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Optimizing lipase production from isolated *Burkholderia* sp.

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ABSTRACT

Lipase produced from microorganisms can be used in many industrial applications, such as detergent formulation, oil/fat degradation, pharmaceutical synthesis, biodiesel and cosmetics production. Due to the increase in demand for lipase, increasing attention has been paid to how to produce it efficiently and economically. In this work, lipase production from an isolated *Burkholderia* sp. C20 was enhanced by using the response surface methodology (RSM) to optimize the medium composition and the fermentation condition. The titer of lipase production was 16.7 U/ml under the optimal conditions with yeast extract of 2.175 g/l and olive oil of 5.54 ml/l. The lipase production was improved to 22.6 U/ml in a fermentor with an aeration rate of 1 vvm and a controlled pH at 6.0. The *Burkholderia* lipase exhibits good thermal and pH stabilities for 21 h incubation and the zymography analysis showed that the molecular weight of the lipase is about 23 kDa.

1. Introduction

Microbial lipase (triacyl glycerol acylhydrolases, E.C.3.1.1.3) catalyzes the hydrolysis of oils/fats to form free fatty acids and glycerol, while the reverse reaction is also driven by lipase in low water content conditions [1]. Lipase can be used as a bio-detergent because that it has the relevant activity and is stable at high temperature and in an alkaline environment [2]. The rising interest in lipase is mainly due to the wide range of industrial applications of the enzyme, including detergent formulation, oil/fat degradation, pharmaceutical synthesis, and cosmetics production [2,3]. The range of uses is because lipase shows activity with regard to hydrolysis, esterification, transesterification, interesterification, alcoholysis and acidolysis [4]. Rising demand for fossil fuels has lead to increases in the price of crude oil and thus it is important to find alternative fuels, such as biodiesel, which can be made from vegetable oils by lipase via transesterification [5]. Lipase can be obtained from various sources, such as plants, microorganisms and animals [6]. To date most lipase has come from microorganisms due to the low production cost. Several microorganisms, such as *Candida rugosa*, *Candida antarctica*, *Burkhoderia cepacia*, and *Pseudomonas alcaligenes*, produce lipase efficiently, and this is now are commercially available [1]. Among these, *Burkholderia* sp. is known to produce lipase with hydrolytic and esterifying activities [7]. In the present work, lipase was produced from an isolated *Burkholderia* sp. obtained in our recent work [8–10].

Since medium composition and fermentation conditions usually play a pivotal role in the production of lipase from microorganisms [11], identification of these is of considerable importance. Literature shows that the response surface methodology (RSM) was an effective tool to optimize the medium composition and the process conditions, thereby being often applied in improving the performance of a fermentation system [12]. In this work, RSM was used to enhance the lipase production performance from the isolated *Burkholderia* sp. C20 strain. There are four major steps in RSM design; namely, two-level factorial design, path of steepest ascent, central composite design, and final model fitting [13]. Based on these steps, RSM was used to optimize the conditions for lipase production from *Burkholderia* sp. in both shake flasks and a 5 l fermentor. The produced lipase was also characterized by zymography and its thermal and pH stabilities was also investigated.

2. Materials and methods

2.1. Bacterial strain and culture conditions

The strain isolated from food wastes and was identified as *Burkholderia* sp. [8]. The culture medium contained (l): olive oil,

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Table 1
Two-level factorial design for lipase production by *Burkholderia* sp. C20.

Run	Pattern	X_1 Hexadecane	X_2 Yeast extract	X_3 Olive oil	Yield (Y1) Lipase production (U/ml)
1	−−−	−1	−1	−1	9.24
2	−−+	−1	−1	+1	16.8
3	−+−	−1	+1	−1	7.77
4	−++	−1	+1	+1	11.46
5	+−−	+1	−1	−1	10.68
6	+−+	+1	−1	+1	15.91
7	++−	+1	+1	−1	9.39
8	+++	+1	+1	+1	11.97

Coded	Olive oil	Yeast extract	Hexadecane
−1	5 ml/l	1 g/l	5 ml/l
+1	10 ml/l	2 g/l	10 ml/l

Item	Olive oil	Yeast extract	Hexadecane
P values	0.0244	0.036	0.2013

10 ml; yeast extract, 2 g; $(NH_4)_2SO_4$, 6 g; HEPES, 2.4 g; KCl, 4.45 g; $CaCl_2$, 0.02 g; $MgCl_2$, 0.2 g. The cultivation conditions were 30 °C and 200 rpm for 30 h. The initial pH of the medium was 6.0.

2.2. Determination of lipase activity

One milliliter of the sample was placed into the emulsified solution (5% gum arabic acid and 10% olive oil) prior to analysis by pH-stat titrimetry (Mettler-Toledo Switzerland, Titrator T50) at pH 9.0 and 55 °C.

