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Specific Enzyme-Catalyzed Hydrolysis and Synthesis in Aqueous and Organic Medium Using Biocatalysts with Lipase Activity from *Aspergillus niger* MYA 135

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Abstract In the present study, the specific hydrolytic activity of three biocatalysts such as the constitutive mycelium-bound lipase, the induced mycelium-bound lipase and the lyophilized induced supernatant from *A. niger* MYA 135 was evaluated in both aqueous and organic media. A direct correlation between activity in water and *n*-hexane was not observed for the same hydrolytic reaction. The *n*-hexane/water activity ratio ($R_{O/A}$) was applied to characterize the activity in organic medium. The three biocatalysts showed $R_{O/A}$ values higher than 1 for hydrolysis of long-chain fatty acid esters, demonstrating a higher specific hydrolytic activity in organic solvent than in water. A different behavior was observed during hydrolysis of middle-chain fatty acid esters, which was higher in aqueous medium ($R_{O/A} < 1$). Transesterifications of different alcohols with various *p*-nitrophenyl derivatives using all three biocatalysts preparations were also evaluated in *n*-hexane. For methanolysis and ethanolysis, the constitutive mycelium-bound lipase displayed an interesting preference for C16 substrate (*p*-nitrophenyl palmitate). The induced mycelium-bound lipase showed high specific transesterification activities in the presence of water-miscible alcohols and middle-chain fatty acid esters (*p*-nitrophenyl caprate and *p*-nitrophenyl laurate), being the highest specific transesterification activity (91.4 ± 1.7 mU/g_{dw}) observed in a reaction mixture containing propanol and *p*-nitrophenyl laurate. Finally, both *p*-nitrophenyl caprate (C10) and *p*-nitrophenyl laurate (C12) were preferentially methanolized by the lyophilized induced supernatant, being this lipase activity the most specific biocatalyst preparation under transesterification conditions. A selectivity-based analysis of each lipase preparation toward transesterification or hydrolysis in organic medium was evaluated as well.

Keywords Lipase · *Aspergillus niger* · Hydrolysis · Transesterification · Organic medium · Aqueous medium · Substrate specificity

1 Introduction

Among all the enzymes that show significant activity in organic media, lipases are of particular interest because of their commercial potential in many applications [1, 2]. Lipases have been widely used as common reagents for ester hydrolysis and synthesis, and lipase-catalyzed reactions are often carried out in organic solvents [3]. However, organic solvents frequently alter enzymatic activity, enzyme stability and substrate specificity [4]. Observations in the three-dimensional structure revealed that organic solvent stability of lipases is their intrinsic property and unique with respect to each lipase [5].

Guidelines have been provided to predict enzyme activity in organic media [6]. For example, the amount of water present in the system should be expressed in terms of its thermodynamic activity instead of its concentration. Halling [7] observed that a change in the reaction solvent or in the support used for immobilization showed no effect on the quantity of water required for maximum activity. In addition, certain enzymes equally accelerated reactions catalyzed in water or organic media [8]. However, very few studies have been carried out to predict activity in organic solvents compared to that in water. Pencreac′h and Baratti

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[9] proved that activity in organic media could not be estimated from that in water.

Lipase specificity has been studied extensively. In most cases, different kinds of specificity or selectivity can be distinguished during hydrolysis or synthesis reactions [10]. Chain-length specificity has been related to structural features of lipases [11, 12], but to date no lipase has been found to be strictly specific for a given chain length. Knowledge about substrate specificity would simplify the choice of a specific biocatalyst for a certain reaction.

Chain-length specificity of lipase could be modified when these enzymes are immobilized [13]. Enzyme immobilization has proven to be a useful technique to enhance enzymatic activity in organic media [14, 15]. Immobilization generally stabilizes the enzyme, enhances lipase properties such as thermostability and activity in a non-aqueous medium and improves flexibility of the enzyme/substrate complex [13]. There exists a variety of methods to immobilize lipases, using many different types of support. Techniques have roughly been classified into five categories: adsorption, covalent binding, entrapment, microencapsulation and naturally immobilized biocatalysts (whole cell biotransformation) [16]. Whole-cell biocatalysts can be readily and cheaply cultured in large quantities, such whole-cell systems could become promising and inexpensive biocatalysts [17]. Thus, among the established whole-cell biocatalyst systems, filamentous fungi have arisen as the most robust whole-cell biocatalyst for industrial applications [18].

