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The Possible Mechanisms Involved in Citrinin Elimination by *Cryptococcus podzolicus* Y3 and the Effects of Extrinsic Factors on the Degradation of Citrinin

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Citrinin (CIT) is a toxic secondary metabolite produced by fungi belonging to the *Penicillium*, *Aspergillus*, and *Monascus* spp. This toxin has been detected in many agricultural products. In this study, a strain Y3 with the ability to eliminate CIT was screened and identified as *Cryptococcus podzolicus*, based on the sequence analysis of the internal transcribed spacer region. Neither uptake of CIT by cells nor adsorption by cell wall was involved in CIT elimination by *Cryptococcus podzolicus* Y3. The extracellular metabolites of *Cryptococcus podzolicus* Y3 stimulated by CIT or not showed no degradation for CIT. It indicated that CIT elimination was attributed to the degradation of intracellular enzyme(s). The degradation of CIT by *C. podzolicus* Y3 was dependent on the type of media, yeast concentration, temperature, pH, and initial concentration of CIT. Most of the CIT was degraded by *C. podzolicus* Y3 in NYDB medium at 42 h but not in PDB medium. The degradation rate of CIT was the highest (94%) when the concentration of *C. podzolicus* Y3 was 1×10^8 cells/ml. The quantity of CIT degradation was highest at 28°C, and there was no degradation observed at 35°C. The study also showed that acidic condition (pH 4.0) was the most favorable for CIT degradation by *C. podzolicus* Y3. The degradation rate of CIT increased to 98% as the concentration of CIT was increased to 20 μg/ml. The toxicity of CIT degradation product(s) toward HEK293 was much lower than that of CIT.

Keywords: Citrinin, *Cryptococcus podzolicus*, degradation, extrinsic factor

Introduction

Citrinin (CIT) is a toxic secondary metabolite produced mainly by some genera of *Penicillium*, *Aspergillus*, and *Monascus*. Studies have shown that CIT is nephrotoxic, hepatotoxic, and carcinogenic to humans and animals [1–3]. CIT commonly affects the kidney, resulting in the damage of the proximal tubules [4]. Furthermore, Gupta *et al*. [5] reported that the liver and bone marrow are among the organs affected by CIT. CIT can interfere with the intracellular redox system and cause the dysfunction of mitochondrial membrane permeability [6–9]. Many agricultural products such as fruits and vegetables are often contaminated with CIT-producing fungi during pre and postharvest phases [10–13]. These contaminated products are potential risk to consumers who consume them. Therefore, it is necessary to adopt appropriate measures to control CIT in these products.

The traditional ways to control mycotoxins include physical [14–16], chemical [17], and biological methods [18–22]. However, although physical methods such as filtration and adsorption could reduce toxins in foods, they also influence

sensorial qualities [23]. Synthetic fungicides can effectively prevent the growth of toxigenic fungi and accumulation of mycotoxins in foods. However, owing to the resistance of toxigenic fungi to synthetic fungicides, environmental pollution as well as the residual effects on human life have necessitated alternative control measures. Over the past decade, biological control has proved to be a viable alternative and emerged as a promising method to control toxigenic pathogens and accumulation of mycotoxins [20, 24]. In light of this, some studies have been conducted with the aim of controlling and/or degrading mycotoxins. It was found that some microorganisms reduced the accumulation of mycotoxins by inhibiting the growth of toxicogenic fungi. For instance, the antagonistic strain *Kluyveromyces thermotolerans* could control the growth of *Aspergillus carbonarius* and *Aspergillus niger* and the accumulation of ochratoxin A (OTA) [19]. In addition, mycotoxin could be eliminated by some microorganisms. Both thermally inactivated and viable *Saccharomyces cerevisiae* could eliminate OTA through its adsorption by the cell wall [25]. OTA could also be degraded directly by *Yarrowia lipolytica* and generates low toxicity products [26]. Cao *et al*. [18] reported that patulin produced by *Penicillium expansum* in apples was significantly reduced by *Pichia caribbica*. In vitro assay indicated that *P. caribbica* could degrade patulin directly.

