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STUDIES OF LIPASE PRODUCTION FROM HALOPHILIC BACTERIA

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Thirty-seven halophilic bacteria were used for studies of lipase production. They were divided to be two groups, moderate halophilic bacteria (10% NaCl, w/v) and halophilic archaea (20% NaCl, w/v). For primary screening of lipase production using Tween 80 agar plate, sixteen moderate halophiles and eleven extreme halophiles were selected as the lipase producers. The lipase production was observed in JCM no. 377 (10% NaCl) and 168 (20% NaCl) as for standard medium. They produced lipase in the range of 2-3.5 unit/ml. The highest lipase producers (seven moderate halophiles and five extreme halophiles) were the representative strains for determination of optimization of lipase production in the medium supplemented with various vegetable oils. Among of selected halophiles, strain BKK1 evaluated the highest lipase production and its lipase activities were 3.35, 3.37, 3.46, 3.89, 3.42 and 4.5 U/ml using 1% (v/v) of cooking oil, lard oil, sunflower oil, soybean oil and palm oil, respectively, as carbon sources and two days incubation. For four days cultivation of strain BKK1 in the medium supplemented with 1% palm oil, the lipase activity increased to be 8.5 U/ml. Therefore, a halophilic strain, BKK1 might be as a capable potential strain as for wastewater treatment.

Keywords: Lipase, Biosurfactant, Cooking oil, Halophilic Bacteria.

Introduction

Lipases are water soluble enzymes and catalyze esterification, inter-esterification, acidolysis, alcoholysis and aminolysis in addition to the hydrolytic activity on triglycerides. This hydrolytic enzyme was produced from various microorganisms such as bacteria, fungi and yeast, due to microorganisms grew rapidly on inexpensive media, produced in the high yield and showed the varieties of catalytic activities. So, microbial lipases, are gaining more attentions and more stable and valuable sources of lipases (Hasan et al., 2009). Lipase producers can be isolated from various natural sources especially oil-contaminated and also fermented foods (Namwong et al., 2011). Thai fermented foods are rich in various nutrients, particularly amino acids and peptides, and contain a high concentration of NaCl, which allow various halophilic micro-organisms to thrive (Lopetcharat et al., 2001; Namwong et al., 2005). They produced halophilic enzymes (protease, lipase, etc.) are stable and active in the presence of NaCl and their enzymatic transformations required to function at low water activities, are expected to be a very powerful tool in applications (Hiraga et al., 2005; Namwong et al., 2006). Therefore, lipase- and biosurfactant-producing halotolerant occurred to thrive in saline environment, may solve such problem and they have not been reported as a wastewater treatment method. The objective of this study was to study the lipase production from halophilic bacteria.

Materials and Methods

Isolation and culture conditions

The halophilic strains were isolated from Fermented fish (Pla-ra) in Chumpron province, Thailand by spread-plate technique on Tween 80 agar plates [composed of (per liter): 100 g NaCl, 1 g Tween 80, 1 g peptone, 0.1 g $CaCl_2H_20$, pH 7.2] incubated at 37 °C for 1-2 weeks (Barrow & Feltham, 1993). Unless otherwise stated, the test strains were grown in liquid or on agar medium of the JCM medium No.377 agar plates [composed of (per liter): 100 g NaCl, 2 g, 5 g casamino acids, 5 g yeast extract, 2 g KCl, 3 g trisodium citrate, 1 g glutamic acid, 20 g $MgSO_4.7H_20$, 0.36 g $FeCl_2.4H_20$, 0.0036 g $MnCl_2.4H_20$, 20 g agar, pH 7.2] (Namwong et al., 2009).

Lipolytic activity analysis

The lipase activity was examined by a spectrophotometric method using p-nitrophenyl palmitate as described previously [9-10]. The assay mixture consisted of 900 µl of buffer (100mM sodium phosphate—10% (v/v) NaCl—0.5% (v/v) Trition-X 100, adjusted to pH 7.0 with 1M NaOH), and 10 µl of 50mM *p*-nitrophenol palmitate in acetonitrile. After incubation at 37 °C for 60 min. the reaction was stopped by adding 250 µl of 0.1M HCl . The activity of enzyme was quantified by UV spectrophotometry at 410 nm. One unit of enzyme activity was defined as the amount of enzyme required to release 1 µmol of p-nitrophenol per min (Matsumiya et al., 2007).

Biosurfactant activity analysis

Surface tension was measured and repeated at least three times using a du Nöuy ring-type tensiometer (Krüss, K10T) at room temperature. An average value was used to express the surface tension of the sample (Ghoavand et al., 2008).

