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Screening, identifying and medium optimization of a lipase-producing filamentous fungus from soil for high chiral resolution of 1-phenylethanol

Yongxian Fan[1], Liming Shang[2] and Junqing Qian[1]*

[1]College of Pharmaceutical Science, Zhejiang University of Technology, Hangzhou 310032, P. R China.
[2]College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou 310032, P. R China.

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The kinetic resolution of racemates by biocatalysis is one of the major routes to manufacture optically pure compounds. Lipase-producing microorganisms were screened from soil for chiral resolution of 1-phenylethanol in the study. One microorganism with high chiral resolution ability was isolated and identified as *Rhizopus stolonifer* by the sequence similarity analysis and construction of phylogenetic tree. The media for the microorganism were optimized using Plackeet-Burman, Steepest Ascent and Box-Benhnken experiments. After optimization, the composition of the optimized medium used for lipase production was as follows: soluble starch (7.60 g/L), yeast extract (22.59 g/L), K_2HPO_4 (5.55 g/)L, NH_4Cl (1.80 g/L), $MgSO_4 \cdot 7H_2O$ (2.40 g/L), NaCl (1.00 g/L), olive oil (25.00 mL/L), Tween-80 8.00 (mL/L), pH 4.59, and the lipase activity increased nearly by 2.04-fold, from 0.46 to 1.40 U/mL. The optimization was significant for the isolation and purification of the lipase and for the research of enzymatic characteristics.

Key words: Screening, lipase, identification, *Rhizopus stolonifer*; optimization, 1-phenylethanol.

INTRODUCTION

Lipase (EC 3.1.1.3) is an ester hydrolase, which catalyzes triglyceride to form diacylglycerol, glycerol ester, glycerol and free fatty acids on oil-water interface. Because of substrate specificity and a variety of different enzymatic properties, such as broad sources, short cycle, wide pH, wide range of temperature, microbial lipase had played a more important role than animal and plant lipases in enzymatic theoretical research as well as practical application, including hydrolysis, esterification, trans esterification, and ester chiral synthesis (Alfonso and Gotor, 2004; Jaeger et al., 1999; Rabtwijk and Sheldon, 2004; Sarda and Desnuelle, 1958). Many microorganisms which can produce lipase had been reported (Shimizu et al., 1998). Recently, the fungal lipases were reviewed, including *Aspergillus*, *Penicillium*, *Rhizopus*, *Candida*, etc. (Singh and Mukhopadhyay, 2012). Rhizopus species is mainly divided into three groups, *R. oryzae*, *R. microsporus*, and *R. stolonifer* but *R. stolonifer* was rarely reported to produce lipase (Singh and Mukhopadhyay, 2012).

The optically active 1-phenylethanol, especially (*R*)-1-phenylethanol, is used as chiral building block and synthetic intermediate in fine chemical, pharmaceutical and agrochemical industries (Suan and Sarmidi, 2004). In pharmaceutical industry, (*R*)-1-phenylethanol is used as an ophthalmic preservative and may also inhibit cholesterol intestinal adsorption and thus decrease high cholesterol level (Suan and Sarmidi, 2004). The other application area of the enantiomers is in the chemical analysis. For different purposes, it is necessary to resolve these

*Corresponding author. E-mail: lilyfan@zjut.edu.cn. Tel: +86-571-88320317.

two enantiomers of 1-phenylethanol. 1-phenylethanol can be resolved both through chemical and biological approaches. There are examples of microbiological way to resolve 1-phenylethanol, such as using immobilized cells of yeast *Pachysolen tannophilus* IFO 1007, and *Hansenula capsulate* IFO 0974 (Hasegawa et al., 1996), cells of *Geotrichum candidum* IFO 4597 (Nakamura et al., 1994), and *Stearothermophilus bacillus* 2027 (Fantin et al., 1993), with *(S)*-1-phenylethanol directionally oxidized to form acetophenone and (*R*)-1-phenylethanol left. As to enzymatic way to resolve 1-phenylethanol, hair mildew lipase (Frings et al., 1999), hares stomach lipase (Legros et al., 1997), goats liver lipase (Saikia et al., 1997), Amano PS lipase (Gutman et al., 1993) and *Pseudomonas* lipases (Ceynowa and Koter, 1999) were used, with (*R*)-1-phenylethanol catalyzed with acyl donor to form (*R*)-phenethyl and unreacted (*S*)-1-phenyl ethanol. The lipase catalysis in kinetic resolution of 1-phenylethanol enantiomers with *C. antarctica* lipase B was investigated by us (Fan et al, 2011) too. Recently, new techniques, such as immobilization (Bai et al., 2012; Kawakami et al.; 2012; Wang et al., 2012) and genetic engineering (Cao et al., 2012; Florczak et al., 2013), were also applied in the resolution of (*R, S*)-1-phenylethanol to improve the production. However, there were few reports about filamentous fungal lipases, such as lipases from *Rhizopus*, *Penicillium* and *Aspergillus*, which were applied in the chiral resolution of 1-phenylethanol.

