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ISOLATION OF A LIPASE-PRODUCING *TRICHOSPORON* SPP AND ENZYME EXTRACTION BY TWO-PHASE AQUEOUS SYSTEM

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SHORT COMMUNICATION

ABSTRACT

A lipase-producing yeast strain isolated from crude cheese and identified as *Trichosporon spp* produced 7.3 U/mL (59.3 U/µg) after 72h of cultivation. Lipase showed optimum activity at pH 7.0-8.0 and 45-50ºC. Extraction by the two-phase aqueous system (PEG-phosphate salts) showed an elevated recuperation (99.8%) of enzymatic activity in the PEG phase.

Key words: *extracellular lipase*, Trichosporon, *aqueous two phase system*, *PEG*

Lipolytic enzymes are frequently used for conferring different flavours of cheeses during cheese production. Most of these enzymes are produced by the milk yeast microbiota (4). Search for good lipase sources is often accompanied by the necessity of alternative ways for enzyme extraction, which can be related to a simple culture-medium design (3) or to the application of selective methods of enzyme separation. The aqueous two phase system (ATPS) is used for separation and purification of biomaterials and it is produced when aqueous solutions of polymers and salts or two polymers are mixed. The most used system is constituted by poly(ethylene) glycol (PEG) and phosphate salts (9). In this work, we report the isolation of lipase-producing yeast identified as *Trichosporon spp* and enzyme extraction by the ATPS PEG-phosphate salts. Thirty yeast isolates from crude cheese were screened for lipase production in Victoria Blue solid medium according to Samad (10). A secondary selection was performed by inoculating a liquid basal medium containing yeast extract (10 g/L), $MgSO_4$ (0.1 g/L), KH_2PO_4 (1.0 g/L) and 1.0% (v/v) triolein with 10^8 cells/mL. Lipase activity was determined by the pNPP method (8). One unit (U) of enzymatic activity was defined as the release of 1 µmol of p-nitrophenol per min. Protein determinations were performed according to Bradford (1). Aqueous two phase system was prepared as described by Queiroz *et al* (9). The isolated *Trichosporon spp* produced up to 7.3 U/mL after 72 h of cultivation. Optimum pH and temperature were pH 7.0-8.0 and 45-50ºC, respectively, similarly to other microbial lipases. Yeasts of the genus *Trichosporon* have been frequently isolated from milk and dairy products (4) and many *Trichosporon* species have been used for lipase production and isolation (5,7). Lipase extraction was tested in different pH values and tie line lengths. The enzyme preferably partitioned to the PEG phase, probably due to the hydrophobic properties of PEG, as also observed by other authors (2). Particularly, best results were shown with PEG 8000, tie line 2.0 at pH 8.0, with coefficient of partition 2.69 (Figure 1). In this system, 99.8% of the lipase activity present in the system was recovered in the PEG phase. Moreover, specific activity was the highest observed (Table 1), with the purified enzyme maintaining its optimal pH and temperature of activity. Nevertheless, the purification factor and yield values achieved suggested that the system must be optimized to reach better yields. Thus, the results indicate that the combination presented