In this work, the reactivity of three lipase preparations such as the constitutive mycelium-bound lipase, the induced mycelium-bound lipase and the lyophilized induced supernatant obtained from *Aspergillus niger* MYA 135 was studied under hydrolytic and synthetic conditions. A selectivity-based analysis of each biocatalyst preparation toward transesterification or hydrolysis in organic medium was also evaluated.

2 Experimental Section

2.1 Microorganism and Maintenance

Aspergillus niger ATCC MYA 135, formerly *A. niger* 419 from the PROIMI culture collection, was used throughout this work. It was maintained by monthly transfers onto glucose–potato agar slants, incubated at 30 °C and stored at 4 °C.

2.2 Fermentation Medium

The fermentation medium was as follows (in g/l): sucrose, 10.0; KH_2PO_4, 1.0; NH_4NO_3, 2.0; $MgSO_4{\cdot}7H_2O$, 2.0; $CuSO_4$, 0.06. The initial pH was adjusted to 7.0 with NaOH.

2.3 Enzyme Production

Fermentation was carried out at 30 °C in 500-ml shake flasks (250 rpm) containing 100 ml of fermentation medium. Culture flasks were inoculated with 10 ml of a conidial suspension (about 10^6 conidia/ml) from a stock culture. After 24 h of incubation, the culture was transferred to another 500-ml shake flask containing either 50 ml 2 % (v/v) olive oil or distilled water and further incubated for 4 days under the same conditions. The mould developed a pelleted growth form. Mycelium was collected and washed with acetone by filtration at 4 °C for 3 min at 6,000 g; cells were used as enzyme source. Calibration curves were generated with wet and dry mycelium developed in medium without olive oil ($R^2 = 0.973$) or supplemented with 2 % olive oil ($R^2 = 0.982$). Supernatant obtained after filtration of olive oil-containing medium was also used as enzyme source. This lipase preparation was lyophilized and protein content was determined according to Bradford [19].

2.4 Specific Hydrolytic Activity in Aqueous and Organic Media

Specific hydrolytic activity in either aqueous or organic medium was measured using the following substrates dissolved in acetone: *p*NPCa (*p*-nitrophenyl caprate), *p*NPL (*p*-nitrophenyl laurate), *p*NPP (*p*-nitrophenyl palmitate) and *p*NPS (*p*-nitrophenyl stearate). In aqueous solvents, about 0.010 g of wet mycelium or lyophilized supernatant was added to 1 ml of 100 mM phosphate buffer (pH 7.0), containing 2 mM *p*-NP derivative, 0.1 % (w/v) gum Arabic and 0.4 % (w/v) Triton X-100 [20]. The molar extinction coefficient of *p*-nitrophenol (*p*-NP) under the given assay conditions was 0.0103 μM^{-1} cm^{-1}. In organic solvents, about 0.010 g of wet mycelium or lyophilized supernatant was added to 1 ml of *n*-hexane containing 2 mM *p*-nitrophenyl derivative [21]. The reaction mixture was shaken (150 rpm) at 37 °C. The absorbance of the supernatant containing *p*-NP was determined at 405 nm. The *p*-NP was extracted with 1 ml 0.25 M Na_2CO_3 from *n*-hexane before measurement. The molar extinction coefficient of *p*-NP under these assay conditions was 0.0205 μM^{-1} cm^{-1}. One unit of enzyme activity was defined as the amount of biocatalyst that released 1 µmol of *p*-NP per min. Specific activity was expressed as milliunits per gram of dry cell weight or protein for mycelium and lyophilized supernatant, respectively.

