
	Inorg. salt	++	Pale beige	Pale beige	Pale beige
SC 13-4	YM	+++	Yellowish white	Yellowish white	Pale beige
	Tyrosine	+++	Yellowish white	Yellowish white	Pale beige
	Oatmeal	+++	Yellowish white	Yellowish white	Pale beige
	Asparagine	+++	Pale beige	Yellowish white	Pale yellow
	Inorg. salt	+++	Yellowish white	Yellowish white	Pale beige
SC 13-5	YM	+++	Pale beige	Yellowish white	Pale beige
	Tyrosine	+++	Pale beige	Yellowish white	Pale beige
	Oatmeal	+++	Pale beige	Yellowish white	Pale beige
	Asparagine	+++	Pale beige	Yellowish white	Pale yellow
	Inorg. salt	+++	Pale beige	Yellowish white	Pale beige
SC 13-7	YM	+++	Gold	Gold	Light brown
	Tyrosine	+++	Yellowish brown	Yellowish brown	Yellowish brown
	Oatmeal	+++	Vivid yellow orange	Gold	Light brown
	Asparagine	+++	Vivid yellow orange	Vivid yellow orange	Vivid yellow orange
	Inorg. salt	+++	Vivid orange	Vivid orange	Light brown
SC 13-9	YM	+++	Deep orange	Deep orange	Pale beige
	Tyrosine	++	Pale yellow	Pale yellow	Pale beige
	Oatmeal	+++	Deep orange	Deep orange	Pale beige
	Asparagine	++	Deep orange	Deep orange	Pale beige
	Inorg. salt	++	Pale yellow	Pale yellow	Pale beige
SC 16-1	YM	+++	Deep orange	Deep orange	Gold
	Tyrosine	++	Light reddish yellow	Light reddish yellow	Light brown
	Oatmeal	+++	Light reddish yellow	Light reddish yellow	Brownish gold
	Asparagine	++	Pale yellow	Pale yellow	Yellowish brown
	Inorg. salt	++	Pale yellow	Pale yellow	Yellowish brown

Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

(Continued)

Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
SC 16-2	YM	+++	Strong yellow	Strong yellow	Gold
	Tyrosine	++	Vivid yellow	Vivid yellow	Light brown
	Oatmeal	+++	Vivid yellow	Vivid yellow	Yellowish brown
	Asparagine	+++	Vivid yellow	Vivid yellow	Yellowish brown
	Inorg. salt	++	Vivid yellow	Strong yellow	Brownish gold
SC 18-2	YM	+++	Medium gray	Medium gray	Reddish brown
	Tyrosine	+++	Medium gray	Medium gray	Reddish brown
	Oatmeal	+++	Medium gray	Medium gray	Brown
	Asparagine	+++	Medium gray	Medium gray	Reddish brown
	Inorg. salt	+++	Medium gray	Medium gray	Brown
SC 19-1	YM	+++	Bright yellow	Bright yellow	Gold
	Tyrosine	+++	Yellowish white	Brownish white	Pale yellow
	Oatmeal	+++	Pale yellow	Light yellow	Pale yellow
	Asparagine	+++	Pale yellow	Light yellow	Vivid yellow
	Inorg. salt	+++	Pale yellow	Light yellow	Pale yellow
KC 19-1	YM	+++	Brownish white	Brownish white	Pale beige
	Tyrosine	+++	Yellowish white	Yellowish white	Pale yellow
	Oatmeal	+++	Yellowish white	Yellowish white	Pale yellow
	Asparagine	+++	Brownish white	Brownish white	Pale beige
	Inorg. salt	+++	Brownish white	Brownish white	Pale beige
KC 19-2	YM	+++	Pinkish white	Pinkish white	Yellowish pink
	Tyrosine	+++	Pinkish beige	Pinkish white	Strong reddish orange
	Oatmeal	+++	Pinkish white	Pinkish white	Yellowish pink
	Asparagine	+++	Pinkish white	Pinkish white	Yellowish pink
	Inorg. salt	+++	Pinkish white	Pinkish white	Pale reddish yellow
KC 20-1	YM	+++	Yellowish white	Yellowish white	Vivid orange
	Tyrosine	+++	Yellowish white	Yellowish white	Pale beige
	Oatmeal	+++	Yellowish white	Brownish white	Pale yellow
	Asparagine	+++	Pale reddish yellow	Pale reddish yellow	Pale reddish yellow
	Inorg. salt	+++	Yellowish white	Yellowish white	Pale yellow

Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

(Continued)

Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
S 1-5-6	YM	+++	Yellowish gray	Yellowish gray	Pale beige
	Tyrosine	+++	Medium gray	Medium gray	Yellowish gray
	Oatmeal	+++	Medium gray	Medium gray	Graynish yellow
	Asparagine	+++	Medium gray	Medium gray	Yellowish gray
	Inorg. salt	+++	Yellowish gray	Medium gray	Pale beige
S 3-1-2	YM	+++	Strong yellow	Strong yellow	Vivid yellow
	Tyrosine	+++	Vivid yellow	Vivid yellow	Strong yellow
	Oatmeal	+++	Vivid yellow	Vivid yellow	Vivid yellow
	Asparagine	+++	Vivid yellow	Vivid yellow	Vivid yellow
	Inorg. salt	+++	Vivid yellow	Strong yellow	Gold
S 3-1-4	YM	+++	Grayish white	Grayish white	Brownish gold
	Tyrosine	+++	Grayish white	Brownish white	Pale beige
	Oatmeal	+++	Grayish white	Yellowish white	Gold
	Asparagine	+++	Grayish white	Brownish white	Light brown
	Inorg. salt	+++	Grayish white	Grayish white	Brownish gold
S 3-1-5	YM	+++	Pale beige	Pale beige	Bright yellow
	Tyrosine	+++	Light yellow	Pale yellow	Pale yellow
	Oatmeal	+++	Pale beige	Pale beige	Bright yellow
	Asparagine	++	Light yellow	Pale yellow	Pale yellow
	Inorg. salt	++	Pale beige	Pale beige	Pale yellow
S 3-1-6	YM	+++	Medium gray	Medium gray	Gold
	Tyrosine	+++	Bluish gray	Medium gray	Bright yellow
	Oatmeal	+++	Medium gray	Light medium gray	Gold
	Asparagine	+++	Bluish gray	Light medium gray	Vivid yellow
	Inorg. salt	+++	Bluish gray	Light bluish gray	Vivid yellow
S 3-2-1	YM	+++	Vivid orange	Pale beige	Pale beige
	Tyrosine	+++	Vivid orange	Vivid orange	Pale beige
	Oatmeal	+++	Vivid orange	Vivid orange	Pale beige
	Asparagine	++	Deep orange	Deep orange	Pale beige
	Inorg. salt	+++	Vivid orange	Vivid orange	Pale beige

Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

(Continued)

Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
S 3-2-2	YM	+++	Yellowish brown	Yellowish brown	Brown
	Tyrosine	+++	Brown	Yellowish brown	Dull yellow
	Oatmeal	+++	Dull reddish yellow	Dull reddish yellow	Yellowish brown
	Asparagine	+++	Yellowish brown	Yellowish brown	Light brown
	Inorg. salt	+++	Yellowish brown	Yellowish brown	Light brown
S 3-2-5	YM	+++	Grayish yellow	Grayish yellow	Light brown
	Tyrosine	+++	Light gray	Light medium gray	Gold
	Oatmeal	+++	Grayish yellow	Medium gray	Grayish yellow
	Asparagine	+++	Grayish yellow	Medium gray	Brownish gold
	Inorg. salt	+++	Grayish yellow	Light medium gray	Gold
S 3-2-6	YM	+++	Light gray	Light gray	Pale beige
	Tyrosine	+++	Light gray	Light gray	Gold
	Oatmeal	+++	Grayish yellow	Grayish brown	Light brown
	Asparagine	+++	Light gray	Light gray	Pale beige
	Inorg. salt	+++	Light gray	Light gray	Pale beige
S 3-3-1	YM	+++	Strong yellow	Strong yellow	Strong yellowish pink
	Tyrosine	+++	Vivid yellow	Vivid yellow	Dull reddish yellow
	Oatmeal	+++	Vivid yellow	Vivid yellow	Dull reddish yellow
	Asparagine	+++	Vivid yellow	Vivid yellow	Dull reddish yellow
	Inorg. salt	+++	Strong yellow	Strong yellow	Strong yellowish pink
S 3.3-2	YM	+++	Yellowish white	Yellowish white	Pale beige
	Tyrosine	+++	Pale beige	Pale beige	Pale beige
	Oatmeal	+++	Yellowish white	Pale beige	Dull yellow
	Asparagine	+++	Yellowish white	Brownish white	Pale beige
	Inorg. salt	+++	Yellowish white	Brownish white	Pale beige
S 3-3-5	YM	+++	Deep purplish red	Deep purplish red	Deep red purple
	Tyrosine	+++	Vivid red purple	Deep purplish red	Deep red purple
	Oatmeal	+++	Deep purplish red	Vivid red purple	Deep red purple
	Asparagine	+++	Vivid red purple	Deep purplish red	Deep red purple
	Inorg. salt	+++	Vivid red purple	Deep purplish red	Deep red purple

Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

(Continued)

Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
S 3-3-6	YM	+++	Medium gray	Medium gray	Grayish brown
	Tyrosine	+++	Medium gray	Light medium gray	Brown
	Oatmeal	+++	Medium gray	Light medium gray	Dark brown
	Asparagine	+++	Medium gray	Light medium gray	Light brown
	Inorg. salt	+++	Yellowish gray	Light medium gray	Brownish gold
S 3-4-4	YM	+++	Medium gray	Medium gray	Yellowish brown
	Tyrosine	+++	Yellowish gray	Medium gray	Grayish brown
	Oatmeal	+++	Yellowish gray	Medium gray	Light reddish brown
	Asparagine	+++	Light gray	Grayish sky	Grayish brown
	Inorg. salt	+++	Dark medium gray	Medium gray	Grayish brown
S 3-7-4	YM	+++	Brownish white	Brownish white	Pale beige
	Tyrosine	+++	Yellowish white	Yellowish white	Pale yellow
	Oatmeal	+++	Brownish white	Brownish white	Pale beige
	Asparagine	+++	Brownish white	Brownish white	Pale beige
	Inorg. salt	+++	Brownish white	Brownish white	Pale beige
S 3-7-5	YM	+++	Brown	Brown	Brown
	Tyrosine	+++	Dull orange	Brown	Brown
	Oatmeal	+++	Yellowish brown	Yellowish brown	Yellowish brown
	Asparagine	+++	Dull orange	Brown	Brown
	Inorg. salt	+++	Yellowish brown	Yellowish brown	Yellowish brown
S 4-1-1	YM	+++	Grayish white	Grayish white	Pale beige
	Tyrosine	+++	Grayish white	Grayish white	Strong yellow
	Oatmeal	+++	Brownish white	Brownish white	Pale yellow
	Asparagine	+++	Yellowish white	Yellowish white	Pale yellow
	Inorg. salt	+++	Yellowish white	Yellowish white	Pale yellow
S 4-2-1	YM	+++	Medium gray	Medium gray	Brown
	Tyrosine	+++	Medium gray	Medium gray	Grayish yellow
	Oatmeal	+++	Medium gray	Medium gray	Light brown
	Asparagine	+++	Bluish gray	Bluish gray	Grayish yellow
	Inorg. salt	+++	Light medium gray	Light medium gray	Grayish yellow

Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

(Continued)

Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
S 4-2-2	YM	+++	Dark yellowish brown	Dark yellowish brown	Dark brown
	Tyrosine	+++	Medium gray	Medium gray	Dark yellowish brown
	Oatmeal	+++	Dark medium gray	Medium gray	Dark yellowish brown
	Asparagine	+++	Medium gray	Medium gray	Dark yellowish brown
	Inorg. salt	+++	Medium gray	Medium gray	Dark brown
S 4-5-2	YM	+++	Light gray	Light gray	Pale yellow
	Tyrosine	+++	Light gray	Light gray	Pale yellow
	Oatmeal	+++	Grayish sky	Grayish sky	Pale beige
	Asparagine	+++	Light bluish gray	Light bluish gray	Pale beige
	Inorg. salt	+++	Light gray	Light gray	Pale yellow
S 4-5-3	YM	+++	Medium gray	Medium gray	Pale beige
	Tyrosine	+++	Medium gray	Medium gray	Pale beige
	Oatmeal	+++	Medium gray	Medium gray	Pale beige
	Asparagine	+++	Medium gray	Medium gray	Pale beige
	Inorg. salt	+++	Medium gray	Medium gray	Pale beige
S 4-6-1	YM	+++	Bluish gray	Bluish gray	Gold
	Tyrosine	+++	Light gray	Light gray	Light brown
	Oatmeal	+++	Bluish gray	Bluish gray	Brown
	Asparagine	+++	Bluish gray	Bluish gray	Brownish gold
	Inorg. salt	+++	Bluish gray	Bluish gray	Gold
S 4-6-2	YM	+++	Light medium gray	Light medium gray	Pale beige
	Tyrosine	+++	Grayish sky	Grayish sky	Pale beige
	Oatmeal	+++	Grayish sky	Grayish sky	Pale beige
	Asparagine	+++	Dark medium gray	Medium gray	Pale beige
	Inorg. salt	+++	Light medium gray	Light medium gray	Pale beige
S 4-9-1	YM	+++	Pale beige	Pale beige	Gold
	Tyrosine	+++	Pale beige	Pale beige	Light brown
	Oatmeal	+++	Pale beige	Pale beige	Gold
	Asparagine	+++	Pale reddish yellow	Pale beige	Brownish gold
	Inorg. salt	+++	Pale beige	Pale beige	Gold

Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

(Continued)

Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
S 38-2	YM	+++	Light medium gray	Light medium gray	Yellowish brown
	Tyrosine	+++	Medium gray	Medium gray	Yellowish gray
	Oatmeal	+++	Medium gray	Medium gray	Yellowish gray
	Asparagine	++	Medium gray	Medium gray	Yellowish gray
	Inorg. salt	+++	Medium gray	Medium gray	Yellowish gray
K 38-2	YM	+++	Pinkish white	Pinkish white	Brown
	Tyrosine	+++	Pinkish white	Pinkish white	Brown
	Oatmeal	+++	Brownish white	Brownish white	Reddish brown
	Asparagine	+++	Pinkish white	Pinkish white	Brown
	Inorg. salt	+++	Pinkish white	Pinkish white	Brown
K 39-4	YM	+++	Grayish white	Grayish white	Medium brown
	Tyrosine	++	Grayish white	Grayish white	Dark brown
	Oatmeal	+++	Grayish white	Grayish white	Medium brown
	Asparagine	++	Grayish white	Grayish white	Dark brown
	Inorg. salt	++	Yellowish white	Yellowish white	Pale yellow
S 39-7	YM	+++	Pinkish white	Pinkish white	Vivid purplish red
	Tyrosine	+++	Pinkish white	Pinkish white	Dark violet
	Oatmeal	+++	Pinkish white	Pinkish white	Deep purplish red
	Asparagine	+++	Pinkish white	Pinkish white	Dark brown
	Inorg. salt	++	Pinkish white	Pinkish white	Vivid purplish red
S 40-1	YM	+++	Light gray	Light gray	Brown
	Tyrosine	++	Light medium gray	Light medium gray	Light Brown
	Oatmeal	+++	Light medium gray	Light medium gray	Light Brown
	Asparagine	++	Light gray	Light gray	Brown
	Inorg. Salt	++	Light gray	Light gray	Brown
S 42-1	YM	+++	Yellowish white	Yellowish white	Pale beige
	Tyrosine	+++	Yellowish white	Yellowish white	Pale beige
	Oatmeal	+++	Yellowish white	Yellowish white	Pale beige
	Asparagine	+++	Yellowish white	Yellowish white	Pale beige
	Inorg. Salt	+++	Yellowish white	Yellowish white	Pale beige

Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

(Continued)

Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
S 42-2	YM	+++	Grayish white	Grayish white	Pale beige
	Tyrosine	+++	Grayish white	Grayish white	Pale beige
	Oatmeal	+++	Grayish white	Grayish white	Pale beige
	Asparagine	+++	Grayish white	Grayish white	Pale beige
	Inorg. Salt	+++	Grayish white	Grayish white	Pale beige
K 45-3	YM	+++	Grayish white	Grayish white	Pale beige
	Tyrosine	++	Grayish white	Grayish white	Dull yellow
	Oatmeal	+++	Grayish white	Grayish white	Dull yellow
	Asparagine	+	Grayish white	Grayish white	Pale beige
	Inorg. Salt	+	Grayish white	Grayish white	Pale beige
K 45-2	YM	+++	Brownish white	Brownish white	Yellowish brown
	Tyrosine	+++	Brownish white	Brownish white	Light brown
	Oatmeal	+++	Brownish white	Brownish white	Gold
	Asparagine	+++	Brownish white	Brownish white	Yellowish brown
	Inorg. Salt	+++	Brownish white	Brownish white	Yellowish brown
S 45-4	YM	+++	Light bluish gray	Light bluish gray	Brown
	Tyrosine	++	Light gray	Light gray	Brown
	Oatmeal	+++	Light gray	Light gray	Brown
	Asparagine	++	Light gray	Light gray	Brown
	Inorg. Salt	++	Light bluish gray	Light gray	Brown
K 45-5	YM	+++	Pale beige	Pale beige	Pale beige
	Tyrosine	+++	Pale beige	Pale beige	Pale beige
	Oatmeal	+++	Pale yellow	Pale yellow	Pale yellow
	Asparagine	+++	Pale yellow	Pale yellow	Pale yellow
	Inorg. Salt	+++	Pale beige	Pale beige	Pale beige
K 45-6	YM	+++	Brownish white	Brownish white	Light brown
	Tyrosine	+++	Brownish white	Brownish white	Light brown
	Oatmeal	+++	Brownish white	Brownish white	Light brown
	Asparagine	+++	Brownish white	Brownish white	Brown
	Inorg. Salt	+++	Brownish white	Brownish white	Light brown

Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

(Continued)

Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
K 45-9	YM	+++	Yellowish white	Yellowish white	Pale beige
	Tyrosine	++	Yellowish white	Yellowish white	Pale beige
	Oatmeal	+++	Yellowish white	Yellowish white	Pale yellow
	Asparagine	++	Yellowish white	Yellowish white	Pale yellow
	Inorg. Salt	++	Yellowish white	Yellowish white	Pale beige
K 45-10	YM	+++	Pale beige	Pale beige	Light brown
	Tyrosine	+++	Pale beige	Pale beige	Brown
	Oatmeal	+++	Pale yellow	Pale yellow	Brown
	Asparagine	++	Pale yellow	Pale yellow	Light brown
	Inorg. Salt	++	Pale beige	Pale beige	Light brown
S 47-4	YM	+++	Grayish white	Grayish white	Pale beige
	Tyrosine	+++	Pinkish white	Pinkish white	Pale yellow
	Oatmeal	+++	Pinkish white	Pinkish white	Pale beige
	Asparagine	+++	Grayish white	Grayish white	Pale beige
	Inorg. Salt	+++	Grayish white	Grayish white	Pale beige
S 48-2	YM	+++	Purplish pink	Purplish pink	Pale beige
	Tyrosine	+++	Purplish pink	Purplish pink	Pale beige
	Oatmeal	+++	Purplish pink	Purplish pink	Pale beige
	Asparagine	+++	Purplish pink	Purplish pink	Pale beige
	Inorg. Salt	+++	Purplish pink	Purplish pink	Pale beige
S 48-4	YM	+++	Pinkish white	Pinkish white	Brownish gold
	Tyrosine	+++	Pinkish white	Pinkish white	Brown
	Oatmeal	+++	Pinkish white	Pinkish white	Light brown
	Asparagine	+++	Pinkish white	Pinkish white	Brown
	Inorg. Salt	+++	Pinkish white	Pinkish white	Brownish gold
S 49-1	YM	+++	Purplish pink	Purplish pink	Vivid purplish red
	Tyrosine	++	Vivid red	Vivid red	Rose
	Oatmeal	+++	Vivid red	Vivid red	Vivid red
	Asparagine	++	Purplish pink	Purplish pink	Vivid red
	Inorg. Salt	++	Purplish pink	Purplish pink	Purplish pink

Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

(Continued)

Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
S 49-3	YM	+++	Brownish white	Brownish white	Pale beige
	Tyrosine	+++	Brownish white	Brownish white	Pale beige
	Oatmeal	+++	Brownish white	Brownish white	Pale beige
	Asparagine	+++	Brownish white	Yellowish white	Pale beige
	Inorg. Salt	++	Brownish white	Yellowish white	Pale beige
S 49-4	YM	+++	Yellowish white	Yellowish white	Brown
	Tyrosine	+++	Yellowish white	Yellowish white	Brown
	Oatmeal	+++	Yellowish white	Yellowish white	Brown
	Asparagine	+++	Yellowish white	Yellowish white	Light brown
	Inorg. Salt	+++	Yellowish white	Yellowish white	Light brown
S 49-6	YM	+++	Grayish white	Grayish white	Pale beige
	Tyrosine	+++	Grayish white	Pinkish white	Pale beige
	Oatmeal	+++	Grayish white	Grayish white	Pale pinkish beige
	Asparagine	+++	Grayish white	Grayish white	Pale beige
	Inorg. Salt	+++	Pinkish white	Pinkish white	Pale beige
S 55-2	YM	+++	Grayish white	Grayish white	Pale beige
	Tyrosine	+++	Grayish white	Grayish white	Pale yellow
	Oatmeal	+++	Grayish white	Grayish white	Pale yellow
	Asparagine	+++	Grayish white	Grayish white	Pale beige
	Inorg. Salt	+++	Pinkish white	Pinkish white	Pale yellow
S 55-4	YM	+++	Brownish white	Brownish white	Light brown
	Tyrosine	+++	Pinkish white	Pinkish white	Light brown
	Oatmeal	+++	Yellowish white	Yellowish white	Pale beige
	Asparagine	+++	Pinkish white	Pinkish white	Light yellowish brown
	Inorg. Salt	+++	Pinkish white	Pinkish white	Light yellowish brown
S 57-1	YM	+++	Grayish pink	Grayish pink	Pale beige
	Tyrosine	+++	Grayish pink	Grayish pink	Pale yellow
	Oatmeal	+++	Grayish pink	Grayish pink	Pale beige
	Asparagine	+++	Grayish pink	Grayish pink	Pale beige
	Inorg. Salt	+++	Grayish pink	Grayish pink	Pale beige

Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

(Continued)

Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
K 57-1	YM	+++	Yellowish white	Yellowish white	Pale beige
	Tyrosine	+++	Yellowish white	Yellowish white	Pale yellow
	Oatmeal	+++	Yellowish white	Yellowish white	Pale beige
	Asparagine	+++	Yellowish white	Yellowish white	Pale yellow
	Inorg. Salt	+++	Yellowish white	Yellowish white	Pale yellow
K 57-3	YM	+++	Brownish white	Brownish white	Light brown
	Tyrosine	+++	Brownish white	Brownish white	Brownish gold
	Oatmeal	+++	Brownish white	Brownish white	Brown
	Asparagine	+++	Brownish white	Brownish white	Light brown
	Inorg. Salt	+++	Brownish white	Brownish white	Light Brown
S 65-3	YM	+++	Gold	Gold	Pale beige
	Tyrosine	+++	Dull yellow	Dull yellow	Pale yellow
	Oatmeal	+++	Gold	Gold	Pale beige
	Asparagine	+++	Pale reddish yellow	Pale reddish yellow	Light reddish yellow
	Inorg. Salt	+++	Gold	Gold	Pale beige
S 68-2	YM	+++	Yellowish white	Yellowish white	Pale beige
	Tyrosine	+++	Yellowish white	Brownish white	Pale yellow
	Oatmeal	+++	Yellowish white	Brownish white	Pale beige
	Asparagine	++	Brownish white	Brownish white	Pale beige
	Inorg. Salt	+++	Yellowish white	Brownish white	Pale beige
S 70-2	YM	+++	Yellowish gray	Yellowish gray	Pale beige
	Tyrosine	+++	Medium gray	Medium gray	Yellowish gray
	Oatmeal	+++	Medium gray	Medium gray	Graynish yellow
	Asparagine	+++	Medium gray	Medium gray	Yellowish gray
	Inorg. Salt	+++	Yellowish gray	Medium gray	Pale beige
S 70-4	YM	+++	Brownish white	Brownish white	Brownish gold
	Tyrosine	++	Brownish white	Brownish white	Brown
	Oatmeal	+++	Brownish white	Brownish white	Light brown
	Asparagine	++	Brownish white	Brownish white	Light Brown
	Inorg. Salt	++	Brownish white	Brownish white	Brownish gold

Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

(Continued)

Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
S 70-5	YM	+++	Light gray	Light gray	Yellowish brown
	Tyrosine	+++	Light gray	Light bluish gray	Light Brown
	Oatmeal	+++	Light gray	Light bluish gray	Yellowish brown
	Asparagine	+++	Light gray	Light gray	Yellowish brown
	Inorg. Salt	+++	Light gray	Light gray	Yellowish brown
S 71-1	YM	+++	Yellowish white	Yellowish white	Pale beige
	Tyrosine	+++	Yellowish white	Yellowish white	Pale beige
	Oatmeal	+++	Brownish white	Yellowish white	Pale beige
	Asparagine	+++	Yellowish white	Yellowish white	Pale beige
	Inorg. Salt	+++	Yellowish white	Yellowish white	Pale beige
S 71-2	YM	+++	Light medium gray	Light medium gray	Pale beige
	Tyrosine	+++	Light medium gray	Light medium gray	Pale beige
	Oatmeal	+++	Medium gray	Medium gray	Pale beige
	Asparagine	+++	Light medium gray	Light medium gray	Pale beige
	Inorg. Salt	+++	Light medium gray	Light medium gray	Pale beige
S 72-10	YM	+++	Purplish pink	Purplish pink	Light brown
	Tyrosine	+++	Purplish pink	Purplish pink	Brown
	Oatmeal	+++	Purplish pink	Purplish pink	Brownish gold
	Asparagine	+++	Purplish pink	Purplish pink	Light brown
	Inorg. Salt	+++	Purplish pink	Purplish pink	Light brown
S 72-11	YM	+++	Yellowish brown	Yellowish brown	Brown
	Tyrosine	+++	Yellowish brown	Light brown	Brownish gold
	Oatmeal	+++	Yellowish brown	Yellowish brown	Brownish gold
	Asparagine	++	Yellowish brown	Yellowish brown	Brown
	Inorg. Salt	++	Yellowish brown	Light brown	Brown
S 72-12	YM	+++	Brownish white	Brownish white	Pale beige
	Tyrosine	+++	Brownish white	Brownish white	Pale beige
	Oatmeal	+++	Brownish white	Brownish white	Pale beige
	Asparagine	+++	Brownish white	Brownish white	Pale beige
	Inorg. Salt	++	Brownish white	Brownish white	Pale beige

Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

(Continued)

Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
S 72-15	YM	+++	Purplish pink	Purplish pink	Pale beige
	Tyrosine	+++	Purplish pink	Purplish pink	Pale beige
	Oatmeal	+++	Purplish pink	Purplish pink	Pale beige
	Asparagine	+++	Pinkish gray	Pale purplish pink	Pale beige
	Inorg. Salt	+++	Pinkish gray	Pinkish gray	Pale beige
S 74-1	YM	+++	Light brown	Light brown	Pale beige
	Tyrosine	+++	Brownish gold	Brownish gold	Pale beige
	Oatmeal	+++	Brownish gold	Brownish gold	Pale beige
	Asparagine	++	Light brown	Light brown	Pale beige
	Inorg. Salt	++	Light brown	Light brown	Pale beige
S 74-5	YM	+++	Purplish pink	Purplish pink	Pale beige
	Tyrosine	+++	Purplish pink	Purplish pink	Pale yellow
	Oatmeal	+++	Purplish pink	Purplish pink	Pale beige
	Asparagine	+++	Purplish pink	Purplish pink	Pale beige
	Inorg. Salt	+++	Purplish pink	Purplish pink	Pale beige
S 74-6	YM	+++	Light medium gray	Light medium gray	Brownish gold
	Tyrosine	+++	Light gray	Light bluish gray	Brownish gold
	Oatmeal	+++	Light medium gray	Light bluish gray	Brown
	Asparagine	+++	Light gray	Light gray	Brown
	Inorg. Salt	+++	Light gray	Light gray	Yellowish brown
S 74-7	YM	+++	Yellowish white	Yellowish white	Pale beige
	Tyrosine	++	Yellowish white	Yellowish white	Pale yellow
	Oatmeal	+++	Brownish white	Yellowish white	Pale beige
	Asparagine	++	Yellowish white	Yellowish white	Pale beige
	Inorg. Salt	++	Yellowish white	Yellowish white	Pale beige
S 75-1	YM	+++	Yellowish white	Yellowish white	Pale beige
	Tyrosine	+++	Brownish white	Yellowish white	Palebeige
	Oatmeal	+++	Brownish white	Brownish white	Light brown
	Asparagine	+++	Yellowish white	Yellowish white	Light brown
	Inorg. Salt	+++	Yellowish white	Yellowish white	Pale beige

Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

(Continued)

Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
S 75-3	YM	+++	Brownish white	Brownish white	Pale beige
	Tyrosine	+++	Brownish white	Brownish white	Pale beige
	Oatmeal	+++	Brownish white	Brownish white	Pale beige
	Asparagine	+++	Brownish white	Brownish white	Pale beige
	Inorg. salt	+++	Brownish white	Brownish white	Pale beige
S 75-4	YM	+++	Vivid red	Vivid red	Vivid red
	Tyrosine	+++	Vivid red	Vivid red	Vivid red
	Oatmeal	+++	Vivid red	Vivid red	Vivid red
	Asparagine	+++	Strong yellowish red	Strong yellowish red	Deep yellowish red
	Inorg. Salt	+++	Vivid red	Vivid purplish red	Strong yellowish red
S 75-5	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
S 76-1	YM	+++	Purplish pink	Purplish pink	Light brown
	Tyrosine	+++	Purplish pink	Purplish pink	Brown
	Oatmeal	+++	Purplish pink	Purplish pink	Brownish gold
	Asparagine	+++	Purplish pink	Purplish pink	Light brown
	Inorg. Salt	+++	Purplish pink	Purplish pink	Light brown
S 76-6	YM	+++	Yellowish white	Yellowish white	Pale beige
	Tyrosine	+++	Brownish white	Yellowish white	Pale beige
	Oatmeal	+++	Brownish white	Brownish white	Pale beige
	Asparagine	+++	Yellowish white	Yellowish white	Pale beige
	Inorg. Salt	+++	Yellowish white	Yellowish white	Pale beige

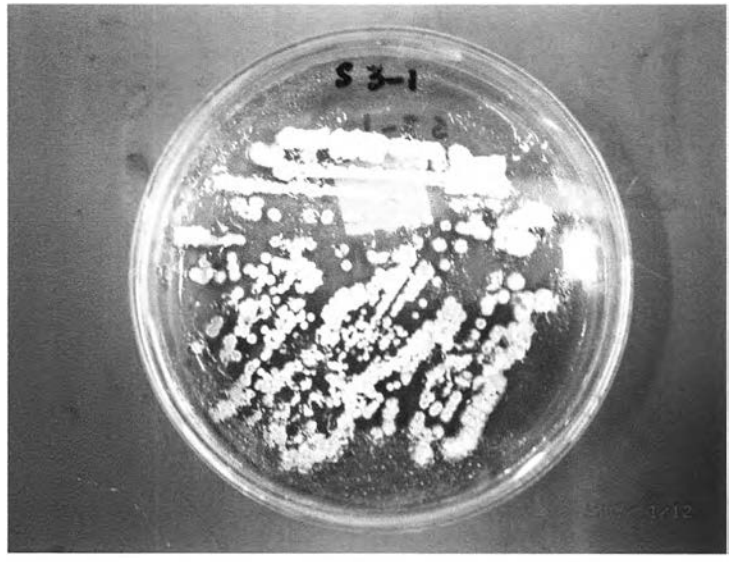
Table 4.4 Cultural characteristics of the strains on different media after 14 days incubation

(Continued)

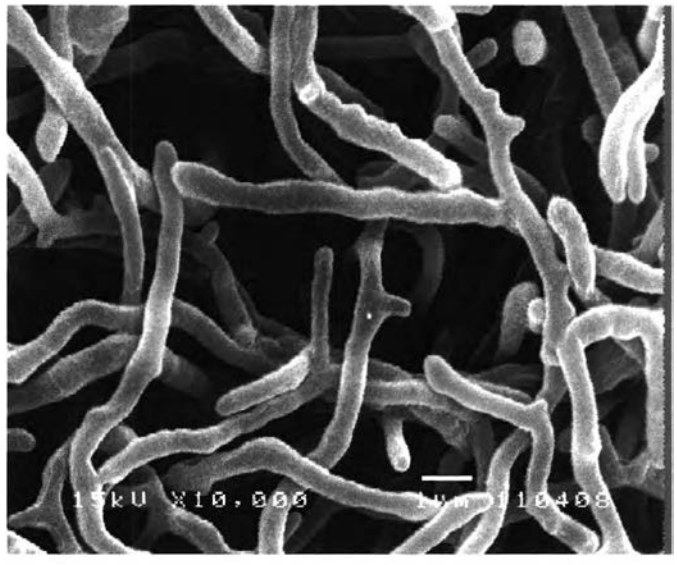
Strain no.	Medium	Growth	Spore color	Colony color	
				Upper colony	Lower colony
S 77-2	YM	+++	Yellowish white	Yellowish white	Pale yellow
	Tyrosine	++	Yellowish white	Yellowish white	Pale yellow
	Oatmeal	+++	Brownish white	Yellowish white	Pale beige
	Asparagine	++	Yellowish white	Yellowish white	Pale yellow
	Inorg. Salt	+++	Yellowish white	Yellowish white	Pale yellow
S 77-3	YM	+++	Brownish white	Brownish white	Brownish gold
	Tyrosine	+++	Brownish white	Brownish white	Brown
	Oatmeal	+++	Brownish white	Brownish white	Light brown
	Asparagine	+++	Brownish white	Brownish white	Light Brown
	Inorg. Salt	+++	Brownish white	Brownish white	Brownish gold

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

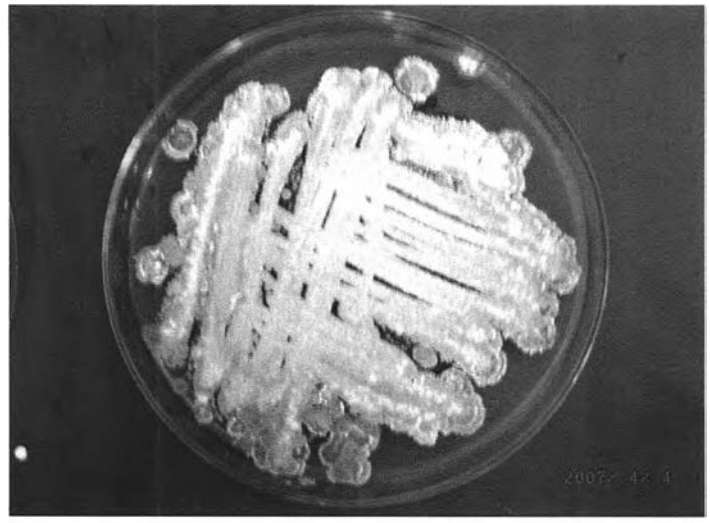


A

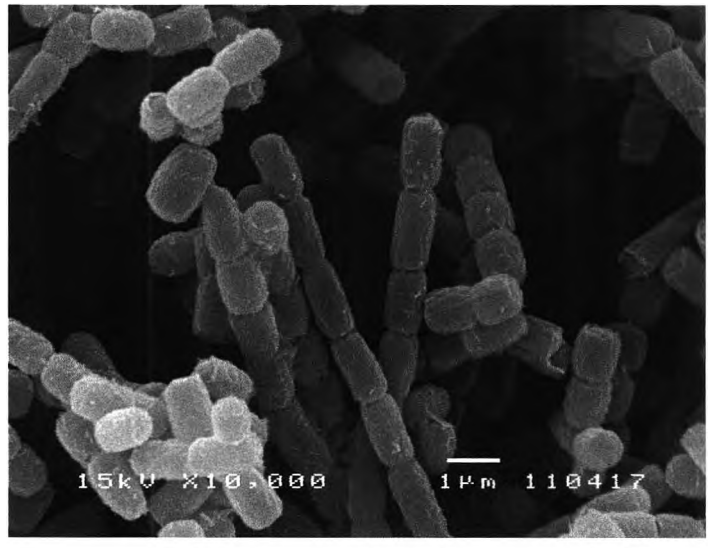


B

Figure 4.1 Colonial appearance (A, velvety and vivid red spore color) and scanning electron micrograph of S3-1 on YMA medium (B) (14 days)



A



B

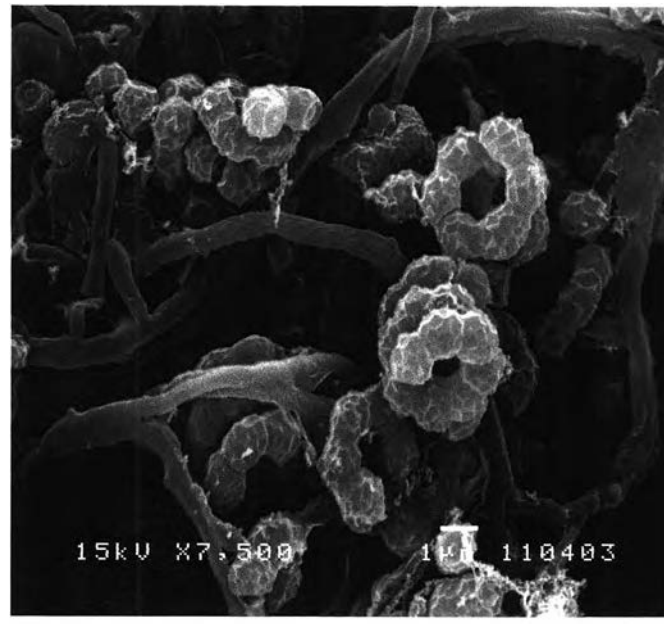
Figure 4.2 Colonial appearance (A, powdery and gray spore color) and scanning electron micrograph of S38-2 , rectiflexibiles of spore chain on YMA medium (B) (14 days)

A

B

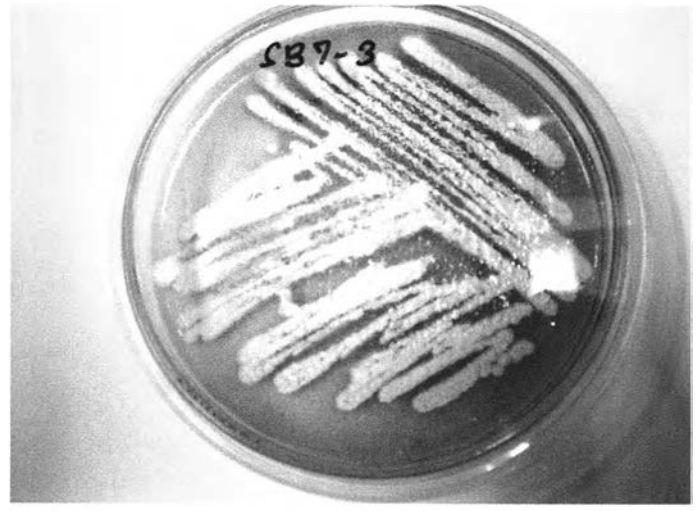
Figure 4.3 Colonial appearance (A, powdery and purplish pink spore color) and scanning electron micrograph of S72-10, rectiflexibiles of spore chain on YMA medium (B) (14 days)

A

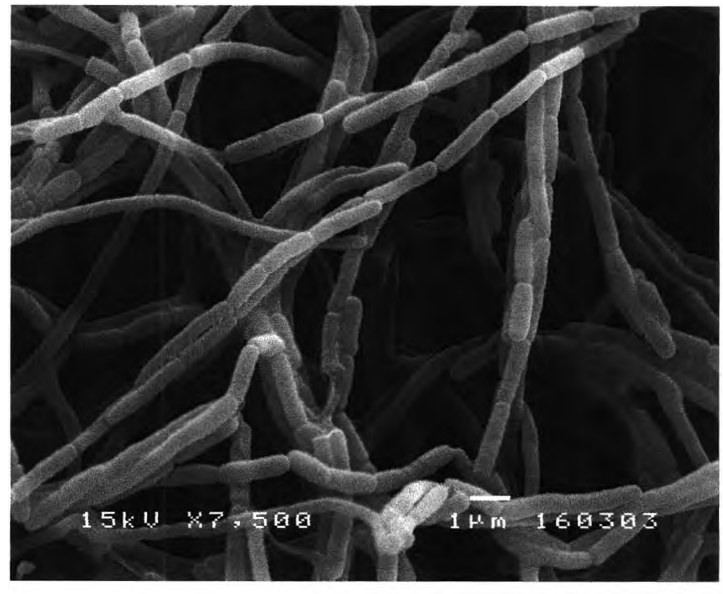


B

Figure 4.4 Colonial appearance (A, powdery and black spore color) and scanning electron micrograph of S75-5, spiral of spore chain on YMA medium (B) (14 days)



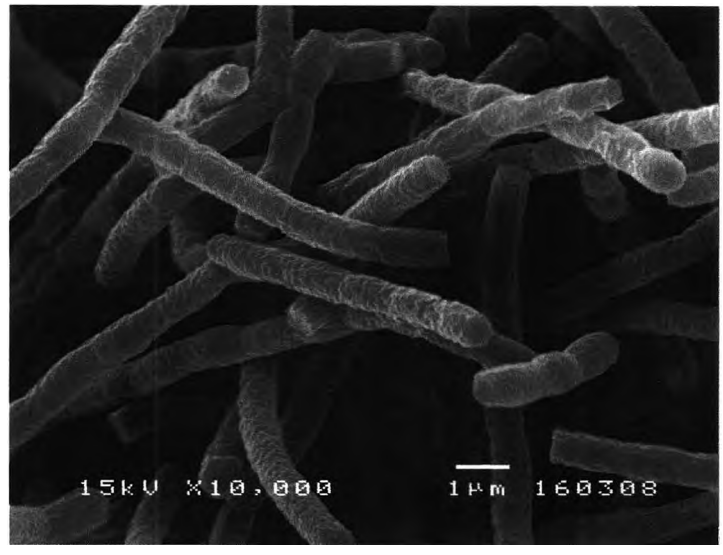
A



B

Figure 4.5 Colonial appearance (A, powdery and pinkish white spore color) and scanning electron micrograph of SB7-3, spore chain on YMA medium (B) (14 days)

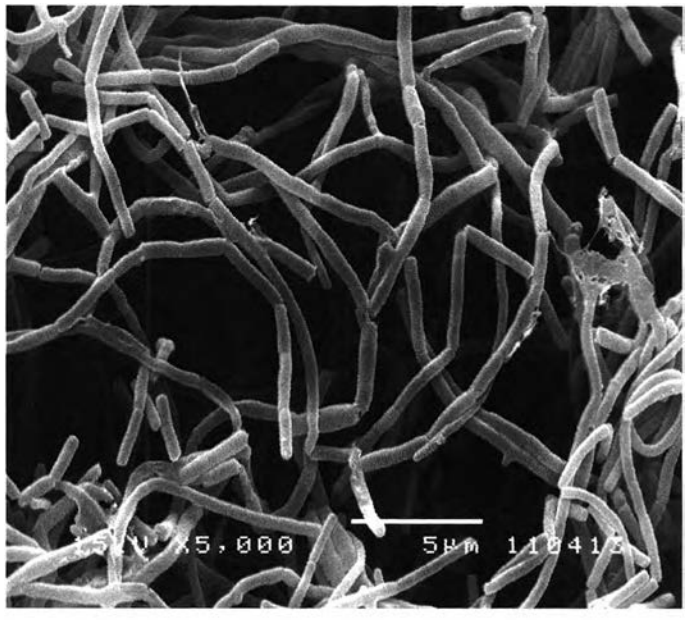
A



B

Figure 4.6 Colonial appearance (A, powdery and pinkish white spore color) and scanning electron micrograph of S39-7, spore chain on YMA medium (B) (14 days)

