CHAPTER IV

RESULTS

1. Isolation of *Streptomyces* strains

Sources of soil samples, province where collected and strain number are shown in Table 9.

Table 9 Source of *Streptomyces* strains

Sample no.	Province	Date of isolation	Strain no.
A3	Krabi	28 June 1995	A3-2
A5	Chaiyaphoom	14 July 1995	A5-1
C3	Chaiyaphoom	14 July 1995	C3-1
C10	Chaiyaphoom	14 July 1995	C10-1
C11	Chaiyaphoom	14 July 1995	C11-1
CB5	Chanthaburi	17 May 1996	CB5-3
			CB5-6
CB12	Chanthaburi	17 May 1996	CB12-1
			CB12-2
			CB12-4
			CB12-8
CPM3	Chon Buri	17 May 1996	CPM3-1
			CPM3-2
L1	Lampang	1 May 1996	L1-2
L4	Lampang	24 May 1996	L4-1
L5	Lampang	24 May 1996	L5-2
			L5-4

Table 9 (Continued)

Sample no.	Province	Date of isolation	Strain no.
KB1	Kanchanaburi	17 May 1996	KB1-1
NK3	Nong Khai	1 April 1996	NK3-1
R1	Ratchaburi	1 May 1996	R1-3
R4	Ratchaburi	10 May 1996	R4-1
			R4-3
R6	Ratchaburi	10 May 1996	R6-2
R7	Ratchaburi	10 May 1996	R7-1
			R7-2
R8	Ratchaburi	10 May 1996	R8-4
019	Chon Buri	17 May 1996	019-2C
			019-3C
020	Chon Buri	17 May 1996	020-5P
024	Chon Buri	17 May 1996	024-P
029	Chon Buri	17 May 1996	029-P
CS1	Chachoengsao	14 June 1997	CS1-1
			CS1-2
SK1	Srakaew	14 June 1997	SK1-1
			SK1-2

2. Screening of antibiotic producing *Streptomyces*

Activity of antibiotic producing *Streptomyces* isolated from soil as screened by streak plate method is shown in Table 10.

Table 10 Antimicrobial activity of *Streptomyces* strains

Code	Inhibition distance (mm)			
	Bacillus subtilis ATCC 6633	*Candida albicans* ATCC 10231	*Escherichia coli* ATCC 25922	*Staphylococcus aureus* ATCC 25923
A3-2	16.5	0	0	6.6
A5-1	17.3	0	0	0
C3-1	5.0	0	0	0
C10-1	12.0	0	0	34.1
C11-1	11.2	0	0	6.8
CB5-3	17.5	21.3	0	24.2
CB5-6	0	0	0	4.3
CB12-1	0	0	0	6.8
CB12-2	17.9	0	0	0
CB12-4	16.4	0	0	9.2
CB12-8	33.3	0	0	0
CPM3-1	23.2	0	0	0
CPM3-2	7.2	0	0	0
L1-2	11.9	0	7.1	0
L4-1	12.3	0	0	0
L5-2	18.7	0	0	0
L5-4	0	0	0	0
KB1-1	17.4	0	0	0
NK3-1	4.7	0	0	5.7
R1-3	8.6	0	0	7.2
R4-1	17.1	0	0	23.3
R4-3	11.8	0	0	0
R6-2	24.3	0	0	0
R7-1	14.6	0	0	9.3

Table 10 (Continued)

Code	Inhibition distance (mm)			
	Bacillus subtilis ATCC 6633	*Candida albicans* ATCC 10231	*Escherichia coli* ATCC 25922	*Staphylococcus aureus* ATCC 25923
R7-2	16.3	0	0	0
R8-4	6.9	0	0	0
019-2C	0	0	15.7	12.5
019-3C	11.8	0	0	0
020-5P	17.6	0	4.6	4.3
024-P	17.8	0	0	0
029-P	10.3	0	0	0
CS1-1	17.4	0	0	0
CS1-2	12.5	0	0	6.4
SK1-1	0	0	0	0
SK1-2	10.4	0	0	18.8

3. Identification of strains

3.1 Morphological and Cultural Characteristics

The characteristic of the spore and hyphae of *Streptomyces* determined by direct light microscopic examination are shown in Table 11.