2.3. Lipase production under different carbon and nitrogen sources and initial pH

Hexadecane, olive oil, and yeast extract were added to the culture medium with the concentration ranging from 0 to 50 ml/l, 0 to 30 ml/l, and 0 to 8 g/l, respectively. NH_4Cl, urea, NH_4NO_3, and $(NH_4)_2SO_4$ with the concentration of nitrogen atom of 40 mM were used to study the effect of nitrogen source on lipase production. The initial pH of medium was 6.0. For the experiments investigating the initial pH effect, the pH of 5.0–9.0 was used. The cultivation conditions were 30 °C and 200 rpm for 30 h. The resulting broth was centrifuged at 4 °C and 9000 rpm for 10 min and the supernatant was used to determine the lipase activity.

2.4. Two-level factorial design and method of path of steepest ascent

The most significant factors affecting lipase production were screened using two-level factorial design. The factors of interest in the investigation were the concentrations of hexadecane, yeast extract, and olive oil. Table 1 shows the two-level factorial design for these three factors. This design was obtained using the JMP software package (version 3.2.2, SAS Institute Inc., Cary, NC, USA). Factors with the lowest *P* values were the most significant ones affecting lipase production. The new units for the range of factors were determined by the method of path of steepest ascent (Table 2) prior to the RSM experiment.

Table 2
Path of steepest ascent experiments for lipase production by *Burkholderia* sp. C20.

Step	−1	0	1	2	3
Olive oil (ml/l)	8.65	7.5	6.35	5.2	4.05
Yeast extract (g/l)	1.05	1.5	1.95	2.4	2.85
Lipase activity (U/ml)	9.2	11.7	15.37	15.76	13.1

2.5. Response surface methodology (RSM)

The RSM design was also conducted using the JMP software package (Table 3). The predicted maximum value of lipase production was decided by the second order model shown as follows (Eq. (1)):

$$Y = \alpha_0 + \sum_{i=1}^{k} \alpha_i x_i + \sum_{i=1}^{k} \alpha_i x_i^2 + \sum_{i<j} \alpha_{ij} x_i x_j \quad (1)$$

where Y is the predicted response; k is the number of factors; α_0 is the design factor of interest; α_i and α_{ij} are the coefficients.

2.6. Lipase production in the fermentor

The culture medium was similar to that described earlier. The pH of the culture medium was controlled at 6.0 by NaOH. A 5 l bench-top fermentor was used to perform the experiments. The agitation speed was 400 rpm. The aeration rate ranged from 0 to 2 vvm. The lipase activity was monitored by pH-stat during cultivation.

2.7. Analysis of Burkholderia lipase by SDS–PAGE and zymography

After 30 h cultivation, the *Burkholderia* sp. C20 culture was centrifuged at 4 °C and 9000 rpm ($9050 \times g$) for 20 min. The supernatant was lyophilized and the dried powder was collected and kept at −20 °C prior to usage. Both the SDS–PAGE and zymograph analysis were performed on a 12% separation gel and a 4% stacking gel, while the separation gel of zymograph contained 1% glyceryl tributyrate. Before loading 10 μl enzyme samples into both gels, 1 g of the lyophilized *Burkholderia* lipase powder was dissolved in 4 ml double distilled water (ddH_2O). The condition for running both gels was 20 mA and 15 min. After the electrophoresis was finished, the zymograph gel was placed into 10 mM Tris buffer of pH 9.0 and incubated at 55 °C for 60 min.

2.8. Determination of lipase stability

For thermal stability experiments, 15 ml crude lipase at pH 7.0 was incubated at the temperature of 40–70 °C. For pH stability, the same volume of crude lipase at a pH of 7.0–10.0 was incubated at 40 °C. The residual lipase activity under different temperatures and pH was measured as a function of time at designated intervals.

Table 3
RSM experiments for lipase production by *Burkholderia* sp. C20.

Run	Olive oil (X_1)	Yeast extract (X_2)	Comment	Lipase production (U/ml)
1	−1	−1	FF	9.09
2	−1	1	FF	13.51
3	1	−1	FF	14.35
4	1	1	FF	11.88
5	0	0	Center-FF	17.03
6	0	0	Center-FF	17.7
7	0	0	Center-FF	15.58
8	−1.41421	0	Axial	11.53
9	1.41421	0	Axial	12.49
10	0	1.41421	Axial	12.01
11	0	1.41421	Axial	12
12	0	0	Central-Ax	17.1
13	0	0	Central-Ax	15.77
14	0	0	Central-Ax	17.11

Coded	Olive oil (ml/l)	Yeast extract (g/l)
−1	7.5	1.5
0	5.775	2.175
+1	4.05	2.85