LWT - Food Science and Technology 97 (2018) 427–433

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Fermentative degradation of Patulin by *Saccharomyces cerevisiae* in aqueous solution

Min Li[a], Weizhu Chen[a], Zhuo Zhang[a], Zhiwei Zhang[b], Bangzhu Peng[a,*]

[a] Key Laboratory of Environment Correlative Dietology, Ministry of Education, College of Food Science & Technology, Huazhong Agricultural University, 430070, Wuhan, China

[b] College of Food Science and Engineering, Qingdao Agricultural University, 266109, Qingdao, China

ARTICLE INFO

Keywords:
Patulin
Saccharomyces cerevisiae
Fermentation
Metabolite
Biodegradation

ABSTRACT

Patulin (PAT) is a toxic metabolite produced by several filamentous fungi common to fruit- and vegetable-based products, which results in a potential food safety hazard. Over the past few years, there have been reports stating that PAT was removed by yeast from some fermented products. However, the interactions between PAT and yeast during fermentation are still unclear. In this paper, we focused on the interactions between PAT and *S. cerevisiae* CITCC 93161 in aqueous solutions during fermentation. The results demonstrated that PAT affected the yeast cells in a time- and concentration-dependent manner, but the yeast exhibited a very strong tolerance to the toxicity of PAT. The yeast cells could completely degraded PAT through the exo- and endo-enzymes synthesized during yeast fermentation, of which the latter exhibited higher activity. Moreover, the PAT biodegradation ability of yeast was not induced by the addition of such toxics to the culture media. Additionally, E-ascladiol was tentatively identified as the product of PAT biodegradation.

1. Introduction

Patulin (PAT) is a toxic secondary metabolite produced by various fungi such as *Penicillium*, *Aspergillus* and *Byssochlamys* (Tannous et al., 2017), which represents a serious health hazard and is a major issue in food safety (Castoria et al., 2011). The toxicity of PAT, including immunotoxicity (Drusch, Kopka, & Kaeding, 2007), genotoxicity (Donmez-Altuntas, Gokalp-Yildiz, Bitgen, & Hamurcu, 2013), mutagenicity (Smith, Duffus, & Small, 1993), and its other effects can cause gastrointestinal disorders and edema and bleeding in various organs in animals (Zhu et al., 2015). Therefore, PAT is a health hazard that results in both short-term and long-term effects.

PAT is widely found in rotten grains, fruits and vegetables, meat and related products, among which contamination is especially found in apples, pears, grapes and other fruits (Neri, Donati, Veronesi, Mazzoni, & Mari, 2010). Due to its thermal resistance, it is difficult to damage or degrade PAT during the processing of fruit juice; thus, it accumulates through the food chain and leads to serious threats to human health. In view of the potential toxicity and dangers of PAT, the provisional maximum tolerable daily intake for PAT set by the Joint FAO/WHO Expert Committee on Food Additives is 0.4 mg/kg body weight/day (Janotová, Čížková, Pivoňka, & Voldřich, 2011). The maximum level of PAT in food has also been limited in many countries worldwide. Regulations of European Commission set the maximum tolerable levels of PAT in fruit-based products and juices at 50 μg/kg, and for baby food at 10 μg/kg (EC Regulation 1881/2006). China also limited that PAT residuals in semi-finished products of apples and hawthorns is lower than 100 μg/kg, and that in fruit juices, jams, fruit wines, canned and preserved products should not exceed 50 μg/kg. In order to eliminate or reduce the food risk associated with PAT, many control methods, mainly including physical, chemical and biological methods, have been developed to remove or degrade PAT from food and raw materials in the past decades (Castoria et al., 2011; Guo, Yue, Hatab, & Yuan, 2012; Zhu et al., 2015).

In recent years, many types of yeast have been found to have the ability to degrade PAT and can be safely applied to food processing (Mccormick, 2013). Meanwhile, live yeasts are themselves sensitive to PAT, which has strong inhibiting effects on a variety of microbes (Bräse, Encinas, Keck, & Nising, 2009). Most yeasts biodegrade PAT when the concentration of PAT is below 200 μg/mL, but at concentrations greater than 200 μg/mL, they have been shown to completely inhibit the ability of fermentative detoxification (Sumbu, Thomart, & Bechet, 1983). Fermentation by live yeast can play an important role in reducing the potential risks of PAT-contaminated fruit juices for wine production. In order to achieve the efficient removal of PAT during yeast fermentation and to extend its applications to non-fermented products, it is crucial to

* Corresponding author. College of Food Science & Technology, Huazhong Agricultural University, 430070, Wuhan, Hubei, China.
E-mail address: bzpeng@mail.hzau.edu.cn (B. Peng).

https://doi.org/10.1016/j.lwt.2018.07.040
Received 18 April 2018; Received in revised form 12 July 2018; Accepted 20 July 2018
Available online 23 July 2018
0023-6438/