On the other hand, because of the selectivity and the cost, there is no one which can be used in the commercial production of (*S*)-1-phenyl ethanol. In order to improve the selectivity and lower the cost, new microorganisms should be found to produce lipase for the resolution. In this paper, lipase-producing microorganisms for high resolution of 1-phenylethanol had been screened and one filamentous fungus was obtained and identified as *R. stolonifer*. The culture conditions of the strain for lipase production were also optimized with surface response method so as to recover the lipase much easier for the investigation of enzyme characteristics in the future work.

MATERIALS AND METHODS

Chemicals

(*R*, *S*)-1-Phenylethyl acetate (≥98%), (*R*)-1-phenylethanol and (*S*)-1-phenylethanol were provided by Sigma. Other organic solvents and chemicals were all purchased from the local market with analytical grade.

Screening of lipase-producing microorganisms

Enrichment of lipase-producing microorganisms

Soil samples were collected from oil-contaminated soils in China. Each sample was prepared as suspending liquid by adding 10 g oil sample and 40 mL sterilized water in 100-mL conical flask. 5 mL liquid was added into 50 mL enrichment medium, containing yeast extract (5.0 g/L), $(NH_4)_2SO_4$ (5.0 g/L), NaCl (0.5 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), KH_2PO_4 (1.5 g/L), K_2HPO_4 (1.0 g/L), and olive oil (10 mL/L). The microorganisms were cultivated at 30°C and 180 rpm. Then, it was transferred to fresh medium after 72 h cultivation. The operation was repeated for three times to obtain the coating seed.

Isolation of lipase-producing microorganisms

1 mL coating seed was diluted and coated on the plates, comprising $(NH_4)_2SO_4$ (2.0 g/L), NaCl (0.5 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), K_2HPO_4 (2.0 g/L), agar (6.0 g/L), pH 7.0, olive oil emulsion polyvinyl alcohol (120 mL/L), and rhodamine B (0.1 mg/mL) 100 mL/L.

The plates were incubated at 30°C for 72 h. The grown microorganisms that showed high ratios of fluorescent color zone diameter to colony diameter were selected as potential lipase producers.

Rescreening of lipase-producing microorganisms

The microorganisms were inoculated into 50 mL rescreened medium, containing glucose (10.0 g/L), peptone (20.0 g/L), $(NH_4)_2SO_4$ (1.0 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), KH_2PO_4 (2.0 g/L), and olive oil (10 mL/L). The microorganisms were cultivated at 30°C and 180 rpm for 72 h and those with high lipase production were selected.

The fermentation broth was freeze-dried. 0.05 g freeze-dried biomass was added to resolve 1-phenylethanol in reaction solution containing 0.02 mol/L 1-phenylethanol (12.5 μ)L, vinyl acetate 12.5 and 5 mL heptane. The reaction was carried out at 40°C and 150 rpm for 40 h.

Identification of lipase-producing microorganisms

The isolated strain with high lipase activity was identified by internal transcribed spacer (ITS) region amplification and sequencing. Then, the evolutionary status and species of the strains were determined through sequence similarity analysis and construction of phylogenetic tree.

Optimization of composition of fermentation medium used for lipase production

The strain stored on the slope at 4°C was transferred to a fresh slope and cultivated at 28°C for 72 h. Then, the activated strain was transferred to 50 mL sterilized seed medium, composing of glucose (10.0 g/L), yeast extract (10.0 g/L), K_2HPO_4 (1.0 g/L), $MgSO_4.7H_2O$ (1.0 g/L), olive oil (15 mL/L) and natural pH, and incubated at 26°C for 24 h on a rotating shaker at 180 rpm. Seed medium was inoculated to 50 mL fermentation medium with the rate of 2%. The composition of fermentation medium was glucose (5.0 g/L), yeast extract (15.0 g/L), K_2HPO_4 (4.0 g/L), $MgSO_4.7H_2O$ (3.0 g/L), NaCl (1.0 g/L), olive oil (20 mL/L), Tween-80 (10 mL/L) and pH 6.4, and incubated at 26°C for 120 h on a rotating shaker at 180 rpm.

The medium was optimized in three steps. Firstly, effects of 8 factors were determined using Plackett-Burman experimental design. Secondly, factors screened by Plackett-Burman experimental design were further optimized using Steepest Ascent Method to get closer to the optimum conditions. Thirdly, the significant factors were optimized using Box-Behnken design.

Analytical methods

The enantiomer contents during the reaction time course were

Table 1. Strains with obvious lipase activities.