Table 11 Morphological charactertics of *Streptomyces* strains on YM medium

Code	Spore	Hyphae	Spore chain	Spore/ Chain	Spore size (μm)	Hyphae size (μm)
A3-2	oval	branch	rectus	10-50	0.75×0.5	0.25
C3-1	cylindrical	branch	flexuous	10-50	0.5×0.25	0.25

Table 11 (Continued)

Code	Spore	Hyphae	Spore chain	Spore/ Chain	Spore size (μm)	Hyphae size (μm)
C11-1	cylindrical	branch	retinaculiaperti	10-50	0.5×0.75	0.4
CB5-3*	cylindrical	branch	spiral	10-50	1.25	0.4
CB5-6	circular	branch	rectus	3-10	2.0	1.0
CB12-2	circular	branch	rectus	3-10	0.5	0.25
CB12-4	circular	branch	spiral	10-50	1	0.3
CPM3-1	circular	branch	retinaculiaperti	3-10	0.75	0.25
CPM3-2	circular	branch	spiral	10-50	0.7	0.7
L4-1	cylindrical	branch	flexuous	10-50	0.5×0.25	0.25
L5-2	circular	branch	flexuous	6-30	0.8	0.5
L5-4	cylindrical	branch	rectus	6-30	0.5×0.25	0.25
KB1-1	circular	branch	spiral	10-50	1	0.25
R1-3	circular	branch	retinaculiaperti	6-30	0.7	0.4
R4-3	cylindrical	branch	rectus	6-30	0.5×1	0.5
R6-2	circular	branch	flexuous	10-50	0.5	0.5
R7-1	circular	branch	flexuous	10-50	0.5	0.5
R7-2	circular	branch	retinaculiaperti	6-30	1	0.4
R8-4	circular	branch	rectus	3-10	1.5	1.0
019-3C	rod	branch	flexuous	10-50	1.25×0.3	0.6
020-5P	circular	branch	spiral	10-50	0.75	0.25
024-P	circular	branch	rectus	6-30	0.75	0.5
029-P	oval	branch	flexuous	6-30	0.75×0.5	0.25
CS1-1	circular	branch	spiral	6-30	1	0.25
CS1-2	circular	branch	spiral	6-30	0.7	0.5
SK1-1	circular	branch	flexuous	10-50	1	0.25
SK1-2	circular	branch	spiral	10-50	1	0.25

* On PCA (dilute 10 %)

The cultural characteristics of *Streptomyces* on YM media are shown in Table 12

Table 12 Cultural characteristics of *Streptomyces* strains on YM media

Code	Growth	Substrate mycelium	Aerial mycelium	Reverse color	Soluble pigment
A3-2	good	dark grayish yellow	light reddish gray	light brownish yellow	-
C3-1	moderate	dark gray	reddish gray	dark grayish yellow	-
C11-1	good	yellow	brownish gray	yellowish brown	-
CB5-3	good	brownish yellow	light gray	yellow	-
CB5-6	low	reddish brown	light reddish brown	dark reddish brown	-
CB12-1	moderate	yellow	yellow	pale yellow	
CB12-2	moderate	yellow	light yellow	pale yellow	-
CB12-4	good	dark olive green	dark olive gray	dark brown	-
CPM3-1	good	pale yellow	white	yellow	-
CPM3-2	good	light brown	white	light yellow	-
L4-1	good	dark gray	reddish gray	dark grayish yellow	-
L5-2	moderate	dark brownish yellow	light reddish gray	brown	-
L5-4	good	brownish orange	white	brownish yellow	-
KB1-1	good	dark olive gray	olive gray	pale yellow	-

Table 12 (Continued)