Currently, there are few reports about the biological control of CIT. Previously, *Trichoderma hamatum* was used to reduce CIT produced by *Penicillium viridicatum* in rice grains [27]. CIT could be degraded by *Klebsiella pneumoniae* from 2.1% in 1 h to 91.33% in 5 h and was completely degraded at 10 h [28]. *Rhizobium borbori* PS45 was also reported to have the ability to degrade CIT cultured in mineral medium [29]. However, to the best of our knowledge, there is little information about the mechanisms of CIT elimination by microorganisms, as well as the influence of extrinsic factors on the degradation of CIT. The aims of this study were to (i) screen for safe yeast strains with the ability to eliminate CIT, (ii) study the mechanisms of CIT elimination by *Cryptococcus podzolicus* Y3, (iii) explore the factors that influence the degradation of CIT under varying conditions, and (iv) investigate the toxicity of the CIT degradation product(s) by *C. podzolicus* Y3.

Materials and Methods

Analysis of CIT by High-Performance Liquid Chromatography with Fluorescence Detection (HPLC–FLD)

CIT was quantified and detected using an Agilent 1100 system (Agilent Technologies, USA) with an Zorbax SB-C18 column (Agilent) and fluorescence detector. The mobile phase used consisted of acetonitrile and 0.03% orthophosphoric acid in the ratio of 45: 55 (v/v) with a flow rate of 1.0 ml/min. The detector excitation (λ_{ex}) and emission (λ_{em}) wavelengths were set at 331 and 500 nm, respectively.

Preparation of the CIT Standard Solution

The CIT stock solution was prepared by dissolving 5,000 μg of CIT in 5 ml of methanol and then stored at −20°C until use. All standard working solutions (0.01, 0.05, 0.1, 0.25, 1, 5, and 10 μg/ml) were prepared freshly by diluting the stock solution with methanol and then analyzed using HPLC-FLD. A CIT standard curve was generated based on the peak and concentration.

Isolation and Screening of Yeasts with the Ability to Eliminate CIT

Soil samples were collected from a vineyard in Zhenjiang (China) and yeasts were screened to determine their ability to eliminate CIT. First, 25 g of the sample was added into 250 ml of sterile saline and diluted by the decimal dilution method as the stock solution. The above stock solution was spread on Rose Bengal medium and cultured at 28°C for 48 h to isolate the yeasts. Single colonies were streaked on the same medium in different petri dishes to purify each isolate. The purified yeasts were maintained at 4°C on nutrient yeast dextrose agar medium (NYDA, 0.8% nutrient broth, 0.5% yeast extract, 1% glucose, and 2% agar). Subsequently, the yeasts isolated were cultured in 250 ml Erlenmeyer flasks containing 50 ml of nutrient yeast dextrose broth (NYDB) at 28°C and 180 rpm for 20 h. Thereafter, the cultures were centrifuged at 6,000 ×*g* for 10 min and the precipitates were washed twice with sterile distilled water to remove the growth medium. The cell pellets were resuspended in sterile distilled water and adjusted to 1×10^8 cells/ml with a hemocytometer for the experiments.

One milliliter of the above yeast suspensions was added into 50 ml of NYDB medium containing 10 μg/ml CIT and cultured at 28°C and 180 rpm. Then, 500 μl of each sample was taken from the cultures at 1, 2, 3, and 4 days, shaken for 30 sec, after which 500 μl of methanol was added. The mixture was then filtered through a 0.22 μm filter and the residual CIT was analyzed by HPLC-FLD. Each treatment was replicated three times and the experiment was repeated twice.

After that, sequence analysis of the internal transcribed spacer (ITS) region was used to identify the yeasts with the ability to eliminate CIT [30].

Yeast

Cryptococcus podzolicus Y3 was preserved in NYDA medium at 4°C. The cell suspension of *C. podzolicus* Y3 was prepared as described above.

Elimination of CIT by *C. podzolicus* Y3

One milliliter of *C. podzolicus* Y3 suspension (1×10^8 cells/ml)

was added into 50 ml of NYDB medium containing 10 μg/ml CIT and cultured at 180 rpm for 48 h at 28°C. Then, 500 μl of the culture was taken into a 1.5 ml microcentrifuge tube at 1, 2, 3, and 4 days, respectively, and vortexed with 500 μl of methanol for 30 sec. The mixture was then filtered through a 0.22 μm filter and stored at −20°C for HPLC analysis. The treatment with sterile water instead of *C. podzolicus* Y3 was used as the control. Each treatment was replicated three times and the experiment was repeated twice.

