CHAPTER V

DISCUSSION

A number of *Streptomyces* strains were isolated from 24 soil samples collected in Thailand. In the course of screening for antimicrobial agent, 30 strains from 35 strains exhibited antibacterial activity against *Bacillus subtilis* ATCC 6633, 15 strains are active against *Staphylococcus aureus* ATCC 25923, 3 strains can inhibit the gram-negative bacterium, *Escherichia coli* ATCC 25922 and only strain CB5-3 showed antifungal activity against *Candida albicans* ATCC 10231.

The isolated *Streptomyces* strains have yellow, gray, brown and black colony, white, cream, yellow and gray spore, and reverse color is cream, yellow, brown, black, green.

Spores have oval, circular, cylindrical and rod shape, form spiral, rectus, flexuous and retinaculiaperti spore chains, produce no soluble pigment. Almost all strains can use arabinose, fructose, rhamnose, sucrose, raffinose, xylose and manitol, except for strains CB5-3, CB5-6, CB12-1, R7-2 and R8-4 which can use some of these carbon sources. Most of isolated strains can hydrolyze gelatin and starch.

Eighteen out of 30 strains can reduce nitrate, 4 out of 30 strains produce melanin pigment. The morphological, cultural and physiological characteristics of all strains indicated that they had many features in common with *Streptomyces* (Goodfellow, 1989)

The strain CB5-3 was selected because of its antifungal activity. The cultural characteristics on various media of this strain show different features. In addition, soluble pigment was not produced when glucose asparagine, inorganic salt starch, nutrient agar, oatmeal agar, sucrose nitrate agar or yeast starch agar was used.

This strain can hydrolyse starch and gelatin, produce melanin pigment, grow on ISP medium with 4% NaCl. Moreover, it can grow at 30, 37 °C, but weakly grow at 42 °C. When tested with litmus milk, it showed peptonization. This strain can use several kinds of carbon source. Based on its morphological, cultural and physiological characteristics, strain CB 5-3 was considered to belong to the species *S. hygroscopicus* (Holt, 1989; Shirling and Gottlieb, 1972). Media No. 1-6, SS and PY were used as fermentation media (see Appendix). The result of fermentation in PY media of CB 5-3 showed high antibacterial activity against *C. albicans* ATCC 10231. This strain indicated the good antifungal activity when grew in the medium adjusted pH to 6.5 to 7.0.

The isobutanol extract from the fermentation broth of strain CB 5-3 showed antifungal activity. This extract was separated by column chromatography. After activity screening test by agar disc diffusion method, it was found that fraction D4 (R_f 0.7 (ethyl acetate : methanol : water = 6 : 4 : 1)) showed antifungal activity. When bioautographic detection of antibiotics in preparation chromatogram was used with crude extract, an active spot with R_f = 0.7 (ethyl acetate : methanol : water = 6 : 4 : 1) was detected.