Code	Growth	Substrate mycelium	Aerial mycelium	Reverse color	Soluble pigment
R1-3	good	dark gray	olive gray	dark olive gray	-
R4-3	good	yellow	light reddish gray	yellow	-
R6-2	low	light grayish yellow	light reddish gray	dark greenish yellow	-
R7-1	moderate	dark reddish brown	white	dark brown	-
R7-2	moderate	yellow	white	pale yellow	-
R8-4	low	dark greenish brown	olive brown	black	-
019-3C	good	grayish yellow	white	yellow	-
020-5P	good	grayish yellow	light reddish gray	brown	-
024-P	good	yellow	reddish gray	cream	-
029-P	good	dark reddish gray	dark reddish gray	yellow	-
CS1-1	good	dark gray	dark olive gray	yellowish brown	-
CS1-2	good	dark gray	pale olive gray	yellow	-
SK1-1	good	dark gray	pale olive gray	dark gray	-
SK1-2	good	dark reddish gray	dark reddish gray	dark brown	-

The cultural characteristics of *Streptomyces* sp. CB 12-4, 020-5P, L5-4 and L4-1 on various media are shown in Table 13.

Table 13 Cultural characteristics of *Streptomyces* strains on various media

Medium	**Growth**	**Substrate mycelium**	**Aerial mycelium**	**Reverse color**	**Soluble pigment**
Inorganic salt starch					
CB12-4	moderate	yellow	light yellow	yellow	+
020-5P	good	dark reddish gray	reddish gray	light brownish gray	-
L5-4	good	dark yellowish gray	reddish gray	light brownish gray	-
L4-1	good	light yellow	dark reddish gray	brown	-
Glucose asparagine					
CB12-4	good	dark gray	dark olive gray	dark olive gray	-
020-5P	good	dark reddish gray	dark reddish gray	yellow	-
L5-4	good	light orange	white	light yellow	-
L4-1	good	dark reddish gray	dark reddish gray	dark grayish yellow	-
Nutrient agar					
CB12-4	low	pale yellow	cream	yellow	-
020-5P	moderate	pale yellow	cream	light yellow	-
L5-4	low	pale yellow	white	yellow	-

The cultural characteristics of *Streptomyces* sp. CB 5-3 on various media was shown in Table 14.

Table 14 Cultural characteristics of *Streptomyces* sp. CB5-3 on various media

medium	Growth	Substrate mycelium	Aerial mycelium	Reverse color	Soluble pigment
Glucose asparagine	good	dark grayish yellow	cream	grayish yellow	-
Inorganic salt starch	good	brownish yellow	white	yellow	-
Nutrient agar	moderate	light yellow	white	light yellow	-
Oatmeal agar	good	olive brown	light greenish yellow	light olive brown	-
Sucrose nitrate agar	moderate	yellow	yellow	yellow	-
Yeast starch agar	moderate	yellow	white	yellow	-

The photograph of *Streptomyces* sp. CB 5-3 cultured on PCA (dilute 10%) is shown in figure 1.

Figure 1 The colonial appearance of *Streptomyces* sp. CB5-3 on PCA (dilute 10 %)

The scanning electronmicrograph of spore of *Streptomyces* sp. CB 5-3 on PCA (dilute 10%) is shown in figure 2.