Uptake of CIT by *C. podzolicus* Y3 Cells

One milliliter cell suspension of *C. podzolicus* Y3 (1×10^8 cells/ml) was added into Erlenmeyer flasks containing 10 ml of NYDB and 10 μg/ml CIT. The samples were then incubated in a rotary shaker at 180 rpm and 28°C for 3 days. The precipitate of the culture was washed three times with sterile distilled water after centrifugation at 4°C and 7,000 ×*g* for 15 min. Then, the cell pellets were ground in a mortar by adding liquid nitrogen, after which the samples were dissolved in 5 ml of sterile distilled water. After that, 500 μl of the samples was pipetted into a 1.5 ml microcentrifuge tube and treated as described above. Each treatment was replicated three times and the experiment was repeated twice.

Adsorption of CIT by Cell Wall of *C. podzolicus* Y3

One milliliter cell suspension of viable *C. podzolicus* Y3 (1×10^8 cells/ml) was added into Erlenmeyer flasks (250 ml) containing 50 ml of NYDB with 10 μg/ml CIT and incubated in a rotary shaker at 180 rpm and 28°C. Samples were collected every 12 h and treated as follows: (i) 500 μl of the sample was pipetted into a 1.5 ml microcentrifuge tube and treated as described above; (ii) 500 μl of the sample was centrifuged at 4°C and 7,000 ×*g* for 5 min and the supernatant was pipetted into a 1.5 ml microcentrifuge tube, and then treated as described above. The treatment with sterile distilled water instead of *C. podzolicus* Y3 was used as the control. Each treatment was replicated three times and the experiment was repeated twice.

The cell suspension of *C. podzolicus* Y3 (1×10^8 cells/ml) was inactivated by boiling in a water bath for 30 min. Then, 1 ml of cell suspension was transferred into Erlenmeyer flasks (250 ml) that contained 50 ml of NYDB with 10 μg/ml CIT and incubated on a rotary shaker at 180 rpm and 28°C. Then, the subsequent treatment was the same as the method for viable *C. podzolicus* Y3. The treatment with sterile distilled water instead of *C. podzolicus* Y3 was used as the control. Each treatment was replicated three times and the experiment was repeated twice.

Degradation of CIT by Extracellular Metabolites of *C. podzolicus* Y3

C. podzolicus Y3 was incubated in 50 ml of NYDB medium in a rotary shaker at 180 rpm and 28°C for 24 h. Then, 25 ml of culture was centrifuged at 4°C and 7,000 ×*g* for 15 min and the supernatant was filtered through a 0.22 μm membrane. CIT was added into both the filtrate and the culture that was not centrifuged at a concentration of 10 μg/ml, and then cultured at 28°C and 180 rpm. Samples (500 μl) were collected respectively into 1.5 ml microcentrifuge tubes every 12 h and treated as described above. CIT treated with sterile distilled water instead of *C. podzolicus* Y3 was used as the control. Each treatment was replicated three times and the experiment was repeated twice.

Degradation of CIT by Extracellular Metabolites of *C. podzolicus* Y3 Stimulated by CIT

C. podzolicus Y3 was cultured in NYDB medium with 10 μg/ml CIT at 28°C for 24 h. Then the culture was treated as described above. CIT treated with sterile distilled water instead of *C. podzolicus* Y3 was used as the control. Each treatment was replicated three times and the experiment was repeated twice.

Effects of Different Culture Media of *C. podzolicus* Y3 on the Degradation of CIT

One milliliter of *C. podzolicus* Y3 suspension (1×10^8 cells/ml) was introduced respectively into 50 ml of NYDB and PDB containing 10 μg/ml CIT. The samples were cultured for 0, 14, 21, 24, 28, 35, and 42 h in a rotary shaker at 180 rpm and 28°C. Then, 500 μl of the culture was taken into a 1.5 ml microcentrifuge tube and treated as mentioned above. Thereafter, CIT was determined by HPLC-FLD. Each treatment was replicated three times and the experiment was repeated twice.