A



B

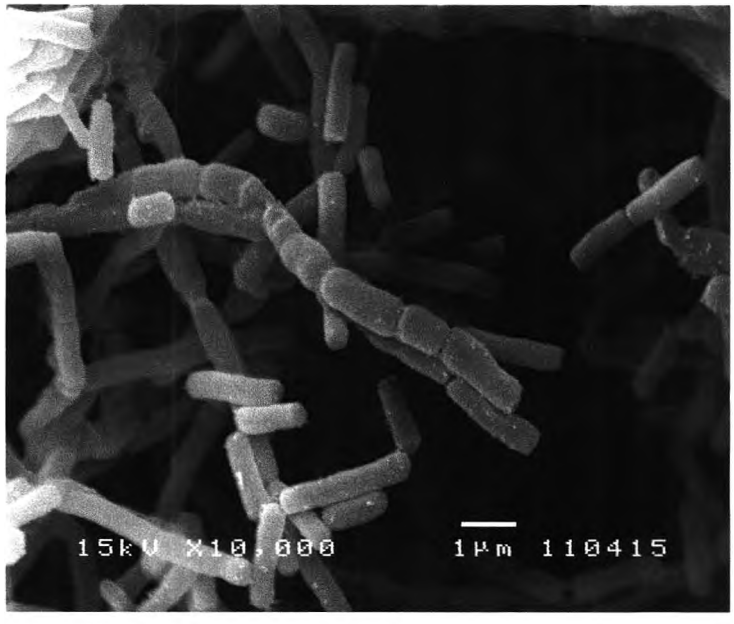
Figure 4.7 Colonial appearance (A, powdery and brownish white spore color) and scanning electron micrograph of KC19-1, spore chain on YMA medium (B) (14 days)

A

B

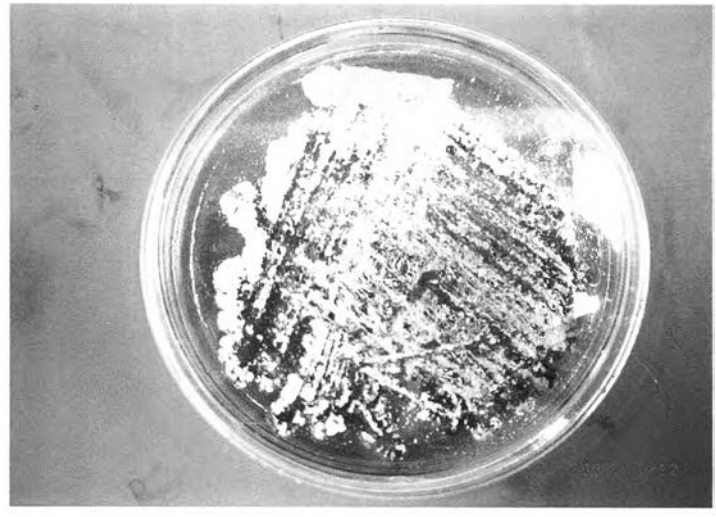
Figure 4.8 Colonial appearance (A, powdery and yellowish white spore color) and scanning electron micrograph of KC20-1, spore chain on YMA medium (B) (14 days)

A

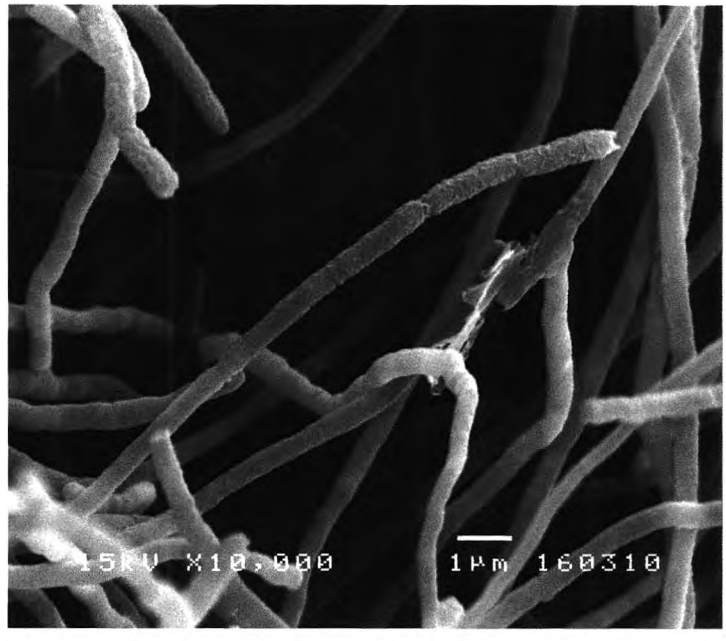


B

Figure 4.9 Colonial appearance (A, powdery and yellowish white spore color) and scanning electron micrograph of K57-1, rectiflexibiles of spore chain on YMA medium (B) (14 days)



A



B

Figure 4.10 Colonial appearance (A, powdery and medium gray spore color) and scanning electron micrograph of SB3-2, rectiflexibiles of spore chain on YMA medium (14 days)

2.2. Physiological and biochemical characteristics

The physiological characteristics of 18 selected strains were shown in Table 4.5. Most strains grew in YMA with 2% and 4% NaCl, at pH 5.0, 7.0, 9.0 and 10 and at 28°C, whereas fewer strains grew in 6% NaCl, at pH 4.0 and at 10°C and 45°C. All strains could not form melanin but all strains coagulated and peptonized skim milk. Most strains liquefied gelatin and hydrolyzed esculin, whereas fewer strains reduced nitrate and hydrolysed starch (Table 4.6). Most strains used glucose, glycerol, arabinose, D-xylose, D-manitol, D-fructose, sucrose, melibiose, rhamnose, and raffinose (Tables 4.7). Seven strains in genus *Amycolatopsis* could produce acid from glucose, adonitol, arabinose, cellobiose, dextrin, *meso*-erythriol, fructose, galactose, meso-inositol, lactose, maltose, mannitol, melezitose, melibiose, methyl D-glucoside, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, and xylose (Table 4.8). Strain SB7-3, KC19-1, KC20-1, K57-1 grew on YMA containing 50 μg/ml of novobiocin, whereas only KC 19-1, KC 20-1, K57-1 grew in YMA containing 100 μg/ml of novobiocin. As mentioned, three strains were resistant to novobiocin 100 μg/ml but *A. keratinophila* KCTC 19104^{T}and *A. albidoflavus* KCTC 9471^T were sensitive as shown in Table 4.9. However, *A. eurytherma* DSM 44348^T, *A. palatopharyngis* 1BDZ ^{T}, and *A. rubida* JCM 10871 ^{T} were resistant to only on novobiocin 5 μg/ml (Huang *et al.*, 2001; 2004; Kim *et al.*, 2002). In addition, strain SB3-2 was sensitive to low concentrations of novobiocin and hence cannot be expected to grow on the novobiocin 50 and 100 μg/ml containing agar medium use by Tajima *et al.*, (2001); Takahashi and Omura (2003). Therefore, the use of novobiocin in the medium for the screening of *Amycolatopsis* and *Kitasatospora* strains should be considered.

2.3 Chemotaxonomic characteristics

On the basis of cell wall peptidoglycan, the selected 18 strains were divided into 3 groups. Group I contained 12 strains which were S1-2, S3-1, SB12-1, S33-3, S38-2, S49-1, S55-4, S71-1, S72-10, S75-3, S75-5 and S76-1. The cell wall component of the strains in this group was LL-isomer of diaminopimelic acid (DAP) which was the same pattern as the genus *Streptomyces*. They had MK-9 (H_6) and MK-9 (H_8) as the predominant menaquinones and the small amounts of MK-9 (H_2) and MK-9 (H_4). Their DNA G+C contents were ranged from 69.0-75.4 mol% (Lechevalier *et al.*, 1977; Goodfellow, 1988; Collin *et al.*, 1977).

Five strains, SB7-3, S39-7, KC19-1, KC20-1, K57-1 (Group II) contained *meso*-diaminopimelic acid which was the same pattern as the genus *Amycolatopsis*. The predominant

menaquinone was MK-9 (H_4) and the small amounts of MK-9 (H_2), MK-9 (H_6), and MK-9 (H_8). Their DNA G+C content ranged from 66.5-73.4 mol% as reported by Lechevalier *et al.*, (1977).

One strains, SB3-2 (Group III) contained LL and *meso* isomer of diaminopimelic acid which had the same pattern as the genus *Kitasatospora*. The predominant menaquinones were MK-9 (H_6) and MK-9 (H_8) and the small amounts of MK-9 (H_4) and MK-10 (H_0). DNA G+C content was 76.1 mol% as reported by Zang *et al.*, (1997) (Table 4.10).

Table 4.5 Physiological characteristics of 18 selected strains

Strain no.	NaCl			pH					Temperature		
	2%	4%	6%	4	5	7	9	10	10°C	28°C	45°C
S 1-2	+	+	+	+	+	+	+	+	+	+	+
S 3-1	+	+	w	+	+	+	+	+	-	+	-
SB 12-1	+	+	w	+	+	+	+	+	-	+	-
S 33-3	+	+	-	-	+	+	+	+	-	+	-
S 38-2	+	-	-	-	+	+	+	+	-	+	-
S 49-1	+	+	-	-	w	+	+	-	-	+	-
S 55-4	+	+	-	-	+	+	+	+	-	+	-
S 71-1	+	+	-	-	-	+	+	-	-	+	-
S 72-10	+	+	-	-	+	+	+	+	-	+	-
S 75-3	+	+	-	-	+	+	+	+	-	+	-
S 75-5	+	+	+	+	+	+	+	+	+	+	+
S 76-1	+	+	-	-	+	+	+	+	-	+	-
SB 7-3	+	+	+	-	+	+	+	+	-	+	-
S39-7	+	+	+	-	+	+	+	+	-	+	-
KC 19-1	+	+	w	-	-	+	+	+	-	+	-
KC 20-1	+	+	+	-	+	+	+	+	-	+	-
K 57-1	+	+	-	-	+	+	+	+	-	+	-
SB 3-2	+	-	-	-	+	+	+	+	-	+	-
KCTC19104^T	+	+	+	-	+	+	+	+	+	+	-
KCTC 9471^T	+	+	+	-	+	+	+	+	+	+	-

+, positive; w, weak; -, negative; *A keratinophila* KCTC 19104^T; *A. albidoflavus* KCTC 9471^T

Table 4.6 Biochemical characteristics of 18 selected strains

Strain no.	NO_3 reduction	Melanin formation	Strach hydrolysis	Esculin hydrolysis	Gelatin liquefaction	Skim milk	
						Coagulation	Peptonization
S 1-2	-	-	+	+	+	+	+
S 3-1	-	-	-	+	+	+	+
SB 12-1	-	-	-	+	+	+	+
S 33-3	-	-	+	-	+	+	+
S 38-2	-	-	+	-	+	+	+
S 49-1	+	-	+	+	+	+	+
S 55-4	-	-	-	-	+	+	+
S 71-1	-	-	+	+	+	+	+
S 72-10	-	-	+	+	+	+	+
S 75-3	-	-	-	+	+	+	+
S 75-5	-	-	+	+	+	+	+
S 76-1	-	-	+	+	+	+	+
SB 7-3	-	-	-	+	+	+	+
S 39-7	-	-	-	+	+	+	+
KC19-1	-	-	-	+	-	+	+
KC20-1	-	-	-	+	+	+	+
K 57-1	-	-	+	+	-	+	+
SB 3-2	+	-	-	+	+	+	+
KCTC 19104^T	+	-	-	+	+	+	+
KCTC 9471^T	-	-	-	+	+	+	+

+, positive; -, negative; *A .keratinophila* KCTC 19104^T; *A .albidoflavus* KCTC 9471^T

Table 4.7 Utilization of various carbon sources of 18 selected strains

Strain no.	None	Glucose	Glycerol	L-Arabinosee	D-Xylose	D-Mannitol	D-fructose	Sucrose	Melibiose	Rhamnose	Raffinose
S 1-2	-	+	+	+	+	+	+	+	+	+	+
S 3-1	-	+	+	+	+	+	+	+	+	+	+
SB 12-1	-	+	+	+	+	+	+	+	+	+	+
S 33-3	-	+	+	-	+	+	+	+	+	-	-
S 38-2	-	+	+	+	-	+	+	-	+	+	+
S 49-1	-	+	w	-	-	-	+	+	-	-	-
S 55-4	-	+	-	+	+	-	+	+	+	+	-
S 71-1	-	+	+	w	+	+	+	-	+	-	-
S 72-10	-	+	+	+	+	-	+	+	+	+	-
S 75-3	-	+	-	-	-	+	+	w	w	-	+
S 75-5	-	+	+	+	+	+	+	+	+	+	+
S 76-1	-	+	+	+	+	-	+	+	+	+	-
SB 7-3	-	+	+	-	+	+	+	+	+	+	+
S 39-7	-	+	+	+	+	+	+	+	-	+	+
KC19-1	-	+	+	+	+	+	+	+	+	+	+
KC20-1	-	+	+	-	+	+	+	+	+	+	+
K 57-1	-	+	+	-	+	+	+	+	+	+	+
SB 3-2	-	+	+	+	-	-	+	-	-	-	-
KCTC 19104^T	-	+	+	+	+	+	+	-	+	+	+
KCTC 9471^T	-	+	+	+	+	+	+	-	+	+	+

+, positive; w, weak; -, negative; *A .keratinophila* KCTC 19104^T; *A. albidoflavus* KCTC 9471^T

Table 4.8 Acid production from various carbohydrates of 7 strains in Group II

Strain no. / Carbon	SB7-3	S39-7	KC19-1	KC20-1	K57-1	KCTC 19104^T	KCTC 9471^T
None	-	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	-
Adonitol	+	+	+	-	+	+	+
L-Arabinose	+	+	+	-	-	+	+
Cellobiose	+	+	+	+	+	+	+
Dextrin	+	+	+	+	+	+	+
meso-Erythriol	+	+	+	-	+	+	+
D-Fructose	+	+	+	+	+	+	+
D-Galactose	-	+	+	+	+	+	+
meso-Inositol	+	+	+	-	+	+	+
Lactose	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+	+
Melezitose	-	+	+	+	+	-	-
Melibiose	-	+	+	+	+	+	-
Methyl D-glucoside	+	+	+	+	+	+	-
Raffinose	-	+	+	+	+	+	-
Rhamnose	-	-	+	+	+	+	-
Salicin	+	-	+	+	+	+	-
Sorbitol	-	-	+	-	+	-	-
Sucrose	-	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+	+