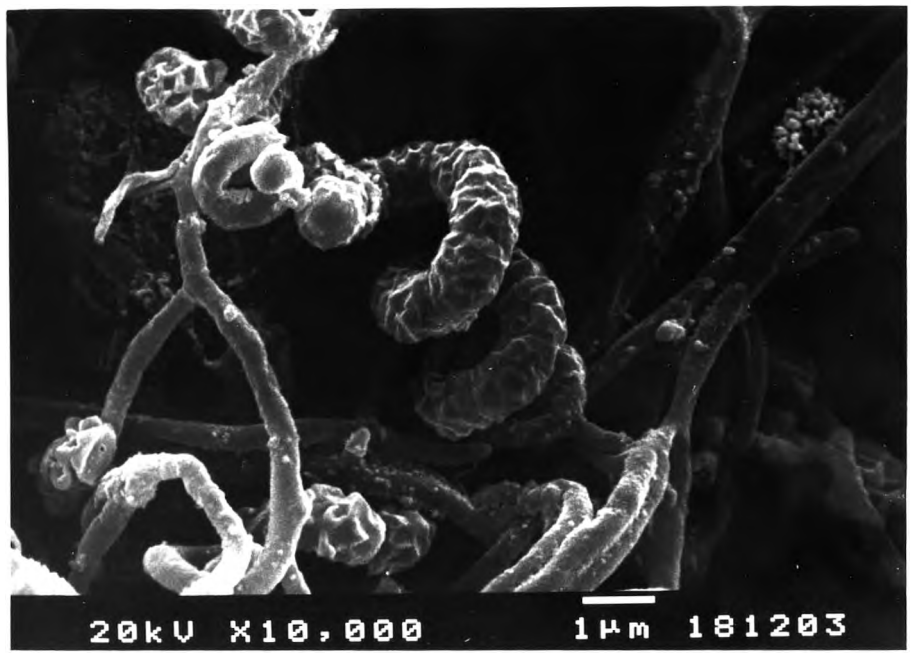

Figure 2 ..canning electronmicrograph of *Streptomyces* sp. CB5-3 on PCA (dilute 10%)

The scanning electronmicrograph of *Streptomyces* sp. CB 5-3 on YM agar is shown in figure 3.

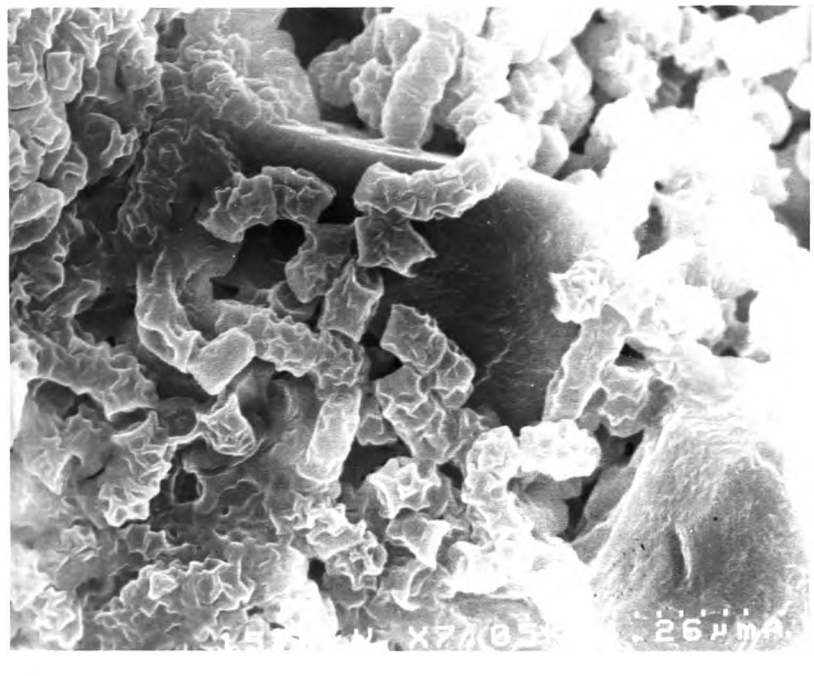

Figure 3 Scanning electronmicrograph of spore-chain of *Streptomyces* sp. CB5-3 on YM agar

Spore chain morphology of the strain CB 5-3: Section spiral: light spiral in dense cluster. Mature spore chains generally contain 10 to 50 spores per chain. Spore surface: Warty, individual spores are poorly delineated.

From the morphological characteristics, strain CB 5-3 was placed it in the genus *Streptomyces.* The morphological, cultural and physiological characteristics of strain CB5-3 indicated that it had many features in common with the *S. hygroscopicus* such as cultures in the Gray series. Scanning electron microscopy studies showed that the spores of culture had rugose surface. Based on morphological, cultural and physiological characteristics, strain CB 5-3 was considered to belong to the species *S. hygroscopicus.*

3.2 Physiological and biochemical characteristics

The utilization of carbon sources of *Streptomyces* isolated in this study are summarized in Table 15.