Effect of the Concentration of *C. podzolicus* Y3 on the Degradation of CIT

One milliliter of *C. podzolicus* Y3 (1×10^4, 1×10^5, 1×10^6, 1×10^7, and 1×10^8 cells/ml) each was pipetted into 250 ml Erlenmeyer flasks containing 50 ml of NYDB medium supplemented with 10 μg/ml CIT and cultured in a rotary shaker at 180 rpm and 28°C. Subsequently, samples were collected and treated as mentioned above. Each treatment was replicated three times and the experiment was repeated twice.

Effect of Culture Temperature of *C. podzolicus* Y3 on the Degradation of CIT

One milliliter of *C. podzolicus* Y3 suspension (1×10^8 cells/ml) was added into 50 ml of NYDB medium containing 10 μg/ml CIT and cultured at 15°C, 28°C, or 35°C in a rotary shaker at 180 rpm. Subsequently, samples were collected and treated as mentioned above. Each treatment was replicated three times and the experiment was repeated twice.

Effect of Culture pH of *C. podzolicus* Y3 on the Degradation of CIT

One milliliter of *C. podzolicus* Y3 suspension (1×10^8 cells/ml) was added into 50 ml of NYDB medium containing 10 μg/ml CIT. The cultures were adjusted to pH 4.0, 6.0, and 8.0 and cultured in a rotary shaker at 180 rpm and 28°C. Subsequently, samples were

collected and treated as mentioned above. Each treatment was replicated three times and the experiment was repeated twice.

Effect of CIT Concentration on the Degradation of CIT by *C. podzolicus* Y3

One milliliter of *C. podzolicus* Y3 suspension (1×10^8 cells/ml) was added into 50 ml of NYDB containing different concentrations of CIT (5, 10, and 20 μg/ml) and cultured at 28°C in a rotary shaker at 180 rpm. Then, samples were collected and treated as mentioned above. Each treatment was replicated three times and the experiment was repeated twice.

Preparation of the Product(s) of CIT Degraded by *C. podzolicus* Y3

One milliliter of *C. podzolicus* Y3 suspension (1×10^8 cells/ml) was added into 50 ml of NYDB containing 20 μg/ml CIT and cultured at 28°C in a rotary shaker at 180 rpm for 42 h. Then, the culture was filtered through a 0.22 μm membrane and the degradation product(s) in the filtrate was (were) extracted with (toluene : ethyl acetate : formic acid = 7:3:1 (v/v/v)). The degradation product(s) in the above extractant was (were) re-dissolved in 5 ml of dimethyl sulfoxide (DMSO) after rotary evaporation.

Cell Line and Cell Culture

The human embryonic kidney (HEK293) cell line was provided by American Type Culture Collection (ATCC, USA). HEK293 cells were cultivated in Dulbeco's modified Eagle's medium containing 10% heat-inactivated FBS and 1% antibiotics (penicillin and streptomycin) at 37°C in a humidified 5% CO_2 incubator (MCO-15AC CO_2 incubator; SANYO, Japan).

Toxicity of CIT and Degradation Product(s) to HEK293

The cytotoxicity of CIT and degradation product(s) toward HEK293 cells was investigated using the MTT method [31]. Cells were seeded into 96-well plates at a concentration of 3×10^4 cells/well. After incubation for 18 h, cells were exposed to medium supplemented with CIT or degradation product(s) at different concentrations for 24 h. After that, the medium was removed carefully and 10 μl of MTT solution (5 mg/ml in phosphate-buffered saline) and 90 μl of FBS-free medium were added into each well and incubated at 37°C for 4 h. Then, the colored formazan crystals were solubilized with 100 μl of MTT stop solution containing 10% SDS and 0.01 M hydrochloric acid. The optical density (OD) was measured using a microplate reader (Infinite M200 Pro spectrophotometer; Tecan, Switzerland) at a wavelength of 550 nm. The cells treated with DMSO instead of CIT or degradation product(s) was used as the control. The survival rate of HEK293 cells was expressed as the percentage of the control for each experiment.

Statistical Analysis

Statistical analyses were performed using SPSS/PC ver. 16.0 (SPSS Inc., USA). The data with a single variable (treatment) were analyzed by analysis of variance. Duncan's multiple-range test was used for means separation, and the independent samples t-test was used for means separation when the group of data was two. Statistical significance was applied at the level of $p < 0.05$.