+, positive; -, negative

Table 4.9 Growth of 18 selected strains on YMA containing novobiocin (μg/ml)

Strain no.	Novobiocin 50 μg/ml	Novobiocin 100 μg/ml
S 1-2	-	-
S 3-1	-	-
SB 12-1	-	-
S 33-3	-	-
S 38-2	-	-
S 49-1	-	-
S 55-4	-	-
S 71-1	-	-
S 72-10	-	-
S 75-3	-	-
S 75-5	-	-
S 76-1	-	-
SB 7-3	w	-
S 39-7	-	-
KC 19-1	+	+
KC 20-1	+	+
K 57-1	+	+
SB 3-2	-	-
A .keratinophila KCTC 19104^T	-	-
A. albidoflavus KCTC 9471^T	-	-

+, positive; w, weak; -, negative

Table 4.10 Diaminopimelic acid, DNA G+C and menaquinone of 18 selected strains

Strain no.	Diaminopimelic acid		DNA G+C (mol%)	Menaquinone (%)				
	meso-DAP	LL-DAP		MK-9 (H_2)	MK-9 (H_4)	MK-9 (H_6)	MK-9 (H_8)	MK-10 (H_0)
S 1-2	-	+	72.5	1.06	2.2	71.5	25.2	-
S 3-1	-	+	74.1	2.7	8.3	30.6	55.9	-
SB 12-1	-	+	73.6	7.3	21.7	41.9	29.0	-
S 33-3	-	+	71.0	4.3	15.1	32.6	48.1	-
S 38-2	-	+	75.4	0.9	1.5	41.6	55.9	-
S 49-1	-	+	73.2	2.8	12.6	27.2	57.5	-
S 55-4	-	+	69.7	3.2	4.2	50.2	42.6	-
S 71-1	-	+	70.7	0.9	20.7	48.7	29.7	-
S 72-10	-	+	69.0	1.9	2.1	56.0	39.9	-
S 75-3	-	+	71.1	5.4	8.0	30.3	56.3	-
S 75-5	-	+	73.0	1.5	17.1	32.6	48.8	-
S 76-1	-	+	69.1	4.1	2.9	45.9	47.0	-
SB 7-3	+	-	66.5	20.4	76.4	1.1	2.1	-
S 39-7	+	-	67.2	0.3	94.5	3.1	2.1	-
KC 19-1	+	-	73.4	3.8	91.2	2.4	2.6	-
KC 20-1	+	-	73.2	16.4	78.4	1.5	3.7	-
K 57-1	+	-	73.1	5.4	88.5	1.5	4.6	-
SB 3-2	+	+	76.1	-	8.1	56.4	28.9	8.6

+, positive; -, negative

Table 4.11 Characteristics of *Streptomyces* strain S3-1

Characteristics	Result
Spore chain	Rectiflexibiles
Spore color	Vivid red
Colonial appearance	Velvety
Upper colony color on YMA	Vivid red
Lower colony color on YMA	Vivid red
Growth on agar medium: Yeast Extract-Malt extract, Tyrosine, Oatmeal, Asparagine and Inorganic salt starch	+
Growth at:	
10°C	-
28°C	+
45°C	-
pH 4-10	+
Growth in NaCl (2-6%)	+
Decomposition of:	
Strach	+
Esculin	+
Gelatin	+
Production of melanin	-
Reduction of nitrate	-
Coagulation of skim milk	+
Growth on sole carbon source (1%w/v) Glucose, Glycerol, Arabinose, D-xylose, D- manitol, D-fructose, Sucrose, Melibiose, Rhamnose, Raffinose	+
Cell wall	LL- diaminopimelic acid
Major menaquinone	MK-9 (H_6) and MK-9 (H_8)
G+C content (%mol)	74.1

+, positive; -, negative

3. 16S rDNA amplification and nucleotide sequence analysis

3.1 16S rDNA sequencing

The PCR products of 18 selected strains were determined for their 16S rDNA nucleotide sequences as illustrated in Appendix C.

3.2 16S rDNA sequences and phylogenetic tree analysis

The almost complete 16S rDNA sequence consisting of about 1300-1500 nucleotides were determined for some type strains of *Streptomyces, Amycolatopsis*, and *Kitasatospora*. Based on 16S rDNA sequences from the selected strains, the phylogenetic tree were constructed from evolutionary distances by using neighbor-joining method in the MEGA program version 2.1.

Phylogenetic analysis of Group I (S1-2, S3-1, SB12-1, S33-3, S38-2, S49-1, S55-4, S71-1, S72-10, S75-3, S75-5, S76-1) strains revealed that they were belonged to the genus *Streptomyces* comparing with some of the type strains validly described, and selected *Micromonospora chalcea* JCM 3082^T as an outgroup (Figure 4.11 and 4.12). The percentage of 16S rDNA sequence similarity of selected *Streptomyces* strains to another strains were showed in Table 4.12 and 4.13. The strains S72-10 and S76-1 were 99.9% related to each other and showed 99.6 and 99.8% similarility with *S. termitum* NBRC 13087^T, respectively. Strain S49-1 showed 99.4% similarity with *S. aureoversilis* NBRC 13021^T. The strains S1-2 and S75-5 were 100% related to each other and shared 16S rDNA nucleotide similarity within 99.8% with *S. hygroscopicus* DSM 41599^T. The strains of *S. hygroscopicus* were reported to produce geldanamycin and 17-0-demethylgeldanamycin (Glasby, 1993). Strains S38-2 showed 99.4% similarity with *S. aureofaciens* NBRC 12483^T which was reported to produced tetracycline (Tortota *et al.*, 1995). Strain S33-3 showed 99.8% similarity with *S. xanthocidicus* NBRC 13469^T. The strains of *S. xanthocidicus* were reported to produce respinomycins (Ubukata *et al.*, 1993). Strain S55-4 showed 99.9% similarity with *S. roseocinereus* NBRC 13829^T. Strains S71-1 showed 99.4% similarity with *S. mycarofaciens* NBRC 13792^T. The strains of *S. mycarofaciens* were reported to produce midecamycin (Harold, 1983). Strains S75-3 showed 99.4% similarity with *S. albospinus* NBRC 13846^T. The strains of *S. albospinus* were reported to produce spinamycin (Wang *et al.*, 1996). The strains S3-1 and SB12-1 were 99.5% related to each other and showed 99.6 and 99.7% similarility with *S. spectabilis* NBRC 13424^T respectively. The strains of *Streptomyces spectabilis* were reported to produce antimicrobial agents such as spectinabilin (Kakinuma *et al.*, 1976), spectinomycin (Yu and Fan, 1994), desertomycin (Ivanova, 1997), spectomycin (Staley and Rinehart, 1994), and streptovaricin (Spasova *et al.*, 1997).

Phylogenetic analysis of Group II strains, SB7-3, S39-7, KC19-1, KC20-1, and K57-1 revealed that they were belonged to the genus *Amycolatopsis* (Figure 4.13). The percentage of 16S rDNA sequence similarity of five selected *Amycolatopsis* strains to another strains were showed in Table 4.14. Strain S39-7 was closely related to *A.albidoflavus* KCTC 9471^T (Lee and Hah, 2001). The two organisms shared 16S rDNA similarity value of 99.2%. Strain SB7-3 showed 99.3% similarity with *A. keratinophila* KCTC 19104^T (Almusallam *et al.*, 2003). The strains KC19-1 and K57-1 were 99.3% related to each other and showed 99.3 and 99.2% similarility with *A. kentuckyensis* NRRL B-24129^T (Labeda *et al.*, 2003), respectively. The strains KC20-1 and K57-1 were 98.3% related to each other and strains KC20-1 and KC19-1 were 98.1% related to each other. Strain KC20-1 showed 98.1% similarity with *A. kentuckyensis* NRRL B - 24129^T. Five selected strains and related *Amycolatopsis* species were differentiated from each other as shown in Table 4.15. S39-7 could produce dark red soluble pigment, acid production from raffinose but no growth at 10 ° C. These characteristics could differentiate it from *A. albidoflavus* KCTC 9471^T. SB7-3 could produce light brown soluble pigment, but no acid production from D-galactose, rhamnose, raffinose and no growth at 10 ° C. These characteristics could differentiate it from *A. keratinophila* KCTC 19104^T. KC19-1 could produce acid from raffinose but not decompose gelatin. KC20-1 could not produce acid from L-arabinose, *meso*-inositol and sorbitol but produce acid from raffinose, while K57-1 could produce acid from raffinose but not from L-arabinose and not liquefy gelatin that differentiated them from *A. kentuchyensis* NRRL B-14129 ^{T}. DNA-DNA hybridization experiment is acknowledged as the superior method for the elucidation of relationships between closely related taxa, such as known strains and species, in which a DNA homology value of about >70% plays a dominant role (Wayne *et al.*, 1987). For further study, selected *Amycolatopsis* strains should be hybridized with closely related type strains for proposed that they possible new species.

Phylogenetic analysis of Group III strain, SB3-2 revealed that it was belonged to the genus *Kitasatospora* (Figure 4.14). The percentage of 16S rDNA sequence similarity of strain SB3-2 and *Kitasatospora* strains was showed in Table 4.16. Strain SB3-2 was closely related *K. putterlickiae* DSM 44665^T (Groth *et al.*, 2003). They shared 16S rDNA similarity value of 98.9%. Strain SB3-2 was differentiated from *K. putterlickiae* and closely related *Kitasatospora* species by melanin pigment and utilization of carbon (Table 4.17). However the DNA-DNA hybridization studies should resolve the taxonomic relationship to separate it as a new species.

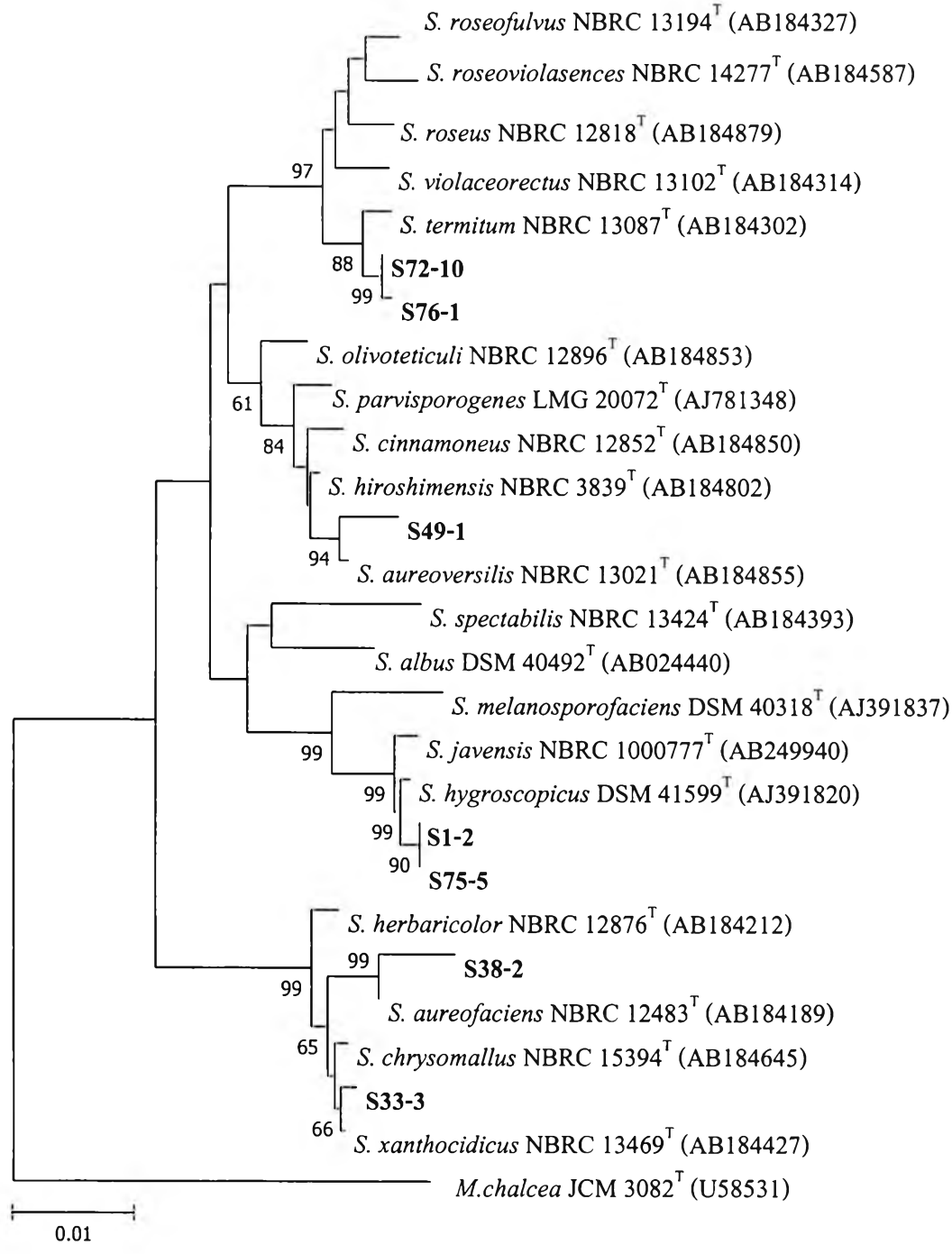


Figure 4.11 A neighbour-joining tree based on 16S rDNA sequences, showing the position of S72-10, S76-1, S49-1, S1-2, S75-5, S38-2, and S33-3. The tree validated by a bootstrap analysis (1000 replications) and values greater than 50% are indicated at the nodes. Bar, 0.01 substitutions per nucleotides position.