Strain number	Lipase activity (U/mL)
2-2	0.23
2-3	0.21
3-2	0.26
9-8	0.46
10-16	0.28

monitored by Model 6890N Gas Chromatography with flame ionization detector and 19091G-B233 (25 m × 0.25 mm) column from Agilent Technologies using high-purity nitrogen as carrier gas, at the following temperature program: 100°C (5)-120°C/5°C/min (12)-200°C /10°C /min(10 min); detector and injector tempera-tures were both set at 250°C. It was splitless in the analysis.

The activity of lipase was detected using the following method. Five milliliter (5 mL) polyvinyl alcohol-olive oil emulsion and 4 mL 0.025 mol/L PBS buffer (pH 7.5) were added into 50 mL conical flask, which was then warmed-up at 40°C for 5 min. 1 mL enzyme solution was added into the conical flask to launch enzymatic reaction. After a reaction of 4 h in water bath shaker at 40°C, 15 mL 95% ethanol solution was added to terminate the reaction and 4 drops of phenolphthalein solution was then added. Free fatty acid generated from enzymatic hydrolysis was then titrated with 0.05 M NaOH solution. As to blank control group, 95% ethanol was added before enzyme solution. One unit (U) of lipase activity was defined as the amount of enzyme required to liberate 1 µmol of free fatty acid per minute under the conditions described above.

$$\text{Lipase activity(U/mL)} = \frac{(V - V_0)}{t \times n} \times M_0$$

Where, V is the volume of NaOH solution consumed in the sample solution (mL), V_o is the volume of NaOH solution consumed in the blank solution (mL), t is the Reaction time (min), n is the volume of enzyme solution (mL) and M is the concentration of NaOH solution

RESULTS AND DISCUSSION

Screening of lipase-producing microorganisms

Through primary screening of 22 soil samples, hundreds of colonies with fluorescent color circle were grown on the plates, among which, 45 strains with larger fluorescence diameters were selected and added into the rescreening medium for further cultivation. After detection of lipase activity, only 5 strains were found to have obvious lipase activities (Table 1).

The chiral resolution experiments to produce (*S*)-1-phenyl ethanol were carried out with the lipases of 5 strains. The results showed that strain 9-8 had higher activity and enantio-selectivity towards the substrate than other strains, with a conversion ratio of 43% and enantiomeric excess of product (e.e.) of 82%, respectively. So, the strain 9-8 was selected for further research, such as identification by ITS region amplification and sequencing.

Identification of strain 9-8

After sequence similarity analysis of obtained sequence (Figure 1) with NCBI sequence data, the strain had a homology of more than 95% with *R. stolonifer* (DQ641318.1), *R. stolonifer* (DQ641317.1) and *R. stolonifer* (AF543526.1) with comparison of the 26s rDNA ITS sequences. Therefore, the strain belonged to *stolonifer* species of *Rhizopus* genus and named *R. stolonifer* ZJUT. Phylogenetic tree was constructed to determine the evolutionary status of the strain (Figure 2).

Selection of carbon source and nitrogen source

Although lipase from *R. stolonifer* ZJUT could resolute the racemic 1-phenyl ethanol, the efficiency was still very low. Furthermore, the characteristics of the lipase were unknown. The pure lipase from *R. stolonifer* ZJUT was needed to study the characteristics. However, the enzyme activity was very low in the broth which caused the isolation and purification of lipase to be hard. Therefore, the conditions of fermentation should be optimized to improve the amount of the lipase.

Effect of carbon sources on the lipase production

For this part of investigation, glucose in fermentation medium employed above was replaced by sucrose, citric acid, corn syrup, maltose, lactose, soluble starch and dextrin, respectively, while other medium components remained unchanged. The results are shown in Figure 3. Among the seven carbon sources, the soluble starch was the best for the lipase production. Therefore, soluble starch (5.0 g/L) combined with olive oil (20 mL/L) was selected as the optimized carbon source and would be employed in the subsequent research.

Effect of nitrogen source on the lipase production

In the experiment, soybean flour, soybean cake powder, yeast extract, soybean flour with $(NH_4)_2SO_4$, soybean flour with NH_4Cl, yeast extract with $(NH_4)_2SO_4$, yeast extract with NH_4Cl, soybean cake powder with $(NH_4)_2SO_4$, soybean cake powder with NH_4Cl, peptone with $(NH_4)_2SO_4$, peptone with NH_4Cl were utilized as the nitrogen sources.

The results are shown in Figure 4. The microorganism preferred to use complex nitrogen sources, among which, yeast extract with the NH_4Cl showed the best result. Therefore, a composition of 15 g/L yeast extract with 1.2 g/L NH_4Cl was selected as the optimum nitrogen source and would be employed in the subsequent research.