2.5 Enzymatic Transesterification of *p*-Nitrophenyl Derivatives with Different Alcohols

Enzymatic transesterification was carried out following the technique of Romero et al. [22]. Briefly, 100 µl of 20 mM

p-nitrophenyl derivative dissolved in acetone, 100 µl of each alcohol and about 0.010 g of wet mycelium or lyophilized supernatant were added to 800 µl of *n*-hexane. The reaction mixture was shaken (150 rpm) for 1 h at 37 °C, and *p*-NP in the supernatant was measured as mentioned above. A reaction mixture without alcohol served as hydrolysis control. In the absence of a biocatalyst, no reaction was observed. One unit of transesterification activity was defined as the amount of biocatalyst that released 1 µmol of *p*-NP per min. Specific transesterification activity was expressed as milliunits per gram of dry cell weight or protein for mycelium and lyophilized supernatant, respectively.

2.6 Statistical Analysis

Statistical analysis was performed using the Minitab (version 14; Minitab Inc) software for Windows. Statistical significance values of the means were evaluated using a one-way analysis of variance. Subsequent comparisons were performed using Tukey's post hoc test. Results were presented as the mean ± SD. Differences were accepted as significant when $P < 0.05$.

3 Results and Discussion

Previously, it was reported an induced extracellular lipolytic extract from *A. niger* MYA 135 that is very stable in the presence of 50 % water-miscible organic solvents. This lipase activity also retains around of 60 and 80 % of its specific hydrolytic activity in aqueous medium after incubation for 1 h at 37 °C with *n*-butanol and *n*-hexanol, respectively [23]. In addition, both the induced and the constitutive mycelium bound lipases are very stable in reaction mixture containing methanol and ethanol. In fact, the constitutive mycelium bound-lipase maintaining almost 100 % of its specific hydrolytic activity in aqueous medium after exposure by 1 h at 37 °C in ethanol [22]. This is an important result because hydrophilic solvents can often destabilize the lipase activity.

On the other hand, under the conditions employed for hydrolysis, the only possible acceptor was water whereas during transesterification both acceptors, alcohols and water, competed in the nucleophilic attack of the acyl-enzyme [24]. Moreover, alcohol, like water, is not only a substrate but also a solvent that may influence thermodynamic parameters of the reaction and enzyme activity. In initial experiments, the performance of both the constitutive and the induced mycelium-bound lipases were evaluated in transesterifications of *p*-nitrophenyl palmitate with different alcohols in the presence of *n*-hexane as a solvent [22, 25]. Additionally, an entrapped extracellular purified extract was able to produce ethyl estearate in a solvent-free system with a transesterification activity of 0.78 ± 0.01 U/l [26].

Thus, taking in mind a future application in food, pharmaceutical and energy industries, the specific hydrolytic activity of three biocatalyst systems such as the constitutive mycelium-bound lipase, the induced mycelium-bound lipase and the lyophilized induced lipase supernatant from *A. niger* MYA 135 was determined against various substrates in both aqueous and organic media. In addition, the selectivity of the three biocatalysts toward transesterification or hydrolysis reaction in organic medium was analyzed as well. A scheme of the reactions assayed is showed in Fig. 1.

3.1 Hydrolysis Reactions in Aqueous and Organic Media

The specific hydrolytic activity of three biocatalyst systems such as the constitutive mycelium-bound lipase, the induced mycelium-bound lipase and the lyophilized induced lipase supernatant was determined against various substrates in both aqueous and organic media. Interestingly, substrate specificity changed depending on the medium in which hydrolysis was carried out. In aqueous medium all three biocatalysts showed highest specific hydrolytic activities with middle-chain fatty acids esters (*p*-NPCa and/or *p*-NPL); while, in organic medium the biocatalysts preparations displayed highest specific hydrolytic activities with long-chain fatty acids esters (*p*-NPP and/or *p*-NPS) (Table 1). However, the constitutive mycelium-bound lipase exhibited the highest specific hydrolytic activity (53.8 ± 2.7 mU/g_{dw}) in organic medium in the presence of *p*-NPS and the induced mycelium-bound lipase exhibited the highest specific hydrolytic activity (37.8 ± 3.8 mU/g_{dw}) in aqueous medium in the presence of *p*-NPL. Concerning the lyophilized induced lipase supernatant, the highest specific hydrolytic activity (5295.0 ± 0.5 mU/$g_{protein}$) was observed in organic medium using *p*-NPP as substrate. Thus, differences in substrate specificity may result from the fact that the catalysis is heterogeneous, except for the case of the hydrolysis in aqueous medium catalyzed by the lyophilized induced lipase supernatant. Therefore, diffusional limitations may change the specificity of lipases toward different substrates.