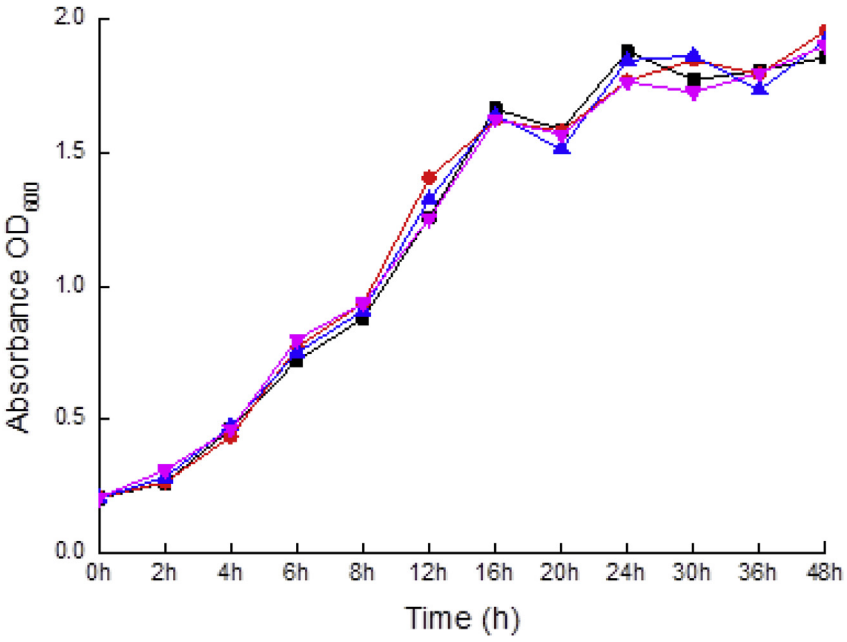

Fig. 1. Effects of PAT concentration on the growth of *S. cerevisiae* CITCC 93161. Yeast cells were inoculated in 50 mL flasks containing 10 mL sterilized YEPD medium with different PAT concentrations (—■— 0μg/L, —●— 100μg/L, —▲— 500μg/L, —▼— 1000μg/L) at 150 rpm and 30 °C.

understand the mechanism of PAT toxicity and its degradation pathway in yeast cells. However, the mechanism of PAT toxicity at a cellular level and that of PAT degradation by yeast are still unclear. The purpose of this work was to investigate the interaction mechanisms of PAT and *S. cerevisiae* cells during fermentation in aqueous solution, focusing on the metabolic mechanism and enzymes involved in PAT biodegradation.

2. Materials and methods

2.1. Materials

L-Malic acid was supplied by Sigma-Aldrich Corp (St Louis, MO, USA). Patulin standard (HPLC grade) was purchased from the Aladdin firm (Shanghai, China). Other chemicals were purchased from the Sinopharm Chemical Reagent Co.Ltd. (Shanghai, China). PAT was dissolved in ethyl acetate to make up a stock solution (100 mg/L) and stored at −20 °C. For each experiment, PAT working solutions were made from this stock solution. An aliquot (10 mL) of the stock solution was dried by nitrogen at room temperature and the residues were immediately dissolved in 10 mL of 0.5% acetic acid solution (pH 4.0) to form the working solution (100 mg/L). Distilled water was used for all experimental processes.

2.2. Preparation of S. cerevisiae cells

Saccharomyces cerevisiae CITCC 93161 (China Center for Type Culture Collection, CITCC, Wuhan, China), which was previously stored at −80 °C was incubated on a YEPD (3.0 g of yeast extract, 3.0 g of malt extract, 5.0 g of peptone, 10.0 g of glucose, 20.0 g of agar and 1.0 L of distilled water) plate before inoculation. The yeast were then inoculated into a 50 mL conical flask containing 10 mL of YEPD liquid medium (YEPD without agar), and the culture was shaken at 150 r/min and 30 °C for 12 h. After shaking, the culture was transferred into a 300 mL conical flask containing 90 mL of YEPD liquid medium and shaken under the same conditions for a new generation. The new culture was then poured into 500 mL conical flask containing 150 mL of YEPD liquid medium and shaken for the third generation. After 3 generations of activation, the yeast cell pellets were separated by centrifugation at 6000 r/min at 4 °C for 15 min, and were then washed 3 times with normal saline. These cell pellets were used as the yeast inoculum for the following experiments.

2.3. Effects of PAT on the growth of S. cerevisiae cells

The turbidity of yeast solutions was measured at 600 nm (OD_{600}) with a spectrophotometer (UV-160, Shimadzu, Japan) and the results were used to evaluate the cell growth of the organisms. Yeast cells in logarithmic phase were inoculated into 50 mL flasks containing 10 mL of sterilized YEPD medium, and the initial values of the OD_{600} were measured to be 0.2 for all samples. Aliquots of PAT working solution were added to the systems separately so that the final concentrations of PAT reached 0 μg/L, 100 μg/L, 500 μg/L and 1000 μg/L. The samples were then incubated for 72 h and the turbidity of each sample was measured at 0 h, 2 h, 4 h, 6 h, 8 h, 12 h, 16 h, 20 h, 24 h, 30 h, 36 h, 48 h, 60 h and 72 h, sequentially. Each experiment was performed independently three times.

2.4. Flow cytometry analyses

The yeast cell pellets obtained from Section 2.2 were added to various PAT solutions (1 mg/L, 10 mg/L and 100 mg/L) and the final concentration of the yeast cells was set to 10 g/L. The solutions were incubated and shaken at 150 rpm at 30 °C for 36 h. During incubation, an aliquot (1.0 mL) of each sample was collected at 0 h, 12 h and 36 h, sequentially, which was then centrifuged at 6000 r/min at 4 °C for 5 min. The residues (i.e., the collected cells) were then washed 3 times using normal saline and incubated with different fluorescent agents. The physiological and biochemical properties of the yeast cells were analysed with a FACSCalibur flow cytometer (Becton–Dickinson, Minnesota, USA), where the fluorescence signals generated by 5000 cells were collected for a single analysis.

2.4.1. Membrane permeability analyses

Damage to the cell membrane permeability induced by PAT was investigated by the flow cytometry analysis of cells double-stained with Hoechst 33342 and propidium iodide (PI) (Zhivotosky & Orrenius, 2001). Yeast cells collected at different incubation times were resuspended in 10 mL of normal saline. An aliquot (1.0 mL) of the solution was loaded into a centrifuge tube and stained subsequently with 10 μL of Hoechst 33342 and 5 μL of propidium iodide (PI) in dark at 37 °C for 15 min. Thereafter, the stained cells were collected by