Plackeet-Burman experiment

Plackeet-Burman design method was a two-level experi-

CGGGTAATCCCACTTGACTTCAGATCATTGATTGAAAAAATGAAAAAAG
AATTTATTTAAAAACTCTCTTTCATTATTCTTTGTTCTCTCCCTTCTTGC
ATAATCGTTATAATACCAAGTAAATAAATTTAATAGAGTATTAACTACAC
AAAAAGGAGAAAAAAAAAAAAAAATTTGAAAAACCCTTTTTTTTTTTTTT
TTTTTTTTT

Figure 1. 26s rDNA ITS sequence.

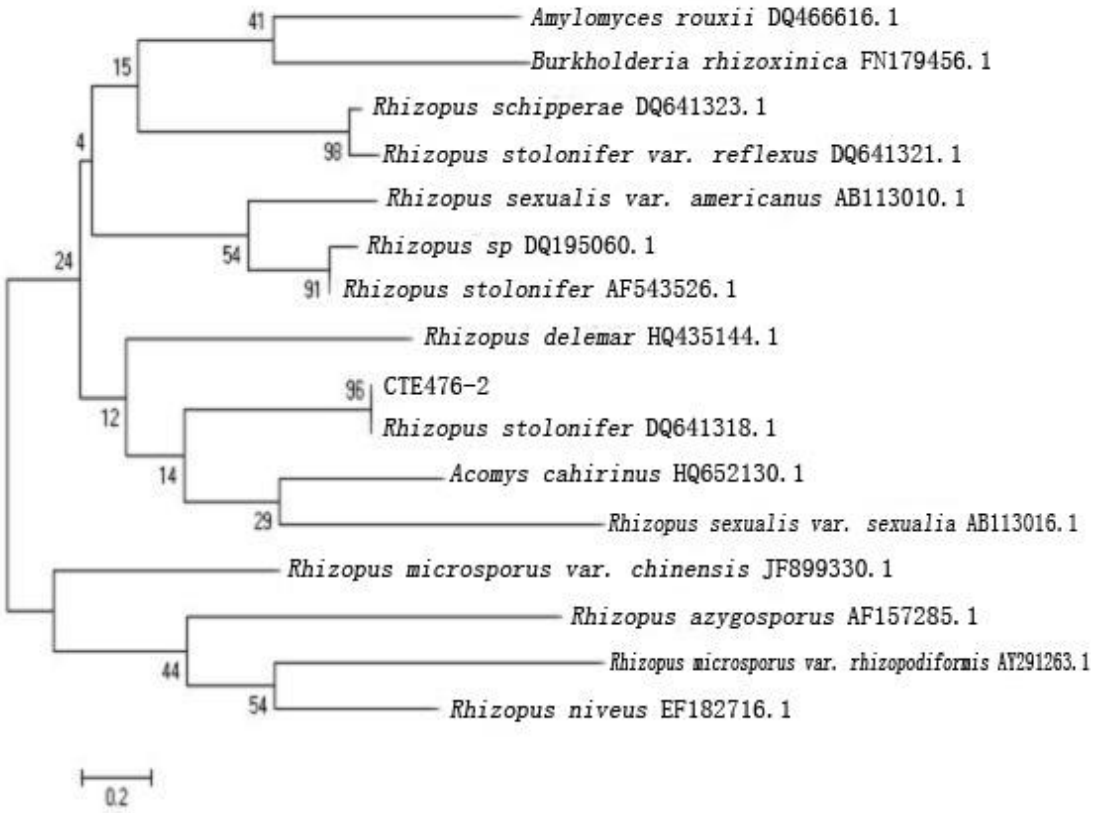

Figure 2. Phylogenetic tree of CTE476-2 (strain 9-8).

mental design method. Based on a non-perfectly balanced block principle, it could estimate the main affecting factors by minimum times of tests.

A set of 12 runs were set to identify the factors that had significant effects on the lipase production. Soluble starch (X_1), yeast extract (X_2), NH_4Cl (X_3), $(NH_4)_2SO_4$ (X_5), olive oil (X_6), $(NH_4)_2SO_4$ (X_7), Tween-80 (X_9) and pH (X_{10}) were selected as the investigated factors and 3 blank groups (X_4, X_8 and X_{11}) were set to examine the experimental error. Factors with credibility of more than 90% were taken as the significant factors. Experimental design and results are shown in Table 2. The levels of factors and the analytical results of main effects are shown in Table 3.

The correlation coefficient of the regression equation R^2 = 92.5% indicated the model was suitable for prediction of lipase production. According to the results (Table 2), three factors of yeast extract (X_2), K_2HPO_4 (X_5) and pH (X_{10}) showed significant effects on lipase production and all were with credibility of more than 90%. Consequently, they could be considered as the main factors for the further research of response surface experiment. Parameters of others factors were as follows: 7.60 g/L for soluble starch, 1.80 g/L for NH_4Cl, 2.40 g/L for $MgSO_4 \cdot 7H_2O$, 1.00 g/L for NaCl, 25.00 mL/L for olive oil, 8.00 mL/L for Tween-80.