Various factors are involved in chain-length selectivity of lipases. Substrate thermodynamic properties determine the chemical reactivity and reaction equilibrium. These properties notably depend on substrate chain-length, temperature, solvent and the composition of the reaction medium [7, 27]. Enzyme structure and molecular dynamics are determinants for substrate specificity. Influence of the lipase structure on the chain-length specificity has been

Reaction	S_1	P_1	S_2	P_2	Reaction medium
Hydrolysis	Ester (p-NP derivate)	(*p*-NP)	H_2O	FA	Aqueous
Hydrolysis	Ester (p-NP derivate)	(*p*-NP)	H_2O	FA	Organic
Transesterification	Ester (p-NP derivate)	(*p*-NP)	Alcohol	Ester	Organic

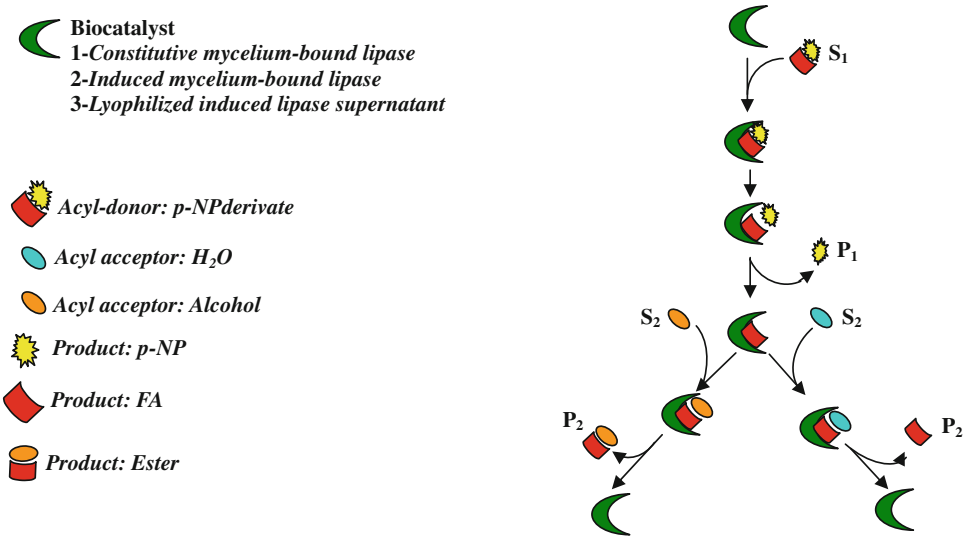

Fig. 1 Mechanisms of hydrolysis and transesterification reactions catalyzed by lipase preparations

Table 1 Specific hydrolytic activities in both aqueous and organic media and $R_{O/A}$ ratio of all three biocatalysts from *A. niger* MYA 135

Biocatalysts/substrate	Specific hydrolytic activity		$R^*_{O/A}$
	Aqueous medium (mU/g_{dw})	Organic medium (mU/g_{dw})	
Constitutive mycelium-bound lipase			
p-NPCa (C10)	33.8 ± 0.5 (e)	25.2 ± 0.3 (d)	0.75
p-NPL (C12)	31.9 ± 4.6 (e)	16.4 ± 0.9 (c)	0.52
p-NPP (C16)	13.1 ± 1.1 (b)	16.5 ± 2.1 (c)	1.26
p-NPS (C18)	7.5 ± 0.6 (a)	53.8 ± 2.7 (f)	7.13
Induced mycelium-bound lipase			
p-NPCa (C10)	20.0 ± 0.8 (c)	15.1 ± 0.4 (b)	0.76
p-NPL (C12)	37.8 ± 3.8 (e)	16.0 ± 1.0 (b)	0.42
p-NPP (C16)	19.5 ± 1.0 (c)	25.9 ± 0.8 (d)	1.32
p-NPS (C18)	12.3 ± 0.2 (a)	21.0 ± 0.2 (c)	1.71
Biocatalysts/substrate	Specific hydrolytic activity		$R^*_{O/A}$
	Aqueous medium (mU/$g_{protein}$)	Organic medium (mU/$g_{protein}$)	
Lyophilized induced lipase supernatant			
p-NPCa (C10)	3122.1 ± 72.8 (e)	2798.8 ± 0.3 (c)	0.90
p-NPL (C12)	4381.9 ± 31.5 (g)	2843.1 ± 0.1 (d)	0.65
p-NPP (C16)	2083.4 ± 35.9 (b)	5295.0 ± 0.5 (h)	2.54
p-NPS (C18)	1174.6 ± 97.1(a)	4089.9 ± 0.2 (f)	3.48