Table 4.12 Percentage similarities of S72-10, S76-1, S49-1, S1-2, S75-5, S38-2, S33-3 and related taxa

Accession no.	% Similarity																										
	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
1.S1-2	100																										
2.S38-2	95.3	100																									
3.S49-1	97.0	95.7	100																								
4.S33-3	95.9	98.7	96.6	100																							
5.S72-10	96.5	96.5	97.6	97.4	100																						
6.S75-5	100	95.3	97.0	95.9	96.5	100																					
7.S76-1	96.4	96.5	97.5	97.4	99.9	96.4	100																				
8.AB184302	96.6	96.8	97.4	97.7	99.6	96.6	99.8	100																			
9.AB184327	96.6	96.1	97.4	97.0	99.2	96.6	99.1	99.0	100																		
10.AB184879	96.6	96.0	97.3	96.9	99.2	96.6	99.1	99.1	99.3	100																	
11.AB184587	96.6	95.8	97.0	96.7	98.8	96.6	98.7	98.6	99.3	99.0	100																
12.AB184314	96.8	96.1	97.3	97.0	98.9	96.8	98.8	98.6	98.9	99.0	99.2	100															
13.AB184189	95.8	99.4	96.4	99.3	97.2	95.8	97.1	97.4	96.7	96.6	96.5	96.6	100														
14.AB184855	97.4	96.1	99.4	97.2	98.2	97.4	98.1	97.9	98.0	97.9	97.5	97.9	96.8	100													
15.AB184802	97.5	95.9	99.2	96.8	97.8	97.5	97.7	97.5	97.6	97.5	97.5	97.8	96.5	99.6	100												
16.AB184850	97.0	95.6	99.0	96.5	97.9	97.0	97.8	97.6	97.7	97.6	97.5	97.9	96.3	99.4	99.6	100											
17.AJ781348	97.5	96.1	98.8	96.8	97.5	97.5	97.4	97.4	97.4	97.4	97.2	97.5	96.7	99.2	99.6	99.2	100										
18.AB184853	96.8	96.5	98.4	97.2	98.4	96.8	98.3	98.3	98.3	98.3	98.1	98.4	97.1	98.8	99.1	99.1	99.1	100									
19.AB184427	96.1	98.8	96.8	99.8	97.4	96.1	97.4	97.7	97.0	96.9	96.7	97.0	99.4	97.2	97.0	96.7	97.0	97.4	100								
20.AB184212	96.1	98.5	96.6	99.5	97.3	96.1	97.2	97.5	96.8	96.8	96.9	97.2	99.1	97.0	97.3	97.0	97.3	97.7	99.7	100							
21.AB184645	96.2	98.8	96.6	99.7	97.4	96.2	97.4	97.7	97.0	96.9	96.7	96.9	99.4	97.0	97.0	96.5	97.1	97.4	99.8	99.5	100						
22.AJ391820	99.8	95.4	97.0	96.0	96.6	99.8	96.5	96.7	96.7	96.6	96.7	96.9	95.9	97.5	97.5	97.1	97.6	96.9	96.1	96.1	96.3	100					
23.AB249940	99.6	95.4	96.9	96.0	96.4	99.6	96.3	96.6	96.6	96.5	96.7	96.9	95.9	97.3	97.4	97.0	97.6	96.9	96.1	96.1	96.3	99.7	100				
24.AB184393	96.9	94.7	96.9	95.6	96.6	96.9	96.6	96.8	96.8	97.3	96.6	96.8	95.4	97.5	97.5	97.3	97.5	96.9	95.6	95.9	95.4	97.0	96.8	100			
25.AJ391837	98.3	95.1	96.8	95.9	96.2	98.3	96.1	96.6	96.3	96.1	96.2	96.1	95.8	97.2	97.3	97.0	97.5	96.7	96.1	96.1	96.2	98.4	98.4	96.7	100		
26.AB024440	97.6	95.0	97.2	95.9	96.9	97.6	96.8	97.0	97.0	97.5	97.3	97.5	95.6	97.6	97.7	97.9	97.8	97.5	96.1	96.2	95.9	97.7	97.9	97.9	97.8	100	
27.U58531	89.1	88.7	88.5	88.7	88.3	89.1	88.2	88.6	87.9	88.0	87.9	88.2	89.3	89.0	89.2	88.9	89.4	88.9	88.9	88.9	89.1	89.2	89.2	89.1	88.7	88.4	100

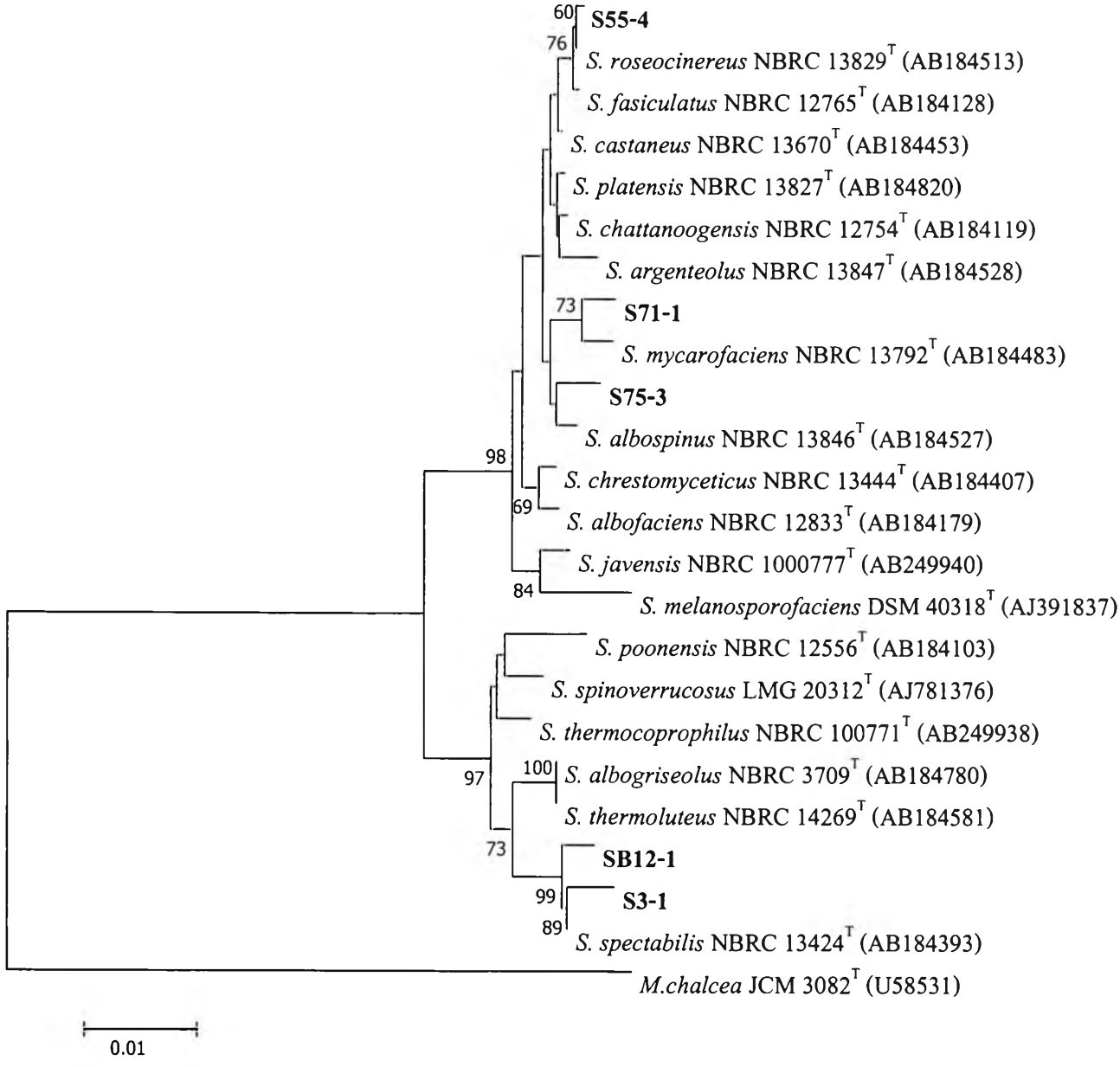


Figure 4.12 A neighbour-joining tree based on 16S rDNA sequences, showing the position of S55-4, S71-1, S75-3, SB12-1, and S3-1. The tree validated by a bootstrap analysis (1000 replications) and values greater than 50% are indicated at the nodes. Bar, 0.01 substitutions per nucleotides position.

Table 4.13 Percentage similarities of S55-4, S71-1, S75-3, SB12-1, S3-1 and related taxa

Accession no.	% Similarity																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1.S71-1	100																							
2.S75-3	99.2	100																						
3.S3-1	97.1	96.6	100																					
4.SB12-1	97.3	96.8	99.5	100																				
5.S55-4	99.1	99.1	96.8	97.0	100																			
6.AB184483	99.4	98.9	97.1	97.3	99.1	100																		
7.AB184119	99.1	99.3	96.9	97.0	99.6	99.0	100																	
8.AB184527	99.3	99.4	96.5	96.7	99.3	99.0	99.5	100																
9.AB184407	98.8	98.8	97.3	97.5	99.3	98.7	99.4	98.9	100															
10.AB184528	98.8	99.0	97.0	97.2	99.2	98.9	99.6	99.3	99.0	100														
11.AB184179	98.8	99.2	97.3	97.5	99.2	98.7	99.3	98.9	99.7	99.0	100													
12.AB184820	99.1	99.3	96.7	96.9	99.6	99.2	99.8	99.7	99.3	99.6	99.3	100												
13.AB184393	97.6	97.0	99.6	99.7	97.2	97.6	97.3	97.0	97.7	97.5	97.7	97.1	100											
14.AB184103	97.0	96.9	98.0	98.1	97.0	97.4	97.0	96.7	97.4	97.4	97.6	97.0	98.4	100										
15.AB249938	97.7	97.4	98.5	98.7	97.6	97.8	97.6	97.3	97.9	98.1	98.1	97.6	98.9	99.0	100									
16.AB184780	97.4	96.9	98.7	98.9	97.5	97.6	97.4	97.0	97.7	97.6	97.7	97.4	99.1	98.6	99.0	100								
17.AB184581	97.4	96.9	98.7	98.9	97.5	97.6	97.4	97.0	97.7	97.6	97.7	97.4	99.1	98.6	99.0	100.0	100							
18.AB184513	99.1	99.1	96.9	97.0	99.9	99.2	99.7	99.3	99.4	99.3	99.3	99.7	97.3	97.1	97.7	97.6	97.6	100						
19.AB184453	99.1	99.3	96.9	97.0	99.8	99.2	99.8	99.5	99.4	99.4	99.3	99.8	97.3	97.0	97.6	97.5	97.5	99.8	100					
20.AB184128	99.2	99.2	97.0	97.1	99.8	99.3	99.6	99.4	99.3	99.2	99.2	99.6	97.4	97.0	97.6	97.5	97.5	99.9	99.8	100				
21.AB249940	98.4	98.7	96.7	96.9	98.8	98.8	98.8	99.0	99.1	98.6	99.1	99.0	97.1	97.3	97.4	97.2	97.2	98.9	99.0	99.0	100			
22.AJ391837	97.8	98.2	96.4	96.5	98.2	98.4	98.3	98.5	98.3	98.6	98.3	98.5	96.8	96.8	97.2	97.0	97.0	98.2	98.4	98.3	98.9	100		
23.AJ781376	97.5	97.1	98.5	98.7	97.6	97.6	97.4	97.2	97.8	97.7	98.0	97.6	98.9	98.9	99.2	99.0	99.0	97.6	97.6	97.6	97.6	97.0	100	
24.U58531	89.1	89.7	89.2	89.4	89.1	89.2	89.3	89.2	89.4	89.6	89.7	89.3	89.6	89.5	89.4	89.5	89.5	89.2	89.3	89.3	89.6	88.9	89.5	100

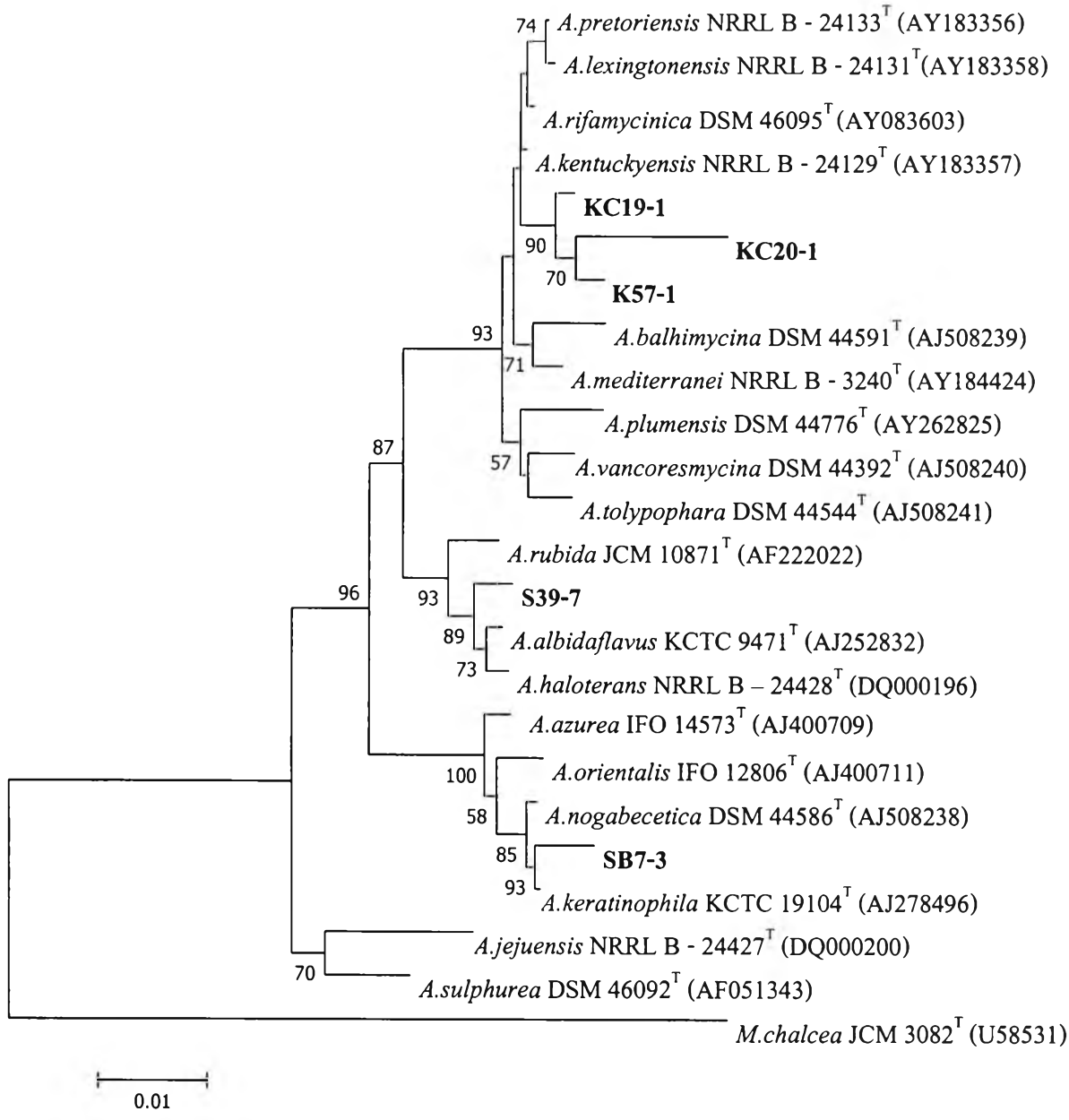


Figure 4.13 A neighbour-joining tree based on 16S rDNA sequences, showing the position of SB7-3, S39-7, KC19-1, KC20-1 and K57-1. The tree validated by a bootstrap analysis (1000 replications) and values greater than 50% are indicated at the nodes. Bar, 0.01 substitutions per nucleotides position.