Steepest Ascent experiment

Steepest Ascent experimental groups were designed according to the results of Plackett-Burman experiment.

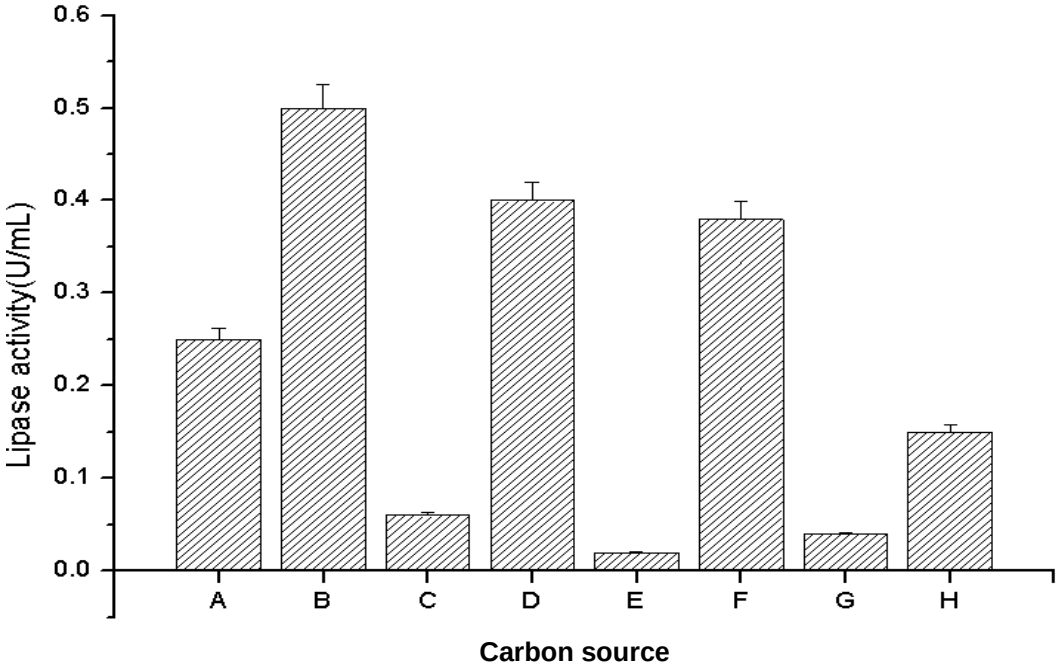

Figure 3. Effects of different carbon sources on lipase production. **A,** Dextrin; **B,** Soluble starch; **C,** Lactose; **D,** Glucose; **E,** Maltose; **F,** Corn syrup; **G,** Citric acid; **H,** Sucrose.