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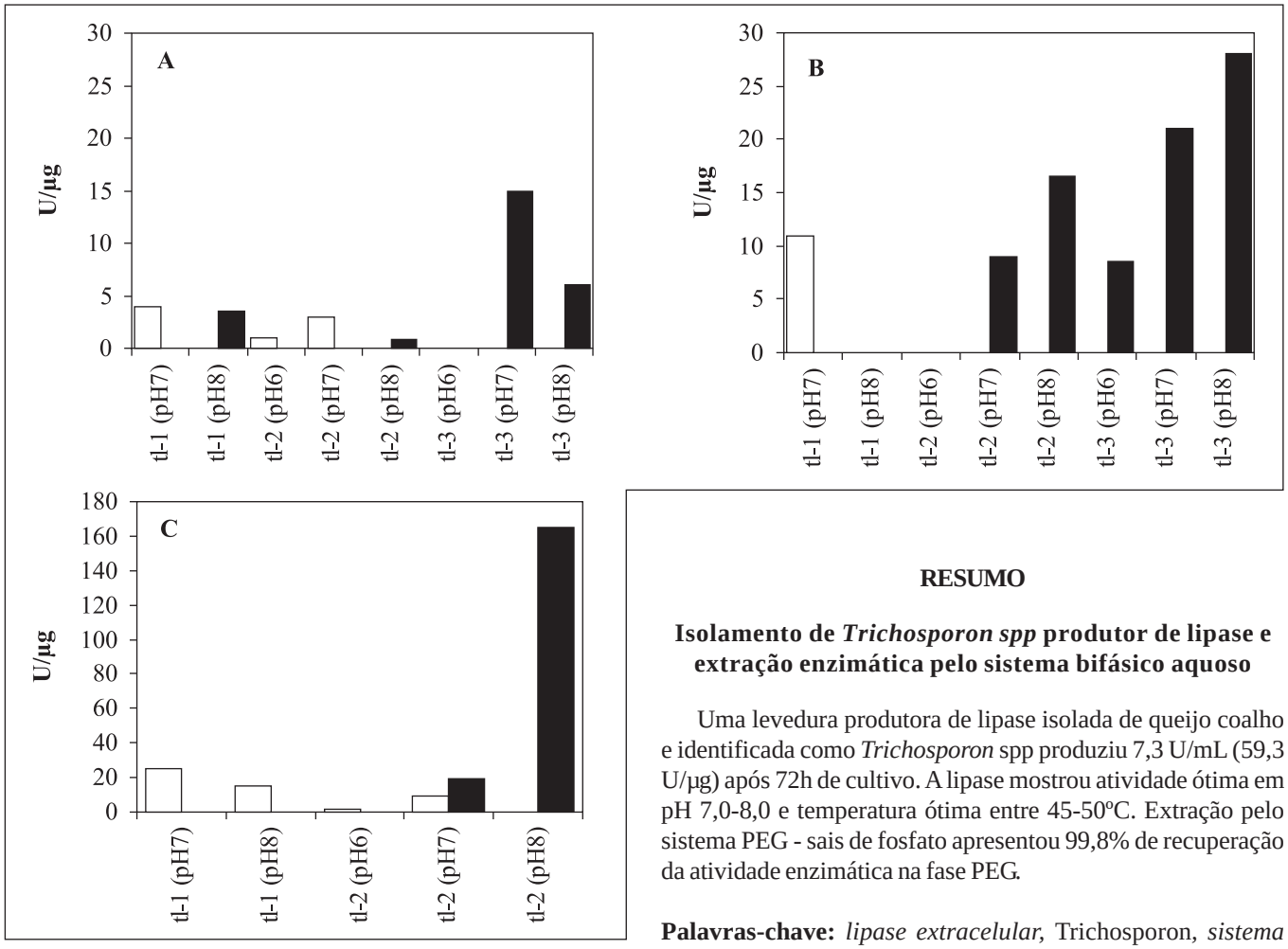

Figure 1. Specific lipase activity in systems containing PEG 1000 (A), PEG 3350 (B) and PEG 8000 (C) in different tie-lines and pH values. **tl**: tie line.

Table 1. Extraction of *Trichosporon spp* lipase with two-phase aqueous system using PEG-8000, tie line 2 and pH 8.0.

	Lipase activity (U/mL)	Protein (µg/mL)	Specific activity	Purification factor (U/µg)	Yield (%)	Coefficient of partition
Crude extract	7.3	0.123	59.3	1	100	1
PEG 8000	1.5	0.009	166.6	2.80	20.5	2.69

here of the *Trichosporon spp* isolate and the PEG/phosphate-salts system has a good potential for high lipase production and rapid enzymatic recovering.

RESUMO

Isolamento de *Trichosporon spp* produtor de lipase e extração enzimática pelo sistema bifásico aquoso

Uma levedura produtora de lipase isolada de queijo coalho e identificada como *Trichosporon* spp produziu 7,3 U/mL (59,3 U/µg) após 72h de cultivo. A lipase mostrou atividade ótima em pH 7,0-8,0 e temperatura ótima entre 45-50ºC. Extração pelo sistema PEG - sais de fosfato apresentou 99,8% de recuperação da atividade enzimática na fase PEG.

Palavras-chave: *lipase extracelular,* Trichosporon, *sistema bifásico aquoso*

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