* $R_{O/A}$, ratio of specific hydrolytic activity in organic medium to specific hydrolytic activity in aqueous medium. For each biocatalyst, values with the same letter are not significantly different ($P > 0.05$)

studied by several authors [28, 29]. The structural flexibility of the enzyme is lower in organic solvents than in aqueous media, which may also altered enzyme activity [4, 30]. This effect is especially important with lipases for which the opening of the lid covering the active site is necessary for good activity [31, 32]. Thus, in organic medium a small amount of water molecules is frequently essential to obtain a sufficient enzyme conformational flexibility [33, 34].

To simulate industrial application, the specific hydrolytic activity in *n*-hexane was determined without any treatment or equilibration at a given water activity. In this connection, the results obtained in this work can be useful in the fatty acid industry where lipase-catalyzed hydrolysis of fats and oils at mild temperature in organic solvent could reduce energy cost, facilitate recovery of the fatty acid product, and yield a product of superior quality [35].

3.2 Comparison of Specific Hydrolytic Activities in Aqueous and Organic Media: The Activity Ratio $R_{O/A}$

Considering that the same hydrolytic reaction was conducted in both aqueous and organic media the activity ratio $R_{O/A}$, defined as the ratio of activity in organic medium to that in aqueous medium, was used to characterize the activity in organic medium [9]. As shown in Table 1, $R_{O/A}$ values from 0.42 to 7.13 were obtained. Although $R_{O/A}$ values for all three biocatalysts were less than one during hydrolysis of middle-chain fatty acid esters and higher than one during hydrolysis of long-chain fatty acid esters, the constitutive mycelium-bound lipase showed the highest $R_{O/A}$ value (7.13) in a reaction mixture containing *p*-NPS as substrate. It means that the constitutive biocatalyst preparation was the most active in hydrolysis reaction conducted in organic medium. In addition, this $R_{O/A}$ value was higher than that reported for a lipase activity from *A. niger* A6 (Amano) [9], which showed a $R_{O/A}$ value of 0.3.

On the other hand, in a useful work, Pencreac′h and Baratti [9] reported the activity ratio $R_{O/A}$ calculated for 26 commercial lipase preparations. They found that this parameter widely varies within the tested biocatalysts. In this connection, the results presented in this work shown that for a given substrate and solvent the $R_{O/A}$ ratio also depend on the way that the biocatalyst was prepared. Additionally, in agreement with those authors, the activity in aqueous medium cannot be predicted from the activity in organic media. This fact was confirmed by studying the relationship between activity in aqueous (A_{HA}) and organic media (A_{HO}) for each biocatalyst. A log/log plot of A_{HO} versus A_{HA} showed a cloudy pattern with no real correlation between the two variables (data not shown); the R^2 values for constitutive mycelium-bound lipase, induced mycelium-bound lipase and lyophilized induced lipase supernatant were 0.39, 0.23 and 0.46, respectively. However, a log/log plot of $R_{O/A}$ of the three biocatalysts versus A_{HA} gave a satisfactory linear relationship (R^2: 0.85 and a slope of −1.4 for constitutive mycelium-bound lipase, R^2: 0.87 and a slope of −1.2 for induced mycelium-bound lipase and R^2: 0.92 and a slope of −1.3 for lyophilized induced lipase supernatant) (Fig. 2). As can be seen, the $R_{O/A}$ values decreased with the A_{HA}, which shows the influence of the substrate specificity besides the possible impact of diffusional limitations.

