Supplementary information

The Solvent Stability Study with Thermodynamic Analysis and Superior Biocatalytic Activity of *Burkholderia cepacia* Lipase Immobilized on Biocompatible Hybrid Matrix of Polyvinyl Alcohol and Hypromellose

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Determination of lipase activity and protein content of crude lipase or protein binding yield of immobilized lipase

The lipase activity of crude and various HY:PVA immobilized lipase was studied in triplicate by spectrophotometrically at 405 nm by hydrolysis of *p*-nitro phenyl acetate (*p*-NPA). In the standard assay condition, reaction mixture consists of 2 mg of crude lipase (or equivalent quantity of the HY:PVA:immobilized lipase) in 1 mL of *n*-heptane solvent. The reaction was started by addition of 1 mL of 12 mM, *p*-NPA dissolved in 2-propanol solution as an enzyme substrate and incubated at 40 ^{o}C for 10 minutes. Later on, 300 µl of reaction mixture was taken out and added to 700 µl of deionised water to extract *p*-nitro phenol (*p*-NP) in aqueous phase. Finally, 400 µl of potassium phosphate buffer solution of pH 8.0 was added to above solution so that, extracted *p*-NP give pale yellow colour which was used to measure the absorbance at 405 nm. The lipase activity was described as µmole of *p*-NP released per minute by per mg of the lipase under the prescribed standard assay condition.

The protein content of the crude lipase or protein binding yield for the immobilized lipase was determined by the Bradford method at 595 nm. A detailed procedure is as follow: At first, initial protein content was determined from crude/free lipase which was used to immobilize into support. Finally after complete removal of immobilized lipase matrix from petri-dish; the petri-dish was rinsed and subjected for determination of un-immobilized amount of protein. Therefore, the amount of protein immobilized on PVA:HY support was the difference between the initial (crude) protein content used to load and un-immobilized amount of protein found after washing petri dishes. BSA was used to construct the internal standard calibration curve; subsequently, % lipase activity, % protein loading, specific activity and % activity yield was determined.

Table ST1 Lipase activity, protein contents and specific activity determination for the crude and immobilized lipase.

Lipase	Lipase activity[a]		Lipase[b] activity (%) (III)	Protein Content[c]		Protein Binding yield[d] (%) (VI)	Specific Activity[e]		Activity yield[f] (%) (IX)
	Crude (I)	Immobil-ized (II)		Crude (IV)	Immobil-ized (V)		Crude (VII)	Immobil-ized (VIII)	
BCL	52.89	82.78	156.54	44.12	42.88	97.18	1.191	1.933	161.04
ROL	19.23	25.92	134.81	32.19	30.65	95.23	0.597	0.844	140.61
RNL	14.55	18.61	127.9	28.81	27.04	93.92	0.505	0.688	136.18

[a]Unit of the lipase activity: U/mg

[b]% lipase activity= immobilized lipase activity / crude lipase activity

[c]Unit of the Protein content: µg/mg

[d]% protein binding yield = protein bound in immobilized lipase / protein content of crude lipase

[e]Unit of specific activity: U/µg

[f]Activity yield (%)= specific activity of immobilized lipase / specific activity of crude lipase ×100

Characterization of the products

GC-MS analysis: MS (70 eV, EI) m/z (%):

Ethyl butyrate (Table 5; entry 1): 43 (80 %), 60 (17 %), 71 (100 %), 88 (54 %), 117 (32 %) (M+);

Butyl butyrate (Table 5; entry 2): 41 (44 %), 43 (61 %), 56 (58 %), 71 (100 %), 89 (59 %), 145 (2 %) (M+);

Isoamyl butyrate (Table 5; entry 3): 41 (32 %), 43 (80 %), 55 (43 %), 71 (100 %), 89 (17 %), 159 (1 %) (M+);

Octyl butyrate (Table 5; entry 4): 41 (38 %), 43 (69 %), 57 (34 %), 71 (100 %), 83 (20 %), 201 (1 %) (M+);

Cyclohexyl butyrate (Table 5; entry 5):43 (91 %), 55 (51 %), 71 (100 %), 82 (87 %), 88 (73 %), 170 (1 %) (M+);

Benzyl butyrate (Table 5; entry 6): 43 (41 %), 65 (18 %), 71 (35 %), 91 (98 %), 108 (100 %), 178 (26 %) (M+);

Cinnamyl butyrate (Table 5; entry 7): 43 (60 %), 71 (100 %), 115 (41 %), 133 (20 %), 204 (11 %) (M+);

Phenethyl butyrate (Table 5; entry 8): 43 (36 %), 71 (26 %), 104 (100 %), 105 (15 %), 192 (1%) (M+);

2-Pyridine methyl butyrate (Table 5; entry 10): 43 (51 %), 65 (28 %), 92 (100 %), 179 (20 %) (M+);

NMR Data:

Benzyl butyrate (Table 5; entry 6) (^{1}H 300 MHz, $CDCl_3$) δ: 0.95 (t, 3H), 1.61-1.74 (sextet, 2H), 2.34 (t, 2H), 5.12 (s, 2H), 7.16–7.38 (m, 5H);

Benzyl butyrate (^{13}C 300 MHz, $CDCl_3$) δ: 173.6, 136.4, 128.7, 128.4, 128.5, 66.2, 36.4, 18.7, 13.8.

Cinnamyl butyrate (Table 5; entry 7) (^{1}H 300 MHz, $CDCl_3$) δ: 0.97 (t, 3H), 1.65–1.77 (sextet, 2H), 2.38 (t, 2H), 4.74 (d, 2H), 6.27-6.36 (m, 1H), 6.68 (d, 1H), 7.22–7.43 (m, 5H);

Cinnamyl butyrate (^{13}C 300 MHz, $CDCl_3$) δ: 173.7, 136.5, 134.4, 129.0, 128.3, 127.0, 123.7, 65.1, 36.5, 18.8, 14.1.

Phenethyl butyrate (Table 5; entry 8) (^{1} H 300 MHz, $CDCl_3$) δ: 0.94 (t, 3H); 1.59-1.71 (sextet, 2H), 2.30 (t, 2H); 2.97 (t, 2H); 4.32 (t, 2H); 7.17-7.35 (m, 5H);

Phenethyl butyrate (^{13}C 300 MHz, $CDCl_3$) δ: 173.6, 137.95, 128.9, 128.4, 126.5, 64.7, 36.15, 35.1, 18.4, 13.7.