CHAPTER 5

DISCUSSION AND CONCLUSION

At present, documents relating to alkaline lipase producing microorganisms isolated in Thai environment have not much been presented, In this study of screening alkaline lipase bacteria at pH 9.0, was conducted by using oil substrate with dye rhodamine B to generate an orange fluorescent halo around the colony instead of clear zone (Kouker and Jaeger, 1987). Due to the simplicity of examination, this technique was performed to screen many strong alkaline producers. Among them, Bacillus strain A30-1 was also screened (Wang and et al.,1995). Alkaline lipase activity of bacteria reported in this thesis was also demonstrated and originated in Thailand.

Two of 350 strains of alkaline lipase producing bacterial isolates, ALP-40.3 and ALP-42.11, were selected. They both were able to produce considerable amounts of alkaline lipases. They were characterized and identified to be ***Pseudomonas species***. These two alkaline lipolytic bacteria were capable to utilize lipid as their substrates. Although lipolytic activity of strain ALP-42.11, based on the size of orange fluorescent halo, is larger than that at strain ALP-40.3 but by examining the lipase activity of strain ALP- 40.3 was higher than that of the other. It may be implied that the quantities of lipase produced by both strains were not corresponding to the activities.

The present study indicated that areas, where oil contaminated soil or lipid waste exit, are suitable lipase producing bacterial source because essentially extracellular lipases are produced by stimulation of inducible substrate as lipids including hydrocarbons. It was found that lipases can

be excreted by hydrocarbon oxidizing bacteria in various media that contain substrates such as olive oil or n-parafins (Gawel and Chen, 1997). Alkaline lipase producing bacteria strain ALP-40.3 and strain ALP-42.11 obviously secrete extracellular lipase on LRA used in the first step of screening.

The result of experiment examining the effect of pH and temperature on growth can be concluded as follows. Growth of both selected bacterial was present in the wide range from slightly acidic condition to alkaline region and shown to be maximum at pH 8.0. For bacterial strain ALP-40.3, however, had no growth at pH 5.0 where as viable cells of strains ALP-42.11 were considerably found. It may be reasonable to say that they are alkalophiles. The result from this study show that temperature at 35°C was suitable for growth of selected bacterial strain ALP 40.3 while the optimum temperature on growth of strain ALP 42.11 was 25°C. However, the temperature between 45°C and 65°C, no growth of them was exhibited. It is possible to say that they are not thermophiles.

In this study, the difference of medium and incubation time on alkaline lipase production by ALP-40.3 and ALP-42.11 can be summarized as follows. A large amount of produced lipases was found when bacterial strain ALP-40.3 and ALP-42.11 were cultivated in LPM with the same pH value and used 18 hrs of incubation time but incubation temperature used was different. It could be concluded that LPM was the appropriate medium for lipase production of both isloates, when compared with LOB. It may be due to favorable nutrient in LPM, which contained gum arabic (emulsifier) for both bacterial strains. The enhancement of extracellular lipase production in the presences of different polysaccharides including gum arabic was also reported with

contained gum arabic (emulsifier) for both bacterial strains. The enhancement of extracellular lipase production in the presences of different polysaccharides including gum arabic was also reported with lipases from ***Serratia*** and ***Bacillus thermocalenulatus*** (Winkler et. al., 1979; Schmidt-Dannert et. al., 1994). Moreover, Winkler et. al. (1979) proposed the theory of detachment for explaining this phenomenon. Therefore, the gum arabic may cause a release of member bound of lipases produced by both of the selected bacterial strains into the LPM.

Some properties of crude alkaline lipase produced by ALP-40.3 and ALP-42.11 on some environmental factors, such as pH, temperature and substrate specificity, were investigated. The results of the present study, the crude ALP-40.2 lipase had an optimum pH 8.0 while the crude ALP-42.11 lipase was 9.0 (**Figure 4.14**). Very recently, many publications on microbial lipases passing high alkalophilic properties have also been reported. One of them which excrete extracellular lipase was ***Pseudomonas pseudoalcaligenes F-11*** (Lin et. al., 1996). Some properties of crude and partially purified lipase from that species were also studied.