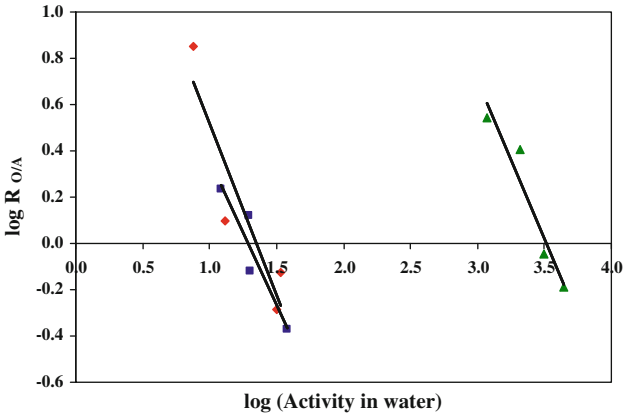

Fig. 2 Log scale plot of $R_{O/A}$ versus activity in water for all three biocatalysts from *A. niger* MYA 135. Each *line* represents the linear regression of all experimental points. Constitutive mycelium-bound lipase (*diamond*), Induced mycelium-bound lipase (*square*) and lyophilized induced supernatant (*triangle*)

3.3 Transesterification Reactions

Transesterifications of different alcohols with various *p*-nitrophenyl derivatives using all three biocatalysts preparations were evaluated in *n*-hexane (Fig. 3). For methanolysis and ethanolysis, the constitutive mycelium-bound lipase displayed an interesting preference for C16 substrate (Fig. 3A). Such mono alkyl esters like methyl and ethyl palmitate are component of biodiesel, an alternative fuel for diesel engines [36]. In addition, transesterifications catalyzed by the induced mycelium-bound lipase in the presence of propanol or butanol and long-chain fatty acid esters (*p*-NPP and *p*-NPS) showed a good performance as well. This is an interesting result because propanol and butanol were also used in the biodiesel production due to the good pouring characteristics of their fatty esters at low temperatures [37].

On the other hand, in general, the induced mycelium-bound lipase displayed high specific transesterification activities in the presence of water-miscible alcohols and middle-chain fatty acid esters (*p*-NPCa and *p*-NPL), being the highest specific transesterification activity

