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Ochratoxin degradation and adsorption caused by astaxanthin-producing yeasts

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Abstract

Ochratoxin degrading and adsorbing activities of *Phaffia rhodozyma* and *Xanthophyllomyces dendrorhous* isolates were tested. *P. rhodozyma* CBS 5905 degraded more than 90% of ochratoxin A (OTA) in 15 days at 20 °C. The data presented indicate that *P. rhodozyma* is able to convert OTA to ochratoxin α, and this conversion is possibly mediated by an enzyme related to carboxypeptidases. Chelating agents like EDTA and 1,10-phenanthroline inhibited OTA degradation caused by *P. rhodozyma* indicating that the carboxypeptidase is a metalloprotease, similarly to carboxypeptidase A. The temperature optimum of this enzyme was found to be above 30 °C, which is much higher than the temperature optimum for growth of *P. rhodozyma* cells, which is around 20 °C. The enzyme responsible for ochratoxin degradation was found to be cell-bound. Besides, both viable and heat-treated (dead) *P. rhodozyma* cells were also able to adsorb significant amounts (up to 250 ng ml^{-1}) of OTA. Heat treatment enhanced OTA adsorbing activities of the cells. Further studies are in progress to identify the enzyme responsible for OTA degradation in *P. rhodozyma*.

Keywords: Adsorption; Carboxypeptidase A; Detoxification; Ochratoxin A; *Phaffia rhodozyma*; *Xanthophyllomyces dendrorhous*

1. Introduction

Mycotoxins are secondary metabolites of fungi, which may cause diseases in animals or humans. Mycotoxin contamination of agricultural products is a serious health hazard throughout the world. One of the most important mycotoxins is ochratoxin A (OTA), which is produced by several *Aspergillus* and some *Penicillium* species (Varga et al., 2003). The occurrence of OTA in several commodities (feeds, foods and beverages) is considered as a serious health hazard in view of its nephrotoxic, teratogenic, hepatotoxic and carcinogenic properties (Varga et al., 2001).

Several strategies are available for the detoxification of mycotoxins. These can be classified as physical, chemical, physicochemical and (micro)biological approaches (Varga and Tóth, 2004). However, physical and chemical methods met with varying degrees of success. Microbes or their enzymes could be applied for mycotoxin detoxification; such biological approaches are now being widely studied (Sweeney and Dobson, 1998; Karlovsky, 1999; Bata and Lásztity, 1999). Such studies led to the identification of several microbes and their enzymes capable of detoxifying OTA, including several bacteria (Wegst and Lingens, 1983, Westlake et al., 1987; Skrinjar et al., 1996; Hwang and Draughon, 1994), yeasts (Böhm et al., 2000; Molnar et al., 2004), filamentous ascomycetes (Varga et al., 2000, 2005; Abrunhosa et al., 2002) and basidiomycetes (Engelhardt, 2002). An adsorption mechanism has also been suggested for OTA removal by lactic acid bacteria (Piotrowska and Zakowska, 2000), yeasts (Bejaoui et al., 2004) and conidia of black aspergilli (Bejaoui et al., 2005).

In this study, *Phaffia rhodozyma* and *Xanthophyllomyces dendrorhous* isolates were examined for their ability to degrade and/or adsorb OTA in a liquid medium. *P. rhodozyma* is a red-pigmented fermentative yeast, first

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isolated from exudates of deciduous trees in Japan and Alaska (Miller et al., 1976). Recent studies revealed that different isolates handled previously as *P. rhodozyma* comprised in fact two astaxanthin-producing yeasts: *P. rhodozyma* and *X. dendrorhous* (Fell and Blatt, 1999; Palágyi et al., 2004). These closely related species are the only known yeasts that produce the pigment astaxanthin. This carotenoid is an important component of fish food since it provides pigmentation, and it may contribute to the successful reproduction of fish and crustacea in man-made environments (An et al., 1991). Astaxanthin also exhibits antioxidant and anti-inflammatory properties, and it was suggested that, protecting body tissues from oxidative damage with daily ingestion of natural astaxanthin might be a practical and beneficial strategy in health management (Johnson, 2003).

In this study, we examined OTA degrading and adsorbing activities of astaxanthin-producing yeast isolates. The data presented indicate that besides producing astaxanthin, *P. rhodozyma* is also able both to detoxify and adsorb OTA at temperatures well above the temperature optimum for growth of *Phaffia* cells.

2. Materials and methods

2.1. Micro-organisms

One strain of *P. rhodozyma* (CBS 5905) and 2 strains of *X. dendrorhous* (CBS 5908, CBS 6938) were tested for their ability to degrade and adsorb OTA under controlled conditions. The strains were maintained on malt extract agar slants.

