Mycopathologia **133:** 119–121, 1996.

Production of lipase free of citrinin by *Penicillium citrinum*

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Received 11 May 1995; accepted in revised form 16 November 1995

Abstract

Lipase (Glycerol ester hydrolase E.C. 3.1.1.3) from a Brazilian strain of *Penicillium citrinum* free of the mycotoxin citrinin has been investigated. Citrinin production was inhibited by using culture medium containing olive oil, soybean oil and corn oil as carbon sources. Potassium concentration and pH play an important role in citrinin production. Potassium concentration lower than 30 mM and pH below 4.5 inhibited the mycotoxin production. *P. citrinum* produced lipase free of extraneous proteins and citrinin when cultured using, as nitrogen source, ammonium sulphate (lipase activity of 7.88 U/mg) and yeast extract (lipase activity of 4.95 U/mg) with olive oil as carbon source. This data is relevant to the larger scale production of lipases for food technology applications, from *Penicillium citrinum*.

Key words: *Penicillium citrinum*, citrinin, lipases

Introduction

Penicillium citrinum, a mold found in contaminated foods, is a potential application source of several industrial enzymes, such as cellulases and lipases, used in detergent and waste treatment applications. *P. citrinum* culture in a solid-state fermentation has been reported to be a good cellulase producer [1]. Over 70% of cellulose was degraded after 12 days with this fungus. Lipase is produced in the presence of olive oil as inducer in a submerged fermentation using starch and peptone as carbon and nitrogen sources respectively [2]. *P. citrinum* also was used as lipase and protease producers, using olive oil and yeast extract as carbon and nitrogen sources respectively [3]. Industrial enzymes are generally commodity products, produced on a large scale, requiring the use of cheap, bulk nutrient components [4]. Since nutrient costs represent up to 50% of production costs, the search for appropriate medium components is continuous [3]. However, *P. citrinum*, which is widely distributed in contaminated food and herbal remedies, produces a mycotoxin with antibiotic activity called citrinin which is a health hazard [5,6]. In animals, it acts as a nephrotoxin, damaging the proximal tubules of the kidneys [7]. Inhibition of citrinin production during growth of *P. citrinum* is an essential development to allow further industrial applications (particularly in the food technology area) of enzymes from this microorganism. In this paper we report the production of lipase free of citrinin using inexpensive media.

Materials and methods

Microorganisms. *P. citrinum* was isolated from olive oil, as described [3].

Cultural conditions. Media: MY: 0.5% yeast extract, 1.0% glycerol or olive oil, at an initial pH of 6.5. MA: 0.75% ammonium sulphate, 30 mM KH_2PO_4, 1.0% olive oil or soybean oil or corn oil, at initial pH of 4.5. MP1: 0.75% ammomum sulphate in 50 mM potassium phosphate buffer at pH 6.5, 1.0% olive oil or soybean oil or corn oil. MP2: 0.75% $(NH_4)_2SO_4$ in 100 mM potassium phosphate buffer, pH 6.5, 1.0% olive oil. The fungus was grown in cotton-stoppered 2-l erlenmeyer flasks with 500-ml culture volume using

Table 1. Citrinin and lipase production by *P. citrinum* in different media (μg/ml of culture) (in 1% oil)

Culture medium	Glycerol		Olive oil		Soybean oil		Corn oil	
	Citrinin μg/mL	Lipase U/mgP	Citrinin μg/mL	Lipase U/mgP	Citrinin μg/mL	Lipase U/mgP	Citrinin μg/mL	Lipase U/mgP
0.5% yeast extract (pH 6.5)	3.89	0.00	2.28	4.95	–	–	–	–
0.75% NH_4 sulphate (pH 4.5) 30 mM K phosphate	–	–	0.00	7.88	1.28	0.80	0.00	0.32
0.75% NH_4 sulphate (pH 6.5) 50 mM K phosphate	–	–	5.91	0.27	13.99	0.11	14.42	0.00
0.75% NH_4 sulphate (pH 6.5) 100 m K phosphate	–	–	9.90	0.38	–	–	–	–

a 10% inoculum. Growth was at 28 °C with orbital shaking (100 rpm) for 3 days.

Citrinin assay. Citrinin was detected in a solvent extract of supernatant by its yellow fluorescence at 360 nm [5] and by TLC spots detected with UV light [8]. Samples of supernatants were extracted with acetonitrile:chloroform (1:2 v/v), after homogenizing for 30 min, separated and evaporated for citrinin determination. Samples were spotted on silica gel plates (silica gel G, Analtech, Inc, USA) and toluene:ethyl acetate:formic acid (5:4:1) was used to develop the plates. Citrinin was quantified using known standards (Sigma Chem. Co).

Lipase assay. Lipase activity was determined spectrophotometrically at 410 nm by the hydrolysis of *p*-nitrophenolpalmitate (pNPP) to *p*-nitrophenol (pNP) and palmitate [9]. One unit of enzyme activity was defined as the cleavage of 1 μmol/min pNPP, pH 8.0 and 37 °C. One unit of lipase activity from *P. citrinum* determined by the pNPP method is equal to one unit by the titration method [3].

Results and discussion

The effect of the culture medium composition is shown in Table 1. The citrinin production by *P. citrinum* decreased 41% when olive oil instead of glycerol was used as carbon source with yeast extract as nitrogen source (MY). The results were in agreement with those obtained with the traditional medium containing glucose and yeast extract used by Vinãs et al. [5] for citrinin production. This could be due to the glycerol–glucose relationship in the metabolism of this fungus. There was no detectable citrinin in the MA medium when yeast extract was replaced by ammonium sulphate in low potassium concentration (30 mM) and olive oil or corn oil as carbon source (Table 1). However, when the initial pH was increased from 4.5 to 6.5 and the potassium concentration from 30 to 50 mM (MP1), using olive oil, soybean oil and corn oil in the presence of ammonium sulphate, an increase in citrinin production was observed. In the presence of olive oil, in 100 mM phosphate buffer (MP2) at pH 6.5 the same behaviour was observed. These results showed that medium pH and potassium concentration have an important effect on the citrinin production by *P. citrinum*. Although the maximal lipase activity has been produced in medium containing olive oil and yeast extract (2850 U/l), the specific lipase activity was greater using ammonium sulphate (7.88 U/mg, 1585 U/l) than with yeast extract (4.95 U/mg proteins) as nitrogen source (Table 1). This result shows that using ammonium sulphate as the sole source of nitrogen produced lipase free of extraneous protein and citrinin.

Acknowledgements

This work was carried out with supported by CAPES, JICA, CNPq, PADCT, FINEP and the Federal University of Pernambuco. Thanks are due to Severino Humberto de Almeida for technical assistance.

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