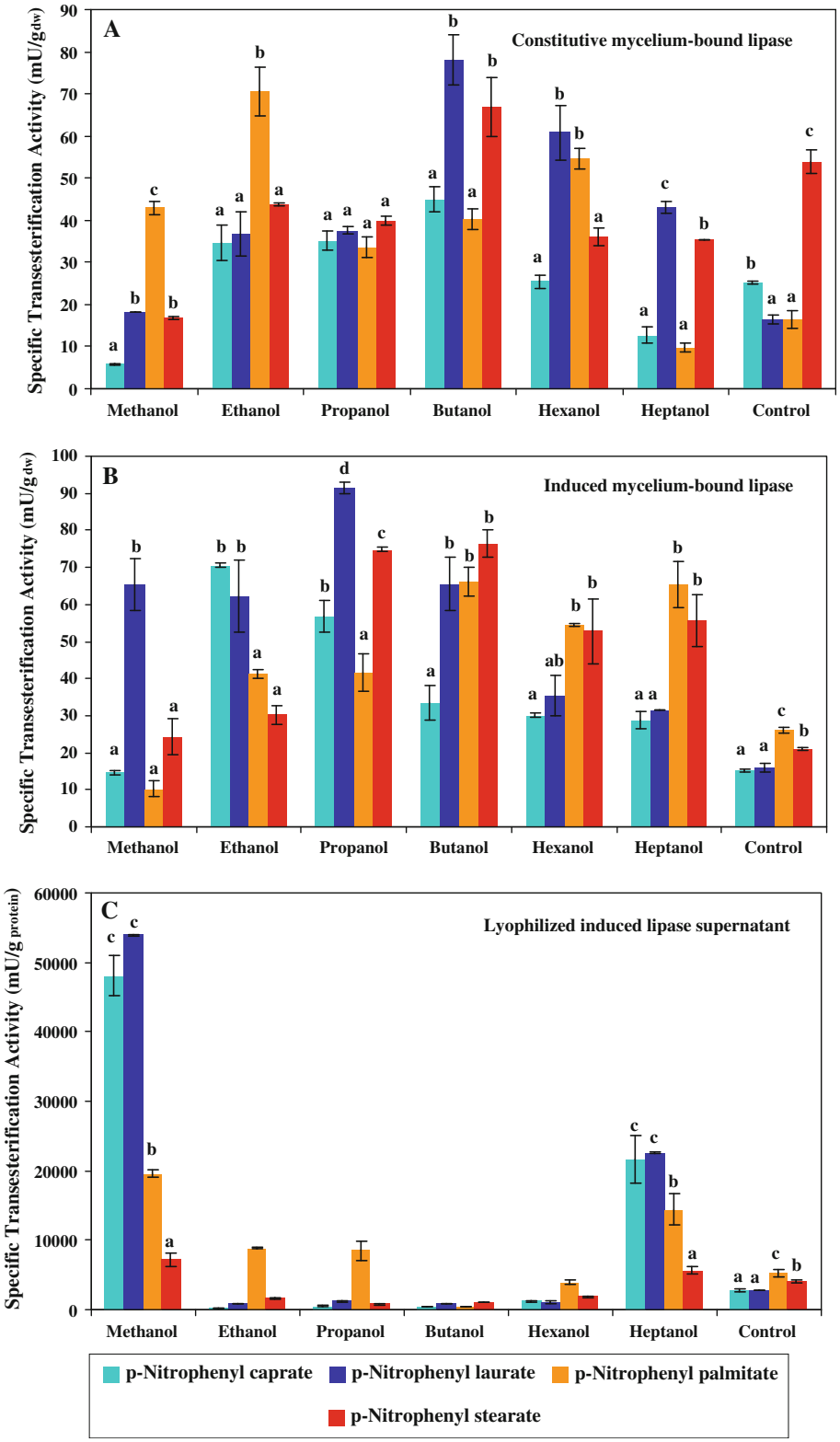

Fig. 3 Specific transesterification activities of all three biocatalysts from *A. niger* MYA 135 determined in reaction mixtures containing *p*-nitrophenyl derivatives and different alcohols. Reaction mixture without alcohol served as hydrolysis control. *Error bars* represent the standard deviation calculated from at least three independent experiments. For each alcohol, *bars with the same letter* are not significantly different ($P > 0.05$)

(91.4 ± 1.7 mU/g_{dw}) observed in a reaction mixture containing propanol and *p*-NPL (C12) (Fig. 3B). Those synthesized esters have applications as flavors constituents in food industry [38]. Besides, it is also interesting to highlight the good performance of this biocatalyst preparation in transesterifications carried out in the presence of water-immiscible alcohols and long-chain fatty acid esters (*p*-NPP and *p*-NPS).

Finally, both *p*-NPC (C10) and *p*-NPL (C12) were preferentially methanolized by the lyophilized induced lipase supernatant (Fig. 3C). Thus, under transesterification conditions, this lipase activity was the most specific biocatalyst preparation.

3.4 Transesterification Versus Hydrolysis in Organic Medium

An important issue in this study was the selectivity-based analysis of each lipase preparation toward transesterification or hydrolysis in organic medium. As displayed in Fig. 3, of the 72 transesterifications tested, 45 exhibited a specific transesterification activity value higher than its corresponding hydrolysis control (reaction mixture without alcohol). However, the selectivity toward a given reaction differed with each biocatalyst preparation. The mycelium-bound lipase showed around sixty percent of selectivity toward transesterification; of 24 transesterifications tested, 15 revealed a specific transesterification activity value higher than its corresponding hydrolysis control. Interestingly, the reactivity toward transesterification was drastically increased in the presence of the induced mycelium-bound lipase; only three reactions with a specific activity value lower than its corresponding hydrolysis control were observed. Concerning the reaction catalyzed by the lyophilized induced lipase supernatant, this biocatalyst preparation exhibited a remarkable specificity toward transesterification, especially in the presence of methanol; of 24 transesterifications tested, 15 reactions displayed a specific transesterification activity value lower than its corresponding hydrolysis control, showing that in most cases water seems to be a better acyl acceptor than the alcohols tested. In this connection, Vaysse et al. [10] reported the chain-length selectivity profile of seven lipases. They found that the chain-length specificity depends on the enzyme and the reaction considered, due to the combined influence of enzyme and substrate properties. According to the results discussed in this work, the substrate specificity also depends on the way that the enzyme was prepared.