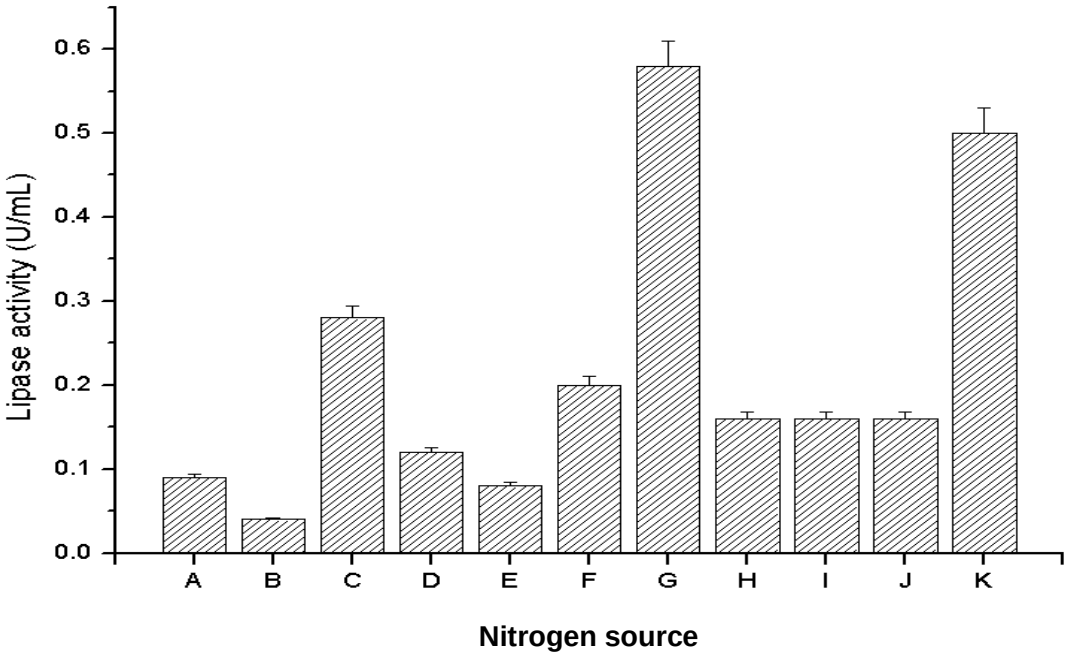

Figure 4. Effects of different nitrogen sources on lipase production. **A,** Soybean flour; **B,** Soybean cake powder; **C,** Yeast extract; **D,** Soybean flour+$(NH_4)_2SO_4$; **E,** Soybean flour +NH_4Cl; **F,** Yeast extract +$(NH4)_2SO_4$; **G,** Yeast extract +NH_4Cl; **H,** Soybean cake powder +$(NH_4)_2SO_4$; **I,** Soybean cake powder +NH_4CL; **J,** Peptone +$(NH_4)_2SO_4$; **K,** Peptone +NH_4Cl.

The factors of yeast extract and K_2HPO_4 had a significant positive effect in 90% confidence interval; therefore, their effects should be increased. pH showed a significantly negative effect in 90% confidence interval. So, its effects

Table 2. Experimental matrix used for the Plackett-Burman statistical design.

Run	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	Y/U
1	-1	1	1	1	-1	1	1	-1	1	-1	-1	1.48
2	-1	-1	-1	1	1	1	-1	1	1	-1	1	1.02
3	-1	1	-1	-1	-1	1	1	1	-1	1	1	1.08
4	-1	-1	1	1	1	-1	1	1	-1	1	-1	1.1
5	1	1	-1	1	1	-1	1	-1	-1	-1	1	1.54
6	1	-1	1	1	-1	1	-1	-1	-1	1	1	1.07
7	1	-1	-1	-1	1	1	1	-1	1	1	-1	1.25
8	1	1	-1	1	-1	-1	-1	1	1	1	-1	1.09
9	-1	1	1	-1	1	-1	-1	-1	1	1	1	1.16
10	1	-1	1	-1	-1	-1	1	1	1	-1	1	1.07
11	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	0.96
12	1	1	1	-1	1	1	-1	1	-1	-1	-1	1.79

Table 3. Analysis of variance for Plackett-Burman experimental design.

Factor		Level		Effect	Significance tests	
Number	Variable	Lowest level (-1)	Highest level (+1)		t-value	Probability > t
X_1	Soluble starch (g/L)	5.0	7.6	0.16833	2.21	0.115
X_2	Yeast extract (g/L)	15.0	19.0	0.27833	3.65	0.036
X_3	NH_4Cl (g/L)	1.8	1.2	0.12167	1.59	0.209
X_5	K_2HPO_4 (g/L)	5.0	4.0	0.18500	2.42	0.094
X_6	Olive oil (mL/L)	25.0	20.0	0.12833	1.68	0.191
X_7	$MgSO_4 \cdot 7H_2O$ (g/L)	3.0	2.4	0.07167	0.94	0.417
X_9	Tween-80 (mL/L)	10.0	8.0	-0.07833	-1.03	0.380
X_{10}	pH	5.5	6.4	-0.18500	-2.42	0.094

Table 4. Design and results of steepest ascent experiment.

Number	Yeast extract ($g.L^{-1}$)	K_2HPO_4 ($g.L^{-1}$)	pH	Lipase activity (U/mL)
1	19.0	5.0	5.5	1.146
2	21.0	5.6	5.0	1.260
3	23.0	6.2	4.5	1.054
4	25.0	6.8	4.0	1.024

should be decreased. The direction and step were set according to the effect sizes of these three factors. The results are shown in Table 4. The optimum condition might be around the one of experimental group 2, whose condition would be then taken as the central point of response surface experiment.

3.6 Response surface experiment

Box-Benhnken methods were employed to design a 3-factors and 3-levels response surface experiment towards lipase production fermentation medium, according to the determined factors and levels in Plackett-Burman and Steepest Ascent Experiment. The response surface experiment included 12 factorial experiments and three center trials. The coding levels of factors and experimental results are shown in Table 5.

Statistical software Minitab was employed to carry out quadratic polynomial regression fitting, the property of polynomial model equation was expressed by R^2, the coefficient of determination and its statistical significance is determined by the T test.

The process of data analysis was conducted through response surface regression (RSREG) procedure, by which the quadratic response surface regression model was established, thus to gain the optimum level of the corresponding factor. The analysis results are shown in

Table 5. Matrix of the Box-Behnken experiment and the corresponding experimental data.