Results and Discussions

Primary Screening of Lipase Production

Thirty-seven halophilic strains were isolated and divided to be two groups based on NaCl concentration for their growth; moderate halophile; B15-5, I15-2, D15-4, G15-2, J15-8, B15-3, B14-2, K15-1, F15-1, F12-1, C15-2, C12-2, A15-1, A14-1, A13-1, A7-1, A11-1, J15-1-2, B15-4-2, KL2-4, KL2-3, NSW13-3 and NSW13-5; growing well in the presence of 10% NaCl, w/v and extrenme halophile; JCM12289, JCM 8876, JCM 10476, JCM 8980, JCM8911, HST01, DB5-2, HST03, PS11-2, IB5-2, HST01-2R, HST03-2R, BKK1 and BKK2; 20% NaCl, w/v. They were initially screened on Tween 80 agar medium supplemented 10-20% NaCl (w/v) and tested for their ability to degrade lipids/fats. Precipitation of free fatty acids with calcium (giving a white zone) was used as an indication to detect the bacterial activity for degrading lipids and producing lipase enzymes. Fig. 1 showed the white zone around the colonies of moderate halophile (KL2-4, Fig. 1a) and extreme halophiles (BKK1, HST03 and HST01-2R, Fig.1b-d) according with previous reports that bacteria produced lipase that will hydrolyze a neutral fat to fatty acid and glycerol (Hasan et al., 2009; Namwong et al., 2011). Based on the white turbid zone around their colonies, sixteen moderate halophiles and eleven extreme halophiles were selected for evaluating the lipase activity in the secondary screening.

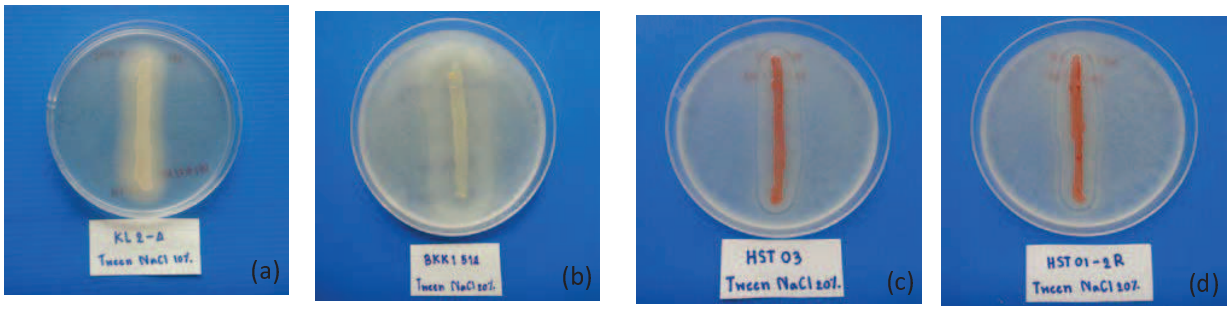

Fig. 1 Primary screening of halophilic bacteria on Tween 80 agar. Moderate halophile (a), Extreme halophiles (b-c).

Secondary sceening of lipase production

Twenty-seven halophililic strains made turbid with emulsified Tween 80 around the bacterial colony revealing the presence of an extracellular lipase. Their growth did not observed in Hydrolysis of lipid medium omitted an energy source. They were selected for determination of lipase activity and biosurfactant formation in JCM no. 377 (10% NaCl) and 168 (20% NaCl) as for standard medium. After cultivated in JCM medium, they also capable to grow and secrete lipase for cleaving of soluble fat contained in yeast extract to be fatty acids and glycerol used as carbon source in order to enhancement of their growth. Table 1 showed the lipase production from moderate halophiles, C15-2 was the highest lipase producer produced 2.5 U/ml without biosurfactant production.

Table 1. Lipase production of moderate halophiles

Strains	Lipase activity (U/ml)
B15-5	2.00
I15-2	1.55
D15-4	2.35
G15-2	2.30
J15-8	1.46
B15-3	2.36
K15-1	1.57
F15-1	1.54
C15-2	2.53
A15-1	1.90
J15-1-2	1.90
B15-4-2	1.76
KL2-4	2.17
KL2-3	1.79
NSW13-3	2.05
NSW13-5	2.10

For extreme halophiles, their growth was very slow when compared with moderate halophiles. After two days cultivation of selected strains (1 day cultivation for moderate halophiles) in the presence of NaCl (20%), halophilic archaea secreted 2.05-3.45 U/ml of lipase activities. The maximal lipase production was from stain BKK1 (3.45 U/ml) under the same condition (Table 2). According to the moderate halophiles, archeal strains did not evaluate any the biosurfactant. The highest lipase producers [7 moderate halophiles (D15-4, G15-2, B15-3, C15-2, KL2-4, NSW13-3 and NSW13-5) and 5 extreme halophiles (JCM12289, HSTO3, HST01-02R, HST03-2R, BKK1 and BKK2)] were the representative strains for determination of optimization of lipase production.