Table 15 Carbon utilization of *Streptomyces* sample

Code	L-arabinose	Sucrose	D-xylose	D-mannitol	D-fructose	Rhamnose	Raffinose
A3-2	+	+	+	+	+	+	+
C3-1	+	+	+	+	+	+	+
C11-1	+	+	+	+	+	+	+
CB5-3	+/-	-	+/-	+	+	+	-
CB5-6	+/-	+	+	+	+	-	+
CB12-1	-	+	+	+	+	-	+
CB12-2	+	+	+	+	+	+	+
CB12-4	+	+	+	+	+	+	+
CPM3-1	+	+	+	+	+	+	+
L4-1	+	+	+	+	+	+	+

Table 15 (Continued)

Code	L-arabinose	Sucrose	D-xylose	D-mannitol	D-fructose	Rhamnose	Raffinose
L5-2	+	+	+	+	+	+	+
L5-4	+	+	+	+	+	+	+
KB1-1	+	+	+	+	+	+	+
R1-3	+	+	+	+	+	+	+
R4-3	+	+	+	+	+	+	+
R6-2	+	+	+	+	+	+	+
R7-1	+	+	+	+	+	+	+
R7-2	-	+	+	+	+	+	+
R8-4	-	-	-	+	-	-	+
019-3C	+	+	+	+	+	+	+
020-5P	+	+	+	+	+	+	+
024-P	+	+	+	+	+	+	+
029-P	+	+	+	+	+	+	+
CS1-1	+	+	+	+	+	+	+
CS1-2	+	+	+	+	+	+	+
SK1-1	+	+	+	+	+	+	+
SK1-2	+	+	+	+	+	+	+

The utilization of carbon sources of *Streptomyces* sp. CB 5-3 are summarized in Table 16.

Table 16 Carbon utilization of *Streptomyces* sp. CB5-3

C-source	7 days	10 days	14 days	conclusion
L-arabinose	+	+	+	+/-
Fructose	++	+	++	+
Rhamnose	+	++	++	+
Sucrose	+/-	+	+	-
Raffinose	+/-	+	+	-

Table 16 (Continued)

C-source	7 days	10 days	14 days	Conclusion
Xylose	+	+	++	+/-
Manitol	++	+	++	+
D-manose	++	++	++	+
Starch	++	+	++	+
Cellobiose	++	++	++	+
D-galactose	++	++	++	+
Maltose	++	++	++	+
Melibiose	++	++	++	+
D-ribose	+	++	++	+
L-sorbose	+/-	+/-	+/-	-
Tehalose	+	++	++	+

The melanin production and biochemical reaction of *Streptomyces* are shown in Table 17.

Table 17 Biochemical characteristics of *Streptomyces*

Code	Nitrate reduction	Starch hydrolysis	Gelatin hydrolysis	Melanoid pigment
A3-2	+	++	+/-	-
C3-1	+	++	+	+
C11-1	+	++	+	-
CB5-3	-	++	+	+
CB5-6	+	-	-	-
CB12-1	-	++	+	-
CB12-2	+	++	+	-
CB12-4	+	++	+	-
CB12-8	+	-	+	-
CPM3-1	-	++	+	-

Table 17 (Continued)

Code	Nitrate reduction	Starch hydrolysis	Gelatin hydrolysis	Melanoid pigment
CPM3-2	-	++	+/-	-
L4-1	-	++	+	-
L5-2	-	++	+	-
L5-4	+	+++	+	-
KB1-1	+	+++	+	-
NK3-1	+	+	+	+
R1-3	-	++	+	-
R4-3	+	++	+/-	-
R6-2	+	++	+	-
R7-1	-	++	+	-
R7-2	+	++	+	-
R8-4	-	+	+	-
019-3C	+	++	+	-
020-5P	+	+	+	-
024-P	+	++	+	-
029-P	-	++	+	-
CS1-1	+	+	+	-
CS1-2	-	++	+	-
SK1-1	+	++	+	+
SK1-2	-	-	+/-	-

The physiological characteristics and biochemical reactions of *Streptomyces* sp. CB5-3 is shown in Table 18.