2.2. Examination of OTA degradation of Phaffia/Xanthophyllomyces isolates

Two millilitres aliquots of PM (0.5% yeast extract, 1% sucrose, 0.5% peptone and 0.2% malt extract) medium containing 7.5 μg ml^{-1} OTA (Sigma-Aldrich Chemical Co., O1877, Budapest, Hungary) were inoculated with dense cell suspensions of the *Phaffia/Xanthophyllomyces* strains and incubated at 20 °C for 14 days in the light. OTA was extracted with 2 ml of dichloromethane, 1 ml of the organic phase was evaporated to dryness and dissolved in 100 μl dichloromethane. Twenty microlitres of the extracts were spotted on thin layer chromatography (TLC) plates and chromatographed as described previously (Téren et al., 1996).

For kinetic studies, 11-day-old cultures of *P. rhodozyma* CBS 5905 were inoculated at two concentrations (2×10^7 and 6×10^8 cell ml^{-1}) into 2 ml aliquots of PM containing 7.5 μg ml^{-1} OTA. The liquid cultures were grown for 1, 3, 5, 7, 11, 13 and 15 days at 20 °C, and the cells were separated from liquid medium by centrifugation (3500 rpm, 10 min). The ferment broths were extracted with 2 ml of dichloromethane, the organic phase was transferred to a clean tube, and liquid–liquid clean-up was performed by mixing it with 2 ml of 1% $NaHCO_3$. After centrifugation, the aqueous phase was acidified to pH 2 and OTA was extracted again with an equal volume of dichloromethane. Yeast cells were suspended in 0.5 ml distilled water, and treated as described above. Five to ten microlitres aliquots of these extracts were applied to high performance TLC (HPTLC) plates, developed and OTA was identified by densitometry as described previously (Varga et al., 1996). Kinetic studies were repeated three times.

To examine the effect of temperature on OTA degradation, a 3-day-old culture of *P. rhodozyma* CBS 5905 was inoculated into 2 ml aliquots of PM containing 5 μg ml^{-1} OTA. The cultures were cultivated for 1 and 3 days at different temperatures (10, 15, 20, 25, 30, 35 °C). After incubation, the cells were separated from liquid medium by centrifugation (3500 rpm, 10 min), and OTA content of the culture broth and cells was determined as described above. Experiments were repeated three times.

To further examine the range of temperature where the OTA degrading enzyme is active, a 3-day-old culture of *P. rhodozyma* CBS 5905 was heat-treated at different temperatures (30, 40, 50, 60, 70, 80, 90, 100 °C) for 15–20 min. After this treatment, *P. rhodozyma* cells were inoculated into 2 ml aliquots of PM containing 5 μg ml^{-1} OTA. The test tubes were incubated for 3 days at 30 °C, and the cells were separated from liquid medium by centrifugation (3500 rpm, 10 min). Cells and liquid medium were extracted as described above. Experiments were repeated three times.

2.3. HPLC analysis

For high performance liquid chromatograpy (HPLC) analysis, the dichloromethane extracts used for kinetic studies were evaporated and redissolved in 100 μl of water–acetonitrile–acetic acid (99:99:2). The HPLC equipment consisted of a S1100 solvent delivery system, S5110 sample injector valve with a 20 μl loop (SYKAM GmbH, Germany) and a Linear Instruments Model LC305 fluorescence detector ($\lambda_{ex} = 334$ nm, $\lambda_{em} = 460$ nm). BST Rutin C18 BD HPLC columns (250 × 4 mm, particle size 10 μm; BioSeparation Techniques, Budapest, Hungary) were used. OTA was eluted with acetonitrile–water–acetic acid (99:99:2) as mobil phase at a flow rate of 1 ml min^{-1} (Varga et al., 2005). OTA and ochratoxin α prepared by enzymatic hydrolysis of OTA as described by Stock (2004) were used as standards. For preparation of ochratoxin α, 1 mg OTA was dissolved in 11 ml buffer (0.1 M NaCl, 0.02 M Tris, pH 7.5) and digested by 62 Units of carboxypeptidase A (from bovine pancreas, Sigma Chemical Co., C9268, Budapest, Hungary) for 2 days at 25 °C. Ochratoxin α was extracted three times with 2 volumes of dichloromethane, the organic extracts were pooled and evaporated. Finally, ochratoxin α was dissolved in water–acetonitrile–acetic acid (99:99:2), and 5–10 μl amounts were used in HPLC analyses.