Table 2. Lipase production of extreme halophiles

Strains	Lipase activity (U/ml)
JCM 12289	2.05
JCM 8876	2.10
JCM 10476	2.65
JCM 8980	3.27
JCM 8911	2.43
HST01	2.36
HST03	2.27
HST01-2R	2.57
HST03-2R	2.39
BKK2	3.51
BKK1	3.45

Optimization of Lipase Production

Shabatai (1991) and Sigurgisledottir et al. (1993) reported that lipase are generally inducible in the presence of different inducers i.e., olive oil, palm oil, oleic acid and Tween 80. Therefore, the enhancement of lipase production, lipase inducers, lard, cooking oil, sunflower oil, soybean oil and palm oil, were added into the standard medium. In the presence of various vegetable oils, lipase production was increased 2-3 folds when compared with lipase production without inducers (JCM no. 377 and JCM no. 168). The biosurfactants was produced (data not shown) to decrease the surface and interfacial tension and to increase of interaction between microbial enzymes and lipids (Desai et al., 1997). Fig. 2 and 3 revealed the lipase production from halophilic bacteria in the presence of various lipase inducers and the range of lipase activities of moderate halophiles and extreme halophiles were 2.25-3.51 U/ml and 3.25-4.29 U/ml, respectively. Among the selected strains, strain BKK1 was the highest lipase production and its lipase activity was 3.35, 3.37, 3.46, 3.89, 3.42 and 4.5 U/ml using 1% (v/v) of cooking oil, lard oil, sunflower oil, soybean oil and palm oil, respectively, as carbon sources (Fig. 3). For further application in wastewater treatment, strain BKK1 was cultured in the JCM. no 168 (without casamino acid) added with palm oil and the lipase production sharply increased after 4 days cultivation (8.5 U/ml) and biosurfactant production was evaluated (data not shown).

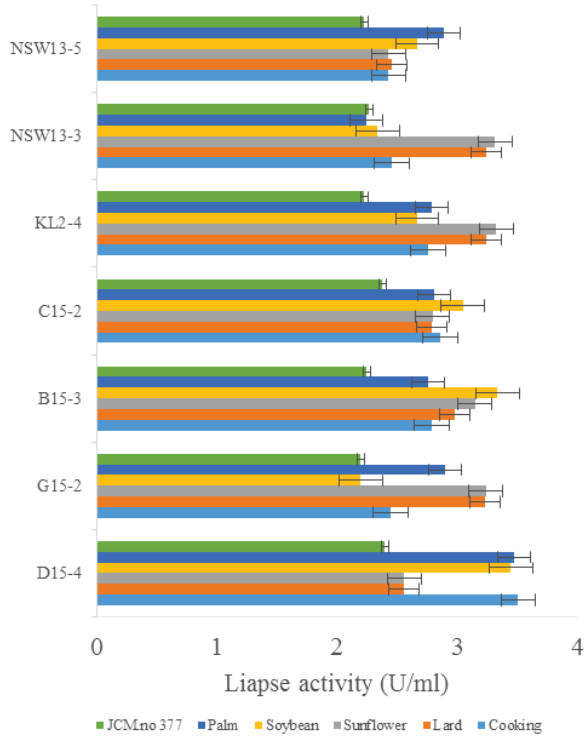

Fig.2 Effect of carbon sources on lipase production from moderate halophile

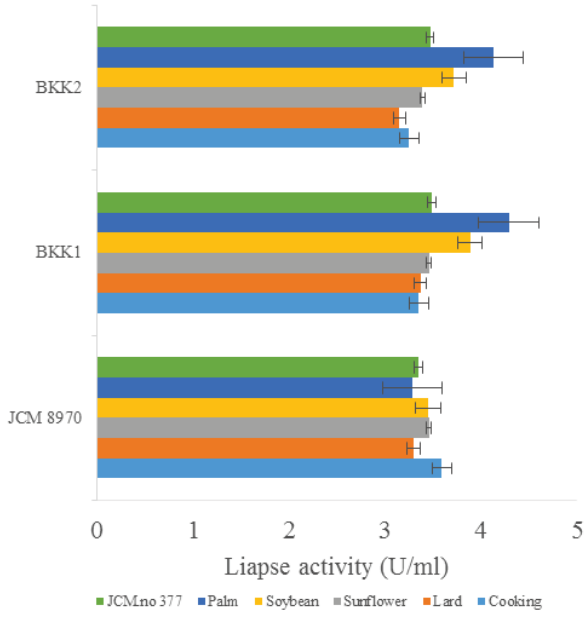

Fig. 3 Effect of carbon sources on lipase production from extreme halophiles

Conclusions

The lipase- and biosurfactant-producing halophilic bacteria, a strain BKK1 might be as a capable potential strain as for in the environment and industrial applications. For the further works, the determination of lipid degradation of various oils without temperature control and additional physical or chemical treatment will be studied for the possibility of the application of srain BKK1 in treatment of household waste.

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