Chapter 5

Discussion

In order to isolate successfully a wide variety of actinomycetes from soil samples it was necessary to eliminate or greatly curtail fungal and bacteria spreaders in the isolation medium without producing an adverse effect on actinomycetes. This could be accomplished in one, or a combination of more than one, of the following ways : 1) control of the medium constituents, 2) addition of inhibitors to the medium, 3) prior treatment of the soil sample.[16]

Fungal contaminants could be virtually eliminated by adding antifungal agents to the isolation medium. In this study, nystatin was added at a level of approximately 50 µg/ml[97] to selective media such as those above have effectively eliminated most undesired contaminants in isolation plates.

The isolation of actinomycetes from cave soil in our primary screening showed 3.81 percent to total microorganisms. For the collecting of soil samples was too shallow,about 6 cm in depth, there was no isolated colonies of actinomyces under anaerobic condition. An early representative study was that of Danish soils, the numbers of actinomycetes varied from none to 13 million per gm and the percent of the total microflora from 0 to 73.[96]

In the antibiotic screening, fifty one strains of actinomycetes out of 104 strains (49.04%) were able to elaborate antibiotic substances

by the streak plate method as compared to Kuroya and Co-workers demonstrated only 360 active ones out of 1,800 strains (20.0%).[98]

The isolated of strain ST-13-2 from Mungkorn-tong cave, that inhibited more clear inhibition distances to all test organisms, was selected for further study. The results of morphological characteristics showed that it belongs to genus *Streptomyces*. The cultural and physiological characteristics of strain ST-13-2 were compared with those of the known species of *Streptomyces* described in "The Actinomycetes, Vol. 2" by Waksman[10] and "Bergey's Manual of Determinative Bacteriology (8^{th} ed)".[11] The results indicate that strain ST-13-2 is closely related to *S. parvullus*. Difference observed between these two strains were as follows : the spore chain of strain ST-13-2 was long open spiral, spore cylindrical, no soluble pigment on nutrient agar and no gelatin liquefaction while the spore chain of *S. parvullus* was long closed spiral, spore spherical, yellow soluble pigment on nutrient agar and slow gelatin liquefaction. These differences were not sufficient to consider strain ST-13-2 as a new species.

The ability of strain ST-13-2 to produce broad spectrum antibiotics in 3 kinds of liquid medium showed that the glucose soybean medium was superior to glucose peptone medium and maltose soybean medium. The medium supplied nutrients for growth, energy, building of all substance and biosynthesis of fermentation products. Of particular importance were the source of carbon and nitrogen in the medium, since microbial cells and fermentation products were composed largely of these elements. A poor choice of medium components could cause limited cellular growth and alter the type and ratios of products. Thus the types and amounts of the nutritive components of a medium

were critical.[83] According to the composition of 3 kinds of liquid medium, it seemed that glucose and soybean powder were suitable for the source of carbon and nitrogen in antibiotic production of strain ST-13-2 in this study.

During microbial growth, pH changes can occur for one of several reasons. Obviously, an acidic or alkaline fermentation product can alter the pH picture. Also, an inorganic salt component of the medium can cause pH changes. The media selected are then studied further in several point of view. The initial pH and temperature were varied so as to determine the effect of pH and product yields.[83] The optimum pH and temperature for antibiotic production of strain ST-13-2 fermented in the glucose soybean medium were pH 7 (before sterilization) at 23°C. The incubation peroid of high yield was between 2 and 6 days.

The antibiotic substances of strain ST-13-2 were determined by thin layer chromatography. The results of bioautography using *S. aureus* showed that they are in water-soluble basic group.[69,92] The Rf values are not any identical with penicillin, kanamycin and other standard known antibiotics used except cloxacillin. The Rf value of cloxacillin was 0.26 in this study. For the chemical detection test on chromatoplate provided no information about the biological activity of the components, this was only through comparison with the bioautograph.

In the laboratory evaluation of antibiotics from strain ST-13-2, the disc-susceptibility of four pathogenic organisms using thirty isolated of each were summarized in table 15 and the range of inhibition

zone provided from the various dilutions of fermented broth from strain ST-13-2 was shown in Table 16.

Table 15 The antibiotic susceptibility test of various pathogenic organisms

Antimicrobial agents	Percentage of sensitive strains			
	S. aureus	*E. coli*	*Ps. aeruginosa*	*K. pneumoniae*
Amikacin	—	—	50.0	—
Cefotaxime	63.0	40.0	6.7	6.7
Ceftriazone	—	96.7	—	63.3
Cloxacillin	100.0	—	—	—
Erythromycin	40.0	—	—	—
Gentamicin	—	80.0	—	30.0
Moxalactam	—	—	10.0	—
Netilmicin	—	—	66.7	—
Penicillin G	0	—	—	—
Tobramycin	—	80.0	—	30.0

Table 16 The range of inhibition zone of antibiotics from strain ST-13-2 against various pathogenic organisms

Dilution	Range of inhibition zone (mm) against 30 isolates of			
	S. aureus	*E. coli*	*Ps. aeruginosa*	*K. pneumoniae*
U_1	12.1-16.4	11.6-16.6	0-22.3	11.3-17.3
U_2	13.5-17.6	12.0-18.0	0-22.8	12.6-18.1
U_3	14.2-18.5	13.6-19.0	0-23.5	13.7-20.7
U_4	15.8-20.0	14.2-20.5	0-26.0	15.0-22.1

The pathogenic bacteria obtained in this study are common causative agents of infectious diseases found in the hospital. In this study, the sensitive strains of *S. aureus*, *E. coli*, *Ps.aeruginosa* and *K. pneumoniae* to cefotaxime are 63.0%, 40.0%, 6.7% and 6.7% respectively. All isolated strains of *S. aureus* are penicillin G resistant.

The susceptibility test of antibiotics from the fermented broth of strain ST-13-2 increase the range of inhibition zone when concentrated broth dilutions were used. They were able to inhibit all isolates of *S. aureus*, *E.coli* and *K. pneumoniae* while they inhibit only some isolates of *Ps.aeruginosa*. The chemotherapy of *Ps. aeruginosa*, that causes the serious infection, is still the great problem in the patient; so the antibiotic substances from strain ST-13-2 may be either solve or support the treatment.

According to the broad spectrum of antibiotic from strain ST-13-2 to all 6 test organisms in secondary screening and the laboratory evaluation, the isolation and the purification of the antibiotics from the fermented broth for physico-chemical properties, structural determination and biological properties are very interesting to the subsequent study.