CHAPTER V

CONCLUSION

In this research, endophytic fungi that can produce starch hydrolyzing enzyme was studies. Twenty five isolated endophytic fungi were obtained from Research Center of Bioorganic Chemistry (RCBC), Faculty of Science, Chulalongkorn University. The fungi were tested for amylase activity. When the starch agar flooded with iodine solution EF6 produce amylase to hydrolyze soluble starch with clear zone around 13-16 mm. The growth rate was measured by amount of dried weight. The maximum growth rate is 10 days. The highest enzyme activity was obtained on the 8^{th} day of incubation and pH of culture filtrate was between 5.0 and 6.0.

The culture filtrate from cultivation EF6 in starch broth with pH 5.5 after 8 days of incubation were performed protein precipitation with $(NH_4)_2SO_4$ at 30%, 60% and 90%. At 90% of $(NH_4)_2SO_4$, there are the highest amount of precipitation protein and amylase activity. The precipitated protein was purified by Ion Exchange Chromatography using Q sepharase as media. The unbound peak (A1) exhibited amylase activity and appeared multiple bands of proteins in SDS-PAGE. The fractions were further purified by Gel filtration using Superdex 75 as media. The chromatogram showed that amylase activity was found in fraction G2 and G3. The purity and component of protein were detected using SDS-PAGE. The results showed that G2 has two bands (G2A and G2B). G3 has single band called G3A. From the result of Native-PAGE, it showed that only G2B and G3A contained amylase activity and molecular weight are around 62 kDa. Consequently, both proteins might be the same amylase.

The amylase from three steps of purification, precipitated with $(NH_4)_2SO_4$, Q Sepharase and Superdex75, represent 3.2 mg total protein from 5 L of culture, 2486 unit of total activity , 776.8 U/mg of specific activity and 14.93 fold of purification over the original culture filtrate with about 10.93% recovery of amylase activity. The K_m and V_{max} value were 2.63 mg and 1.25 mM/min, respectively.

In addition, the characterization of purified amylase suggested that most active is the range from 50-60°C. After 30 minutes of incubation, amylase was found to be stable lower than 50°C. The activity decrease rapidly when the enzyme was incubated at temperature higher than 55°C, retained about 43% activity at 60°C. The optimum pH of amylase activity was 5.0-6.0.

Purified enzyme (G3) was identified by using Tandem Mass Spectrometry (ESI-Q-TOF). Purified enzyme may be glucoamylase due to partial amino acid sequences matched with the partial amino acid sequence of glucoamylase (3.2.1.3) from *Amorphotheca resinae* and statistically significant.