In addition, the relation between the transesterification and the hydrolysis in organic medium was also evaluated in more detail. Each transesterification value was subtracting to that measured for its respective hydrolysis control. This transesterification value was used to analyze the ratio of transesterification to hydrolysis in organic medium (A_T/A_{HO}) for each biocatalyst (Table 2). Thus, in general, the constitutive mycelium-bound lipase showed selectivity toward synthesis when either *p*-NPL (C12) or *p*-NPP (C16) was used as acyl donors. On the contrary, specific hydrolytic activity was favored when either *p*-NPCa (C10) or *p*-NPS (C18) was used as acyl donor, which agrees with the highest specific hydrolytic activities observed in organic medium containing these *p*-nitrophenyl derivatives (Table 1). On the other hand, the induced mycelium-bound lipase exhibited the highest A_T/A_{HO} ratio in the presence of *p*-NPL (C12) and propanol. Only with methanol as acyl acceptor and *p*-NPP as acyl donor the reaction seems to be completely shifted to hydrolysis; in fact, the highest specific hydrolytic activity in organic medium was observed in the presence of *p*-NPP (Table 1). Finally, the lyophilized induced lipase supernatant showed a clear preference toward transesterification in reaction mixtures containing methanol as acyl acceptor and middle-chain fatty acids esters as acyl donors, showing an A_T/A_{HO} ratio of 16.2 for *p*-NPCa and 17.9 for *p*-NPL (Table 2).

4 Conclusions

Three lipase preparations such as the constitutive mycelium-bound lipase, the induced mycelium-bound lipase and the lyophilized induced supernatant obtained from *A. niger*

Table 2 Selectivity of all three biocatalysts from *A. niger* MYA 135 toward transesterification or hydrolysis in organic medium

A_T/A_{HO} [a]												
Biocatalysts	Constitutive mycelium-bound lipase				Induced mycelium-bound lipase				Lyophilized induced lipase supernatant			
Substrate	*p*-NPCa	*p*-NPL	*p*-NPP	*p*-NPS	*p*-NPCa	*p*-NPL	*p*-NPP	*p*-NPS	*p*-NPCa	*p*-NPL	*p*-NPP	*p*-NPS
Methanol	–	0.1	1.6	–	<0.1	3.0	–	0.2	16.2	18.0	2.7	0.8
Ethanol	0.4	1.5	3.1	–	3.7	2.9	0.4	0.4	–	–	0.7	–
Propanol	0.4	1.3	1.0	–	2.8	4.7	0.6	2.6	–	–	0.6	–
Butanol	0.8	3.7	1.5	0.2	1.2	3.1	1.6	2.6	–	–	–	–
Hexanol	<0.1	2.7	2.3	–	1.0	1.2	1.1	1.5	–	–	–	–
Heptanol	–	1.6	–	–	1.0	1.0	1.5	1.7	6.7	6.9	1.7	0.4

Each transesterification value was subtracting to that measured for its respective hydrolysis control. This transesterification value was used to analyze the ratio A_T/A_{HO}

[a] A_T/A_{HO}, ratio of specific transesterification activity to specific hydrolytic activity in organic medium

MYA 135 were evaluated based on their reactivity under both hydrolytic and synthetic conditions. The specific hydrolytic activity of all three biocatalyst was determined against several *p*-nitrophenyl derivatives in both aqueous and organic media. Interestingly, substrate specificity changed depending on the medium in which hydrolysis was carried out. Concerning the specific transesterification activity, the induced mycelium-bound lipase was the biocatalyst most reactive, whereas the lyophilized induced supernatant was the biocatalyst most specific. Finally, an important issue in this study was the selectivity-based analysis of each lipase preparation toward transesterification or hydrolysis in organic medium. It was found that the substrate specificity also depends on the way that the enzyme was prepared. Thus, knowledge about substrate specificity of a lipase preparation would simplify the choice of a specific biocatalyst for a certain reaction and optimize the industrial process.

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