Results

Screening and Identification of Yeasts with the Ability to Eliminate CIT

The analysis of CIT using HPLC-FLD showed that a strain Y3 had the ability to eliminate CIT. The strain Y3 was identified based on sequence analysis of the ITS region and 5.8S rRNA. Similarity analysis of the gene sequence indicated that Y3 is closely related to *Cryptococcus* sp. with a 99%

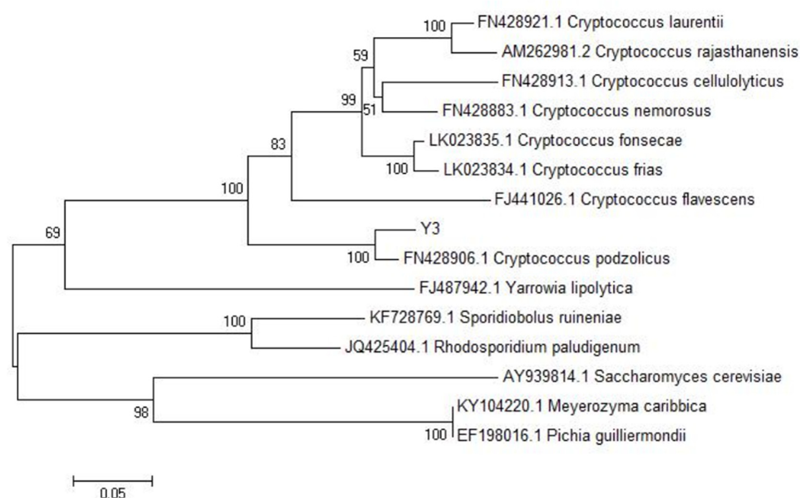

Fig. 1. Phylogenetic tree drawn on the basis of neighbor-joining analysis of the ITS sequence of strain Y3.

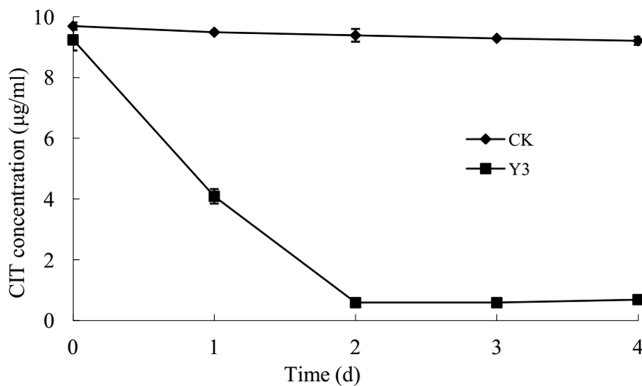

Fig. 2. The ability of *Cryptococcus podzolicus* Y3 to eliminate citrinin (CIT).

Y3: CIT treated with *C. podzolicus* Y3 in NYDB; CK: CIT treated with sterile water. Values are the means of three independent experiments. Error bars represent the standard errors of the means.

homology. A phylogenetic tree was constructed, which showed that Y3 is *Cryptococcus podzolicus* (Fig. 1).

The results shown in Fig. 2 indicated that *C. podzolicus* Y3 could eliminate CIT. The concentration of CIT in NYDB decreased from 9.3 μg/ml at day 0 to 0.6 μg/ml at day 2. The degradation rate of CIT for the first 2 days was 94%. Afterwards, the CIT remained low throughout the test period. On the contrary, the concentration of CIT in the control remained at 9.5 μg/ml throughout the 4 days. These results indicated that *C. podzolicus* Y3 has the ability to eliminate CIT in NYDB medium.

Uptake of CIT by *C. podzolicus* Y3 Cells and Adsorption of CIT by the Cell Wall

There was no CIT detected in the extracts of *C. podzolicus* Y3 ground with liquid nitrogen. This indicated that uptake by cells was not involved in CIT elimination by *C. podzolicus* Y3.