Table 4.14 Percentage similarities of SB7-3, S39-7, KC19-1, KC20-1, K57-1 and related taxa

Accession no.	% Similarity																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1.S39-7	100																							
2.KC19-1	97.4	100																						
3.SB7-3	96.6	96.5	100																					
4.KC20-1	96.0	98.1	95.1	100																				
5.K57-1	97.1	99.3	96.1	98.3	100																			
6.DQ000196	99.0	97.7	96.6	96.1	97.5	100																		
7.AJ508240	98.1	98.8	95.7	97.4	98.6	98.4	100																	
8.AJ508238	97.4	97.0	99.2	95.6	96.7	97.2	96.2	100																
9.AJ400711	97.7	96.6	98.6	95.2	96.4	97.6	96.5	99.3	100															
10.AJ400709	97.6	97.1	98.6	95.6	96.9	97.4	96.4	99.3	99.3	100														
11.DQ000200	97.0	95.5	95.2	93.8	95.1	96.5	96.0	95.7	96.0	96.1	100													
12.AJ508241	97.6	98.6	95.7	97.2	98.3	97.7	99.2	96.2	96.4	96.6	96.1	100												
13.AY183356	97.6	99.2	96.5	97.7	98.8	97.4	98.8	96.9	96.5	97.2	96.0	98.9	100											
14.AY183358	97.7	99.2	96.6	97.8	98.9	97.5	98.7	97.0	96.6	97.4	95.9	98.8	99.9	100										
15.AF051343	97.4	96.2	95.7	94.7	96.0	97.4	96.4	96.4	96.7	96.7	97.8	96.1	96.5	96.6	100									
16.AY262825	97.2	98.3	95.2	97.0	98.1	97.4	98.7	95.7	96.1	96.1	95.5	98.8	98.7	98.6	96.0	100								
17.AJ508239	97.0	98.4	95.9	97.1	98.2	97.2	98.2	96.4	96.0	96.7	95.2	98.3	98.9	98.8	96.1	98.1	100							
18.AY083603	97.6	99.2	96.4	98.0	99.0	97.6	99.0	96.9	96.5	97.2	96.0	99.0	99.8	99.6	96.5	98.8	98.9	100						
19.AY184424	97.2	98.9	96.2	97.6	98.7	97.5	98.7	96.7	96.4	97.1	95.6	98.9	99.2	99.0	96.1	98.4	99.0	99.4	100					
20.AY183357	97.5	99.3	96.2	98.1	99.2	97.7	99.2	96.7	96.4	97.1	95.9	99.2	99.6	99.5	96.4	98.9	99.0	99.9	99.5	100				
21.AJ278496	97.1	97.1	99.3	95.6	96.6	97.1	96.2	99.8	99.0	99.0	95.6	96.2	97.1	97.2	96.2	95.7	96.4	96.9	96.7	96.7	100			
22.AF222022	98.9	97.7	96.9	96.2	97.5	99.0	98.1	97.4	97.1	97.5	96.4	97.5	97.7	97.8	96.9	97.0	97.4	97.7	97.6	97.8	97.4	100		
23.AJ252832	99.2	97.6	96.6	96.0	97.4	99.6	98.3	97.2	97.6	97.4	96.6	97.6	97.5	97.6	97.4	97.2	96.9	97.7	97.4	97.6	97.1	98.9	100	
24.U58531	88.2	88.3	88.3	87.1	87.9	88.1	88.1	88.6	88.6	88.8	89.2	87.8	88.8	88.6	89.6	87.9	88.5	88.8	88.8	88.6	88.8	88.6	88.1	100

Table 4.15 Differential characteristics of S39-7, SB7-3, KC19-1, KC20-1, K57-1 and the closest *Amycolatopsis* species

Characters	S39-7	KCTC 9471^T	SB7-3	KCTC 19104^T	KC19-1	KC20-1	K57-1	NRRL B-14129 ^{T}
Soluble pigment	Dark red	-	Light brown	-	-	-	-	Faint brownish
Acid production								
L-Arabinose	+	+	+	+	+	-	-	+
Cellobiose	+	+	+	+	+	+	+	+
D-Galactose	+	+	-	+	+	+	+	+
meso- Inositol	+	+	+	+	+	-	+	+
Lactose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Mannitol	+	w	+	+	+	+	+	+
Raffinose	+	-	-	+	+	+	+	-
Rhamnose	-	-	-	+	+	+	+	+
Sorbitol	-	-	-	-	+	-	+	+
D-Xylose	+	+	+	+	+	+	+	+
Decomposition of								
Casein	+	+	+	+	+	+	+	+
Gelatin	+	+	+	+	-	+	-	+
Growth at								
10 ° C	-	+	-	+	-	-	-	-
45 ° C	-	-	-	-	-	-	-	-

+, positive; w, weak; -, negative; *A. keratinophila* KCTC 19104^T; *A. albidoflavus* KCTC 9471^T; *A. kentuchyensis* NRRL B -14129 ^{T}.

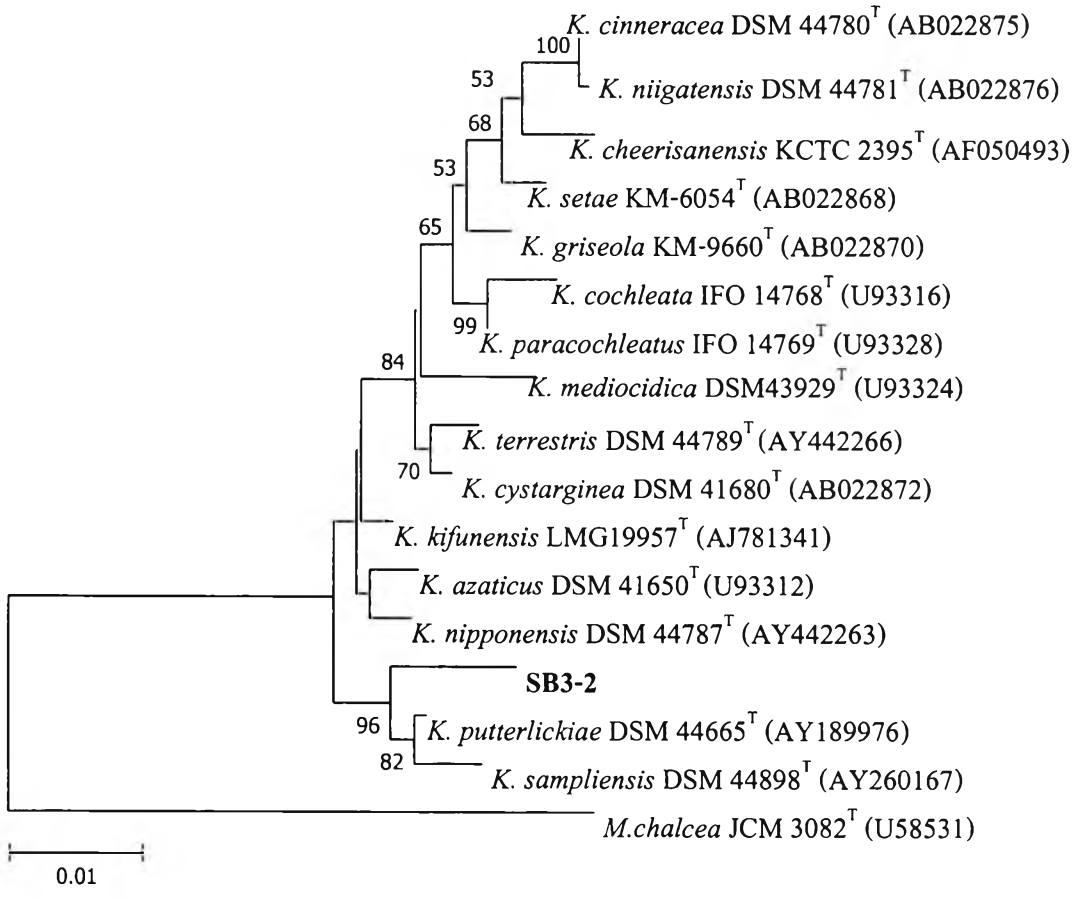


Figure 4.14 A neighbour-joining tree based on 16S rDNA sequences, showing the position of SB3-2. The tree validated by a bootstrap analysis (1000 replications) and values greater than 50% are indicated at the nodes. Bar, 0.01 substitutions per nucleotides position.

Table 4.16 Percentage similarities of SB3-2 and related taxa.

Accession no.	% Similarity																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1.SB3-2	100																
2.AY189976	98.9	100															
3.U93312	98.3	98.8	100														
4.AB022875	96.5	97.3	97.7	100													
5.AB022868	97.0	97.7	98.1	99.3	100												
6.AY442266	97.6	98.2	98.6	98	98.6	100											
7.AB022872	97.8	98.4	98.6	98.2	98.8	99.5	100										
8.AB022876	96.4	97.2	97.7	99.9	99.3	98	98.1	100									
9.U93324	97.3	97.9	98.3	97.9	98.4	98.6	98.7	97.8	100								
10.AJ781341	98.2	98.8	99.2	97.8	98.4	98.9	99.2	97.7	98.4	100							
11.U93316	96.8	97.4	97.9	98.3	98.1	98.7	98.7	98.2	98.0	98.1	100						
12.AY442263	98.0	98.6	99.3	97.7	98.3	98.8	99	97.6	98.3	99.4	98.0	100					
13.U93328	97.3	98.0	98.4	98.8	98.6	99.3	99.3	98.7	98.6	98.6	99.5	98.5	100				
14.AB022870	97.2	98.0	98.3	99.1	98.8	98.7	99.0	99.0	98.5	98.4	98.9	98.1	99.5	100			
15.AF050493	96.5	97.1	97.6	99.0	98.7	98.0	98.3	99.0	97.7	97.9	98.2	97.6	98.7	99.0	100		
16.AY260167	98.3	99.4	98.3	97.1	97.1	97.6	97.8	97.0	97.3	98.2	97.3	98.0	97.8	97.8	97.0	100	
17.U58531	88.3	89.4	89.3	88.8	88.6	88.7	89.0	88.7	88.2	89.5	88.6	89.2	89.0	89.1	88.8	89.1	100

Table 4.17 Differential characteristics of SB3-2 and the closely related *Kitasatospora* species

Characteristic	SB3-2	*K. putterlickiae* DSM 44665 ^{T}	*K. azatica* IFO 13803 ^{T}	*K. kifunesis* DSM 41654 ^{T}
Aerial mycelia	R	R	R	R
Melanin formation	-	+	-	+
Nitrate reduction	+	+	+	-
Starch hydrolysis	-	-	+	+
Gelatin liquefaction	+	+	-	-
Utilization of carbon				
L-Arabinose	+	-	+	+
D-Fructose	+	+	+	-
D-Mannitol	-	-	-	+
D-Sucrose	-	+	-	+
D-Xylose	-	-	+	+
G+C content (mol%)	76.1	ND	70.5	ND

+, positive; - negative; R, Rectiflexibiles; ND, no data

3.3 DNA-DNA relatedness of *Amycolatopsis* strains

The strains SB7-3 showed DNA relatedness values of 39.2% similar to *A.keratinophila* KCTC 19104^T, and S39-7 showed DNA relatedness values of 67.1% similar to *A.albidoflavus* KCTC 9471^T (Table 4.17) The values are well lower than 70% recommended by Wayne *et al.*, (1987), so they could be classified as a new species.

Table 4.18 DNA-DNA relatedness of strains SB7-3, S39-7, KC19-1, K57-1 and related *Amycolatopsis* species

Strain no.	Percentage DNA complementary with Labeled DNA		
	S39-7	KCTC 19104^T	KCTC 9471^T
SB7-3	5.3	39.2	4.2
S39-7	100	6.0	67.1
KC19-1	1.0	1.0	2.6
K57-1	4.5	3.7	16.5
A. keratinophila KCTC 19104^T	16.6	100	2.2
A. albidoflavus KCTC 9471^T	59.2	10.4	100

4. Distribution of actinomycetes in soils

The 18 selected strains were isolated from various soils samples colltected from Chiangrai, Nan, Phatthalung, Songkhla, Chaiyaphum, and Trat provinces and they were found to be *Streptomyces, Kitasatospora,* and *Amycolatopsis*. The known species, *Streptomyces hygroscopicus* (1 strain) and *S. spectabilis* (1strain) were distributed in soil from Chiangrai. *Streptomyces xanthocidicus* (1 strain), *S. spectabilis* (1 strain), *Amycolatopsis* sp. nov. (3 strains) and *Kitasatospora* sp. nov. (1 strain) were distributed in soil from Nan. *Streptomyces aureofaciens* (1 strain) and *Amycolatopsis* sp. nov. (1 strain) were distributed in soil from Phatthalung. *Streptomyces aureoversilis* (1 strain) was distributed in soil from Songkhla. *Streptomyces roseocinereus* (1 strain) and *Amycolatopsis* sp. nov. (1 strain) were distributed in soil from Chaiyaphum. *Streptomyces albospinus* (1 strain), *S. hygroscopicus* (1 strain), *S. mycarofaciens* (1 strain), and *S. termitum* (2 strains) were distributed in soil from Trat (Table 4.19).