Table 18 Physiological and biochemical characteristics of *Streptomyces* sp. CB5-3

Physiological and biochemical characteristics	Result
ISP 2 +NaCl 4%	+, -
ISP 2 +NaCl 7%	-, -
ISP 2 +NaCl 10%	-, -
ISP 2 +NaCl 13%	-, -
growth at 30 °C	+
growth at 37 °C	+
growth at 42 °C	+/-
growth at 55 °C	-
litmus milk	peptonization

4. Antibiotic production

The selected strain CB 5-3, was fermented in 8 various liquid media. The PY medium produced the highest activity of antibiotic against *Candida albicans* at day 14 (Table 19).

Table 19 Antibiotic production in different media

Medium	Colour		Growth	Zone diameter
	Before	After		(mm)
medium No. 1	light yellow	greenish yellow	+++	0, 0
medium No. 2	brown	dark brown	+	0, 0
medium No. 3	light yellow	yellowish orange	++	0, 14.0
medium No. 4	light yellow	olive yellow	+	15.0, 15.9
medium No. 5	light brown	light brown	++	0, 0
medium No. 6	yellow	dark olive	+	14.4, 12.0

Table 19 (Continued)

Medium	Colour		Growth	Zone diameter (mm)
	Before	After		
PY	light yellow	brown	++++	23.6, 22.9
SS	brownish orange	dark orange	+	0, 0

Only the strain CB 5-3 isolated from Chanthaburi could inhibit *Candida albicans*, a test organism causing Candiasis and other diseases. So it was selected for further study and cultured for antibiotic producing.

Streptomyces sp CB5-3 was tested with test organism as shown in Table 20.

Table 20 Antimicrobial activity of *Streptomyces* sp. CB 5-3 (mm)

Test organism	inhibition distance(mm)	
	I	II
Bacillus cereus ATCC 9634	14.8	15.2
Bacillus subtilis ATCC 6633	20.5	14.5
Staphylococcus aureus MRSA I	21.7	17.9
Staphylococcus aureus MRSA II	15.0	12.1
Staphylococcus aureus ATCC 25923	28.0	20.4
Staphylococcus citreus (PCU)	24.6	18.8
Staphylococcus epidermidis ATCC 1622	22.6	17.5
Micrococcus luteus ATCC 9341	13.5	15.2
Alcaligenes sp.	0	0
Enterococcus faecalis (PCU)	14.5	16.3
Escherichia coli ATCC 25922	0	0
Pseudomonas aeruginosa ATCC 27853	0	0
Salmonella typhimurium (PCU)	0	0
Candida albicans ATCC 10231	18.7	23.9
Microsporum gypseum (PCU)	23.1	26.9

Table 21 Effect of pHs on activity against *Candida albicans* ATCC 10231 of *Streptomyces* CB5-3

Initial pH	Inhibition zone (mm)
6.0	16.1
6.25	19.6
6.5	22.3
6.75	20.5
7.0	20.5

5. Extraction

The crude isobutanol extract was detected for active spot by bioautographic technique, showed an active spot with R_f value = 0.7 (ethyl acetate : methanol : water, 6 : 4 : 1).

The fraction extracted from fermentation broth of CB-5-3 strain was tested by agar disc diffusion method using *Candida albicans* ATCC 10231 as test organism.

Table 22 Activity of fractions against *Candida albicans* ATCC 10231

Zone diameter (mm)							
A (100 mg/disc)		**B (10 mg/disc)**		**C (2 mg/disc)**		**D (trace)**	
A1/5	16.9	B1/1-2	14.7	C4/1-6	13.3	D1/4	6.7
A1/24-41	13.8	B1/3-4	8.0	-	-	D2/1-4	28.3
A2/2	17.5	B2/2-3	18.5	-	-	D3/1-5	26.8
A2/3-4	12.5	B3/2	12.6	-	-	D4	15.4
A2/41-50	15.0	B4/2	11.8	-	-	-	-
-	-	B4/3	6.3	-	-	-	-