Number	Yeast extract (g.L^{-1})		K_2HPO_4 (g.L^{-1})		pH		Lipase activity (U/mL)
	X_1	CodeX_1	X_2	CodeX_2	X_3	CodeX_3	
1	20	-1	5.6	0	5.5	1	1.192
2	24	1	5.0	-1	5.0	0	0.894
3	22	0	6.2	1	5.5	1	1.444
4	24	1	5.6	0	5.5	1	0.836
5	22	0	5.0	-1	5.5	1	1.100
6	22	0	5.6	0	5.0	0	1.375
7	20	-1	6.2	1	5.0	0	0.802
8	20	-1	5.6	0	4.5	-1	0.710
9	24	1	6.2	1	5.0	0	0.940
10	20	-1	5.0	-1	5.0	0	0.802
11	22	0	5.0	-1	4.5	-1	1.169
12	24	1	5.6	0	4.5	-1	1.204
13	22	0	6.2	1	4.5	-1	1.146
14	22	0	5.6	0	5.0	0	1.375
15	22	0	5.6	0	5.0	0	1.375

Table 6. Analysis of variance for Box-Behnken experimental design.

Number	Coefficient	Standard deviation	T-value	P>|t|
Constant	1.37500	0.02848	48.284	0.000
X_1	0.04600	0.01744	2.638	0.046
X_2	0.04587	0.01744	2.631	0.046
X_3	0.04288	0.01744	2.459	0.057
X_1X_1	-0.37238	0.02567	-14.507	0.000
X_2X_2	-0.14313	0.02567	-5.576	0.003
X_3X_3	-0.01713	0.02567	-0.667	0.534
X_1X_2	0.01150	0.02466	0.466	0.661
X_1X_3	-0.21250	0.02466	-8.617	0.000
X_2X_3	0.09175	0.02466	3.720	0.014

Table 6.

Ternary quadratic equation of enzyme production about factors of yeast extract, K_2HPO_4, and pH was as follow:

$$Y=1.3750+0.04600X_1+0.04587X_2+0.04288X_3-0.37238X_1^2-0.14313X_2^2-0.01713X_3^2+0.1150X_1X_2-0.2125X_1X_3+0.09175X_2X_3.$$

In the equation, X_1, X_2 and X_3 represented the coding levels of factors of yeast extract, K_2HPO_4 and pH, respectively. The regression equation analysis of variance test of significance showed that the equation F value was greater than $F_{0.01}$, which means that the lack of fit was insignificant in the model, while the regression was significant. In addition, the model coefficient of determination R2 was 0.9855, indicating that the fitting degree of equation was good, and there was a high degree of correlation between the predicted value and measured value. Thus, it could be applied to predict the theoretical enzyme activity. To obtain the optimum concentration of the medium, first-order partial derivatives about respective variables were sought towards the obtained fitting and regression equation. By setting the value as 0, the ternary equations could be obtained. By solving the equations, model extreme points could be gained: X1 = 0.29, X2 = -0.09, X3 = -0.82, which meant that when the concentrations of yeast extract and K_2HPO_4 were 22.59 and 5.55 g/L, respectively, and pH was 4.59, the theoretical maximum enzyme activity was 1.40 U/mL.

Response surface plots and the corresponding contours were obtained when applying software Minitab according to the regression equation above as shown in Figure 5A, B and C. Every response surface represented the interaction between two independent variables while the third variable was maintained at optimum level. It could be concluded from the result that there were signi-