Table 4.19 Distribution of actinomycetes strains

Province	Strain	Closest species	% similarity of 16S rDNA	Identification
Chiangrai	S1-2	*S. hygroscopicus*	99.8	*S. hygroscopicus*
Chiangrai	S3-1	*S. spectabilis*	99.6	*S. spectabilis*
Nan	SB12-1	*S. spectabilis*	99.7	*S. spectabilis*
Nan	S33-3	*S. xanthocidicus*	99.8	*S. xanthocidicus*
Nan	KC19-1	*A. kentuckyensis*	99.3	*Amycolatopsis* sp. nov.
Nan	KC20-1	*A. kentuckyensis*	98.1	*Amycolatopsis* sp. nov.
Nan	SB7-3	*A. keratinophila*	99.3	*Amycolatopsis* sp. nov.
Nan	SB3-2	*K. putterlickiae*	98.9	*Kitasatospora* sp. nov.
Phatthalung	S38-2	*S. aureofaciens*	99.4	*S. aureofaciens*
Phatthalung	S39-7	*A. albidoflavus*	99.2	*Amycolatopsis* sp. nov.
Songkhla	S49-1	*S. aureoversilis*	99.4	*S. aureoversilis*
Chaiyaphum	S55-4	*S. roseocinereus*	99.9	*S. roseocinereus*
Chaiyaphum	K57-1	*A. kentuckyensis*	99.2	*Amycolatopsis* sp. nov.
Trat	S75-3	*S. albospinus*	99.4	*S. albospinus*
Trat	S75-5	*S. hygroscopicus*	99.8	*S. hygroscopicus*
Trat	S71-1	*S. mycarofaciens*	99.4	*S. mycarofaciens*
Trat	S72-10	*S. termitum*	99.6	*S. termitum*
Trat	S76-1	*S. termitum*	99.8	*S. termitum*

5. Fermentation of the selected strains and antimicrobial activity

Antimicrobial activities of 18 selected strains were shown in Table 4.20 and 4.21. Sixteen strains exhibited antimicrobial activity against *Staphylococcus aureus* ATCC 6538, 15 strains against *Bacillus subtilis* ATCC 6633, 6 strains against *Escherichia coli* ATCC 25922, 12 strains against *Micrococcus luteus* ATCC 9341, 11 strains against *Pseudomonas aeruginosa* ATCC 27853, 5 strains against *Candida albicans* ATCC 10231, 2 strains against all tested microorganisms. Seven strains against Methicillin resistant *S. aureus* (MRSA) 266, 6 strains against both MRSA 269 and 643. Strains SB7-3, S39-7, KC19-1, KC20-1, K57-1 which were identified as a new species in genus *Amycolatopsis* could produce antimicrobial substances especially in strain S39-7 that showed antimicrobial activity against Methicillin resistant *Staphylococcus aureus* 266, 269, 643. In addition, strain SB3-2 which was identified as a new species in genus *Kitasatospora* showed antimicrobial activity against *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 9341, and *Pseudomonas aeruginosa* ATCC 27853. The studies of antimicrobial substances from *Amycolatopsis* and *Kitasatospora* strains were interesting to further studies on the fermentation, extraction, purification, and structure elucidation. Strain S3-1 which was identified as *S. spectabilis* was selected for secondary metabolite fermentation study because this strain exhibited good antimicrobial activity against *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 9341, *Pseudomonas aeruginosa* ATCC 27853 and Methicillin resistant *Staphylococcus aureus* 266, 269, 643. Moreover, there had no reports to study on antimicrobial substances from *S. spectabilis* in Thailand. While S1-2 and S75-5 identified as *S. hygroscopicus* were similar in antimicrobial activity as the strain produced geldanamycin that already reported by Jongrungruanchok *et al.*, (2006). Therefore, the strain S3-1 was selected for secondary metabolite fermentation study.

Table 4.20 Antimicrobial activity of 18 selected strains

Strain no.	Inhibition zone (mm)					
	S. *aureus* ATCC 6538	*B. subtilis* ATCC 6633	*E. coli* ATCC 25922	*M. luteus* ATCC 9341	*Ps. aeruginosa* ATCC 27853	*C.albicans* ATCC10231
S 1-2	27	20	11	31	8	11
S 3-1	26	22	-	31	9	-
SB 12-1	23	22	-	31	8	-
S 33-3	17	15	-	-	-	-
S 38-2	8	16	-	21	10	-
S 49-1	12	19	11	30	8	-
S 55-4	17	12	-	-	-	9
S 71-1	18	-	-	12	-	15
S 72-10	20	20	-	-	9	-
S 75-3	-	15	9	-	-	10
S 75-5	27	21	12	31	9	11
S 76-1	19	18	-	-	8	-
SB 7-3	14	11	-	15	-	-
S 39-7	19	22	20	22	9	-
KC 19-1	15	-	-	-	-	-
KC 20-1	11	11	-	19	15	-
K 57-1	-	-	12	14	-	-
SB 3-2	15	12	-	20	9	-

Table 4.21 Antimicrobial activity of 18 selected strains against methicillin resistant *S. aureus* (MRSA)

Strain no.	Inhibition zone (mm)		
	MRSA 266	MRSA 269	MRSA 643
S 1-2	18	17	17
S 3-1	15	16	15
SB 12-1	14	15	14
S 33-3	12	11	11
S 38-2	-	-	-
S 49-1	9	-	-
S 55-4	-	-	-
S 71-1	-	-	-
S 72-10	-	-	-
S 75-3	-	-	-
S 75-5	18	18	17
S 76-1	-	-	-
SB 7-3	-	-	-
S 39-7	14	12	12
KC 19-1	-	-	-
KC 20-1	-	-	-
K57-1	-	-	-
SB 3-2	-	-	-

6. Extraction and Fractionation of the extract of *S. spectabilis* S3-1

The crude ethyl acetate extract from the fermentation broth of S3-1 was detected for active spot by bioautography technique and showed an active spot with R_f value 0.8 (solvent system 15%MeOH in CH_2Cl_2).

Crude extract of S3-1 was purified by quick column chromatography and flash column chromatography to give 13 fractions as shown in Scheme 4.1.

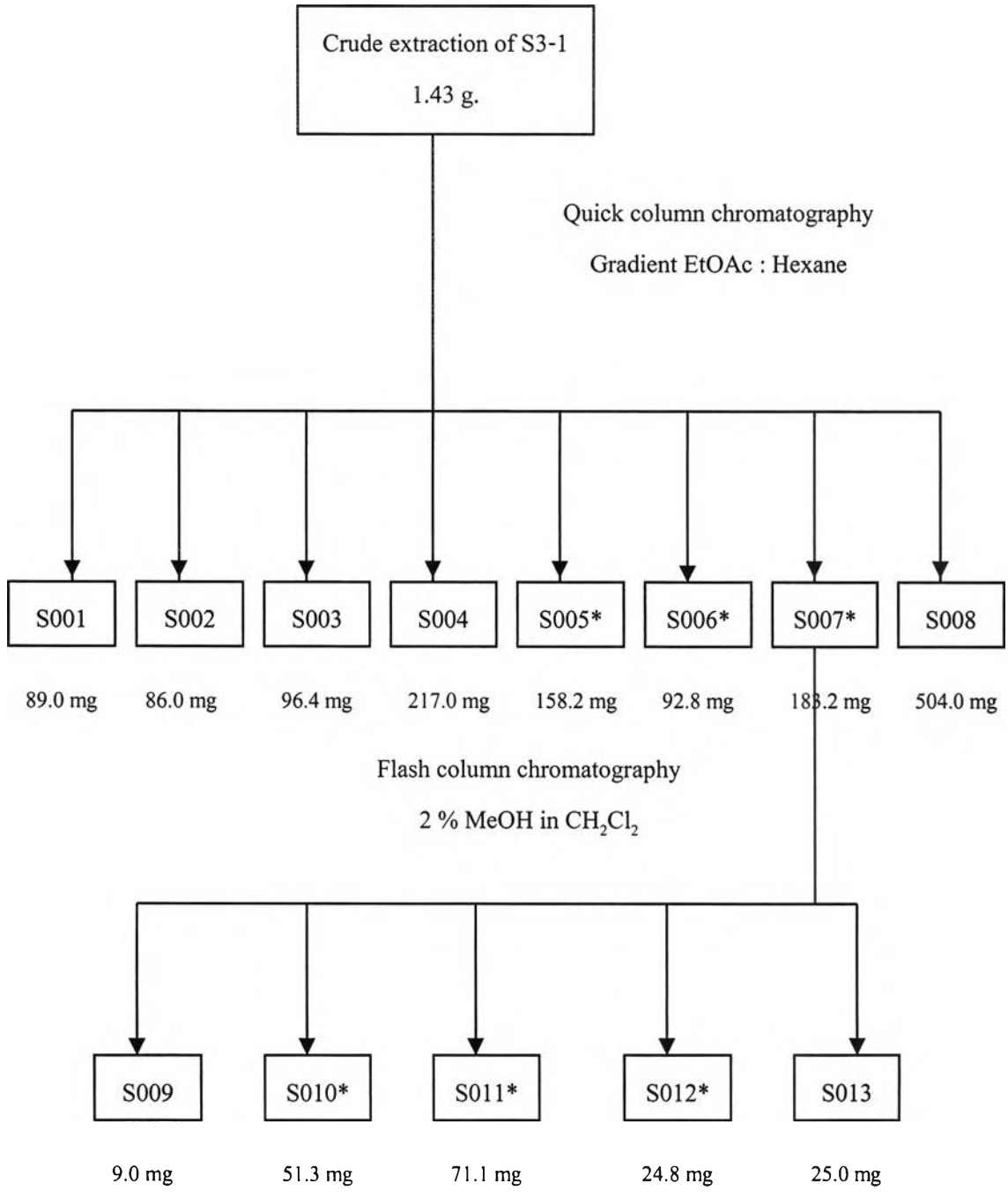
The fractions from the ethyl acetate extract were tested by agar disc diffusion method using *S. aureus* ATCC 6538, Methicillin resistant *S. aureus* 266, 269, 643, *B. subtilis* ATCC 6633, *M. luteus* ATCC 9341 and *Ps. aeruginosa* ATCC 27853 as tested microorganisms. The active fractions (fraction codes S005, S006, S007, S010, S011, and S012) against test microorganisms were shown in Table 4.22. The fraction code S010 which contained a yellow spot at R_f value 0.8 (solvent system 15%MeOH in CH_2Cl_2) showing the highest antimicrobial activity were submitted to measure proton NMR spectrum (Figure 4.15).

The proton MNR spectrum of S010 revealed that S010 was a mixture of several components containing the characteristics of olefinic protons in the region of chemical shifts at 5.5-8.5 ppm, the chelated hydroxyls at about 12 ppm, the methoxy signals at 3.5-4.0 ppm, the heteroatom-connected methine or methylene protons at 3.0-5.5 ppm and methyl protons at 0.9-2.5 ppm.

This study, the strain S3-1 which identified as *S. spectabilis* produced active fractions that exhibited antimicrobial activity as mentioned above however, the strains of *S. spectabilis* were reported to produce a number of antimicrobial agents such as spectinabilin (Kakinuma *et al.*, 1976), spectinomycin or actinospectacin (Yu *et al.*, 1994), desertomycin (Ivanova, 1997), spectomycin (Staley *et al.*, 1994), and streptovaricin (Spasova *et al.*, 1997). The active fractions of strain S3-1 isolated from soil in Chiangrai, Thailand, are still interesting for further purification and structure elucidation of the active compounds.

Table 4.22 Antimicrobial activity of fractions

Fraction code	Inhibition zone (mm)						
	S. *aureus* ATCC 6538	MRSA 266	MRSA 269	MRSA 643	*B. subtilis* ATCC 6633	*M. luteus* ATCC 9341	*Ps. aeruginosa* ATCC 27853
S001	-	-	-	-	-	-	-
S002	-	-	-	-	-	-	-
S003	-	-	-	-	-	-	-
S004	-	-	-	-	-	-	-
S005	24	12	11	12	18	31	8
S006	26	13	14	14	19	31	9
S007	26	15	15	15	22	31	9
S008	-	-	-	-	-	-	-
S009	-	-	-	-	-	-	-
S010	26	15	15	15	21	31	9
S011	20	11	11	10	14	30	-
S012	12	8	8	8	11	15	-
S013	-	-	-	-	-	-	-



* positive antimicrobial activity

Scheme 4.1 Chromatography of crude extraction from *S. spectabilis* S3-1

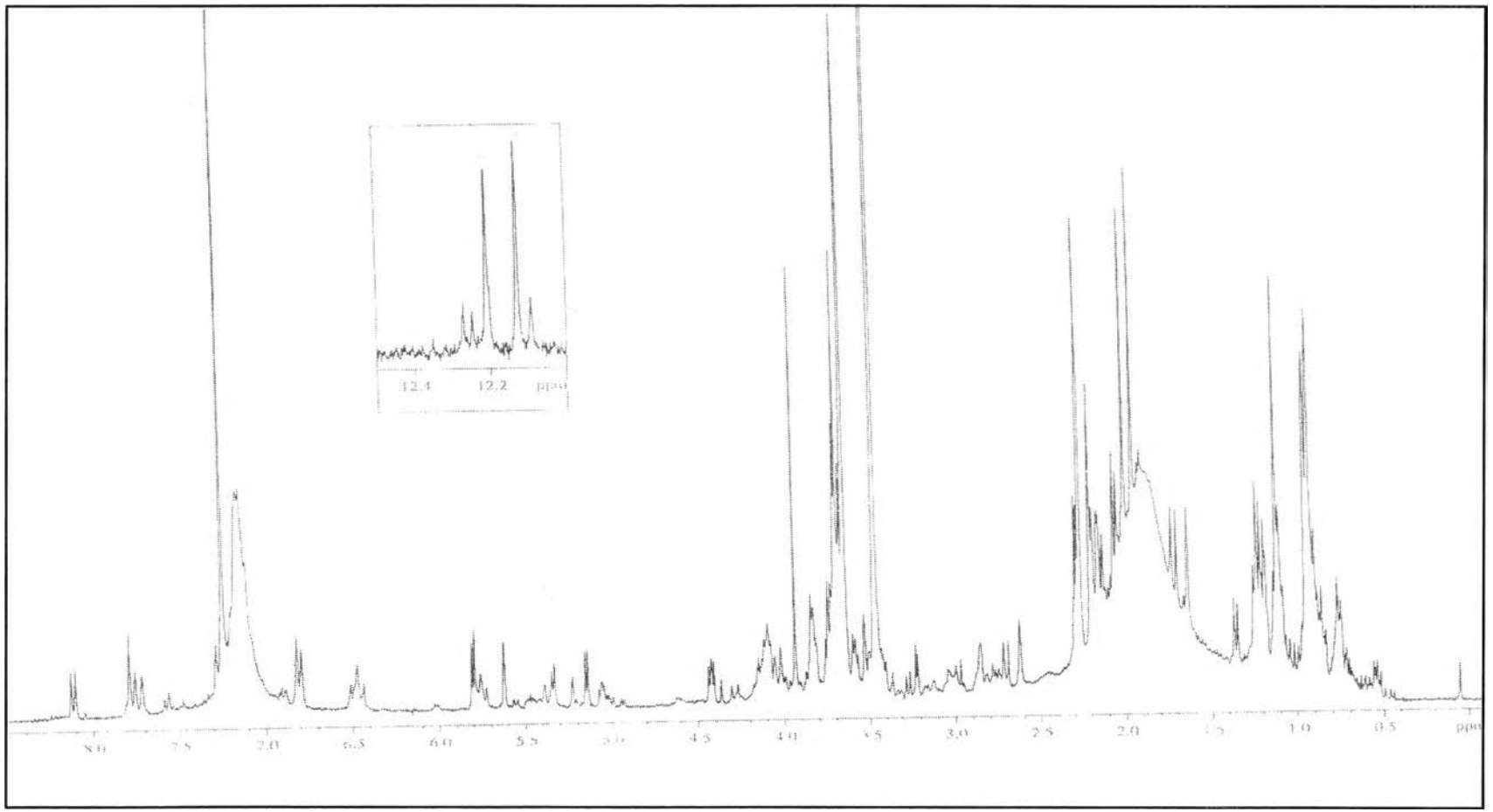


Figure 4.15 The 300 MHz proton MNR spectrum of fraction code S010 in $CDCl_3$