The stability of crude ALP-40.3 and ALP-42.11 lipases was shown in the alkaline region between pH 8.0–10.0 (**Figure 4.15**). Thus it can be said that both bacterial strain were able to produce alkaline lipases.

Generally, lipases excreted from ***Bacillus*** and ***Pseudomonas*** strains seem to be relatively thermostable (Wang et, al., 1995; Schmidt-Dannert et. al., 1994; Lin et. al., 1996; Nishio et. al., 1987). The results of the present study, the optimum temperature of crude ALP-40.3 and ALP-42.11 lipases can be summarized as follows. The temperature optimum on ALP-40.3 lipase activity was at 45°C and that of ALP-42.11 lipase activity was shown at 65°C. However, the stability of both of the selected

bacterial strains was quite similar because the residual activity of lipases from them was decreased at the same point (75°C, **Figure** 4.17).

From this study, the alkaline lipases activities produced by ALP-40.3 and ALP-42.11 were 13639.33 and 700.67 unit/ml, respectively. Comparing to the former investigations, the lipase activities were higher than those reported in some other researches (**Table 5.2**).

Because of the unique properties of the lipases produced by the selected bacterial isolates, these extracellular lipases should be further studied on the other aspects such as the resistance of the lipase to hydrogen peroxide and alkaline protease including purification. These properties allows possibility of these alkaline lipases to use for industrial applications such as in laundry detergents, or even treatment of lipid waste.

In conclusion, this research has reported general properties and characteristics of alkaline lipase producing bacteria. Because of research demand in country to find out the alkaline lipase producing bacteria with high activity including unique properties of the lipase produced. These advantages would provide valuable applications. Moreover, the further investigation at the molecular level on these enzymes would be worth for development of new biotechnology.

A general conclusion which this study can be concluded from date reported here and some characteristics of the selected bacterial isolate ALP-40.3 and ALP-42.11 were concluded in Table 5.1.

Table 5.1 Some characteristics of the selected bacterial isolates ALP-40.3 and ALP-42.11.

Some characteristics	The selected bacterial isolates	
	ALP-40.3	ALP-42.11
1.Extracellular enzyme production	Positive	Positive
2.Optimum condition of growth		
2.1 Optimum pH	8	8
2.2 Optimum temperature	35°C	25-35°C
3. Appropriate medium and incubation time for lipase production	LPM, 18 hrs	LPM, 18 hrs
4. Crude alkaline lipase activity (unit/ml)	13.64	0.70
5.Optimum condition for lipase activity		
5.1 Optimum pH and pH stability	8 and 8-10	9 and 8-10
5.2 Optimum temperature and thermostability	45° and 15-65°C	65° and 15-65°C
6. Preference of substrate	olive oil	palm oil

Table 5.2 Activity of lipases produced fromvarious bacteria compared to the former investigators.

Organisms	Total activity of crude lipase (unit/ml)	Properties of enzyme	References
Pseudomonas nitroreducens	500	Thermostable and alkaline	Watwnabe et. al., 1977
Pseudomonas thermotolerans	30	Thermostable and alkaline	Watwnabe et. al., 1977
Pseudomonas fragi 22.39B	305,000	Thermostable and alkaline	Nishio et. al.,1987
Pseudomonas sp. KWI-56	3,040,000	Thermostable	Iisumi et. al., 1990
Bacillus **thermocalenulatus**	1.1	Thermostable	Schmidt-Dannert et. al., 1994
Bacillus Strain A 30-1 (ATCC 53841)	0.8	Thermostable and alkaline	Wang et. al.,1995
Pseudomonas pseudoalcaligenes F-111	37,000	Thermostable and alkaline	Lin et. al., 1996
Aeromonas Sorbia LP400	0.50	Alkaline	Lotrakul and Dharmsthiti, 1997
Pseudomonas sp. (APL-40.3)	1.33	Thermostable and alkaline	This study
Pseudomonas sp. (APL-42.11)	0.70	Thermostable and alkaline	This study