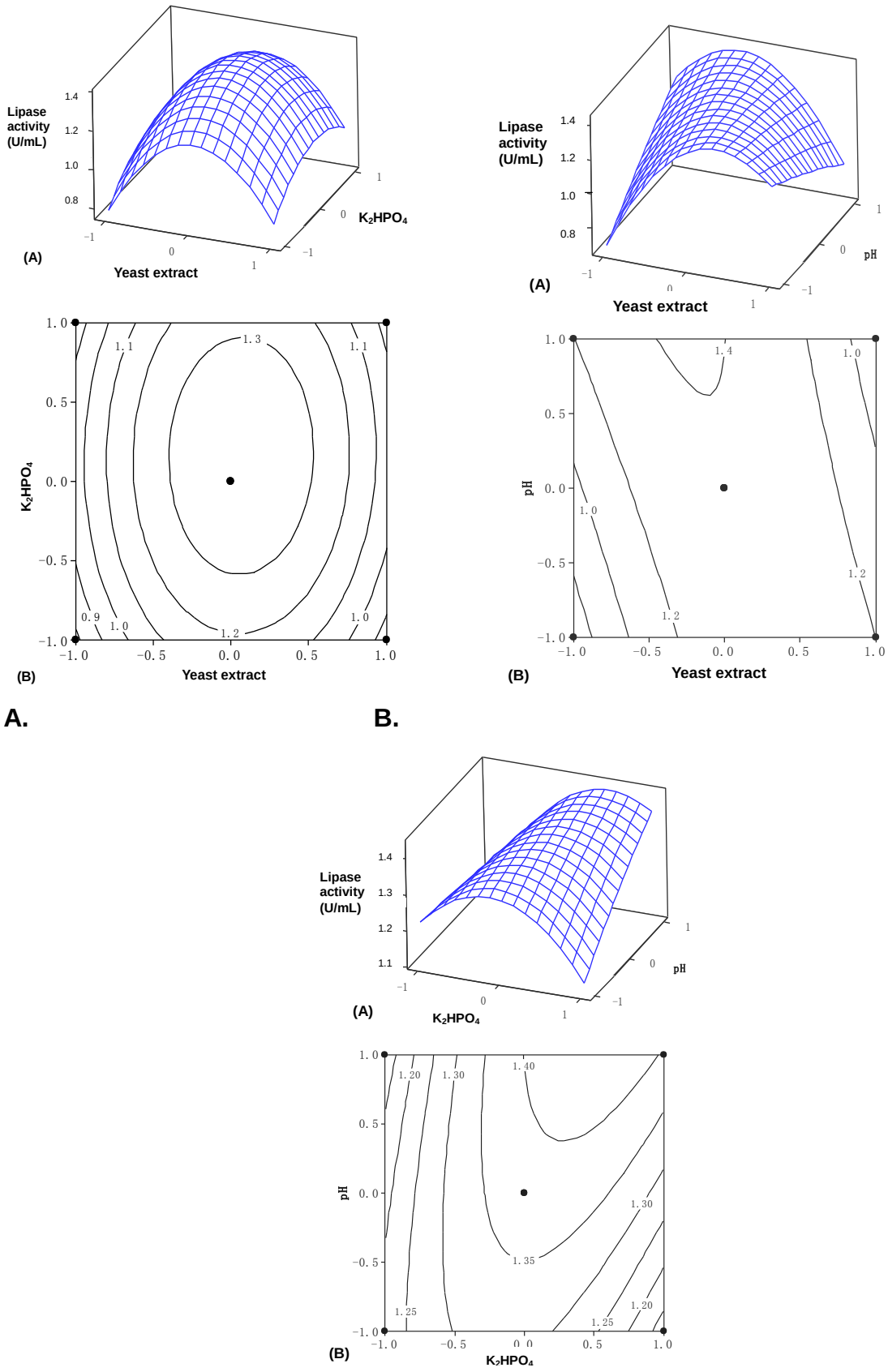

Figure 5. Response surface plot and the corresponding contour concerning effects on enzyme activity. **A.** Yeast extract and K_2HPO_4 concentration; **B.** Yeast extract concentration and pH; **C,** K_2HPO_4 concentration and pH.

ficant correlations between factors of yeast extract concentration, K_2HPO_4 concentration pH, and lipase activity, respectively. Among them, concentration of yeast extract showed the most significant impact on the lipase activity.

The enzyme activity increased significantly along with yeast extract concentration increasing in a range of 20 to 24 g/L. When yeast extract concentration was too high, the cells grown rapidly and was not favorable for the accumulation of metabolites. At the same time, the excessive yeast extract was also unfavorable for the transfer of oxygen and nourishment. High concentration of K_2HPO_4 would also bring a negative effect to the metabolism of cells.

Verification test

The verification test was carried out under the optimized conditions. The measured data of shake flask experiment was extremely close to the theoretical prediction, which meant that the model could predict the actual fermentation situation well, indicating that the response surface method in the optimization of lipase activity was feasible.

Conclusions

Due to the low efficiency and high cost, no lipases are used in the enzymatic resolution of racemic 1-phenylethanol in large scale. In order to find potent lipase-producing microorganisms for the resolution in large scale, microbes were isolated from the soil for the screening. After rescreening for the enzymatic resolution of racemic 1-phenylethanol, 5 microbes were screened and one of them, which had a relative higher activity and enantioselectivity towards the substrate than other strains, with a conversion ratio of 43% and enantiomeric excess of product (e.e.) of 82%, respectively, was identified. Because of the low enzyme activity in the broth which caused the isolation and purification of lipase to be hard, the fermentation conditions were optimized. The optimum composition used for lipase production was soluble starch (7.60 g/L), yeast extract (22.59 g/L), K_2HPO_4 (5.55 g/L), NH_4Cl (1.80 g/L), $MgSO_4{\cdot}7H_2O$ (2.40 g/L), NaCl (1.00 g/L), olive oil (25.00 mL/L), Tween-80 (8.00 mL/L), pH 4.59, and the lipase activity increased nearly by 2.04-fold, from 0.46 to 1.40 U/mL. Further tests are being conducted to investigate the isolation and purification of the lipase, then characteristics of enzyme with the pure lipase, and optimization of catalysis conditions for enzymatic resolution of 1-phenylethanol racemates.

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