The concentration of CIT in the supernatant of heat-inactivated *C. podzolicus* Y3 culture was almost the same as that in the uncentrifugated culture throughout the entire cultivation stage (Fig. 3A). This result suggested that CIT was not adsorbed by the cell wall of heat-inactivated *C. podzolicus* Y3. Furthermore, the concentration of CIT in the culture and its supernatant was the same as that in the control and was constant during the entire incubation period (Fig. 3A). It indicated that heat-inactivated *C. podzolicus* Y3 could not detoxify CIT. As shown in Fig. 3B, the concentration of CIT in the supernatant was almost as high as that in the culture throughout the test period, and decreased significantly as the time increased. However, the concentration of CIT in the control was constant throughout the process. These results indicated that CIT was eliminated quickly by viable

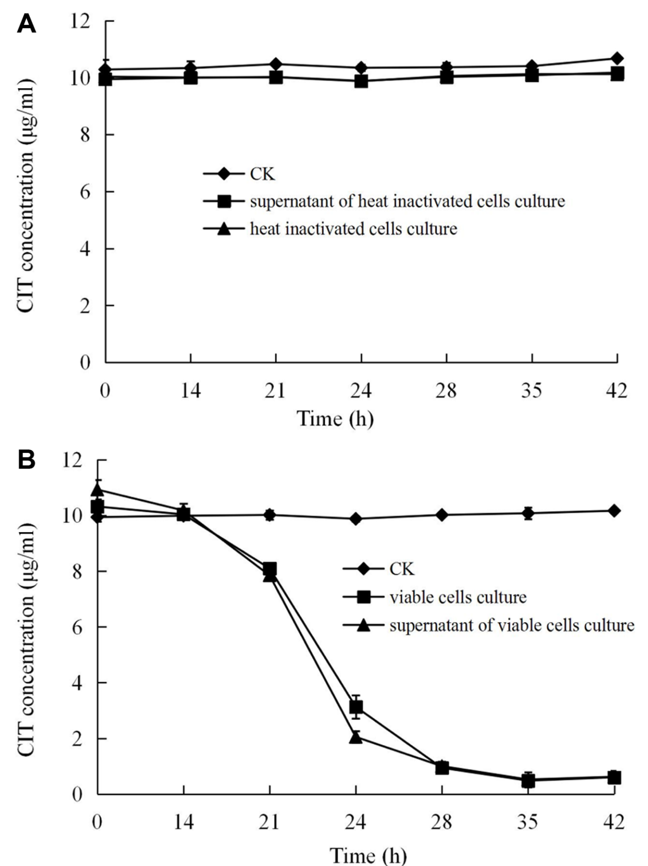

Fig. 3. Adsorption of citrinin (CIT) by cell wall of *Cryptococcus podzolicus* Y3.

(**A**) Adsorption of CIT by cell wall of heated-inactivated *C. podzolicus* Y3; (**B**) Adsorption of CIT by cell wall of viable *C. podzolicus* Y3.

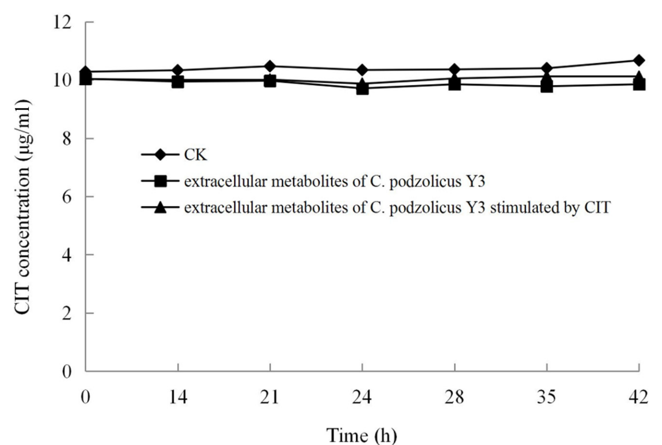

Fig. 4. The degradation of citrinin (CIT) by extracellular metabolite of *Cryptococcus podzolicus* Y3.

C. podzolicus Y3, and adsorption of CIT by the cell wall was not involved in CIT elimination.

Degradation of CIT by Extracellular Metabolites of *C. podzolicus* Y3

Fig. 4 shows that the concentrations of CIT in the cultures treated with extracellular metabolites of *C. podzolicus* Y3 supplemented with or without CIT were not different from the control throughout the experiment. These results suggested that the extracellular metabolites of *C. podzolicus* Y3 whether with or without the addition of CIT have no degrading action on CIT.

Effects of Different Culture Media of *C. podzolicus* Y3 on the Degradation of CIT

As shown in Fig. 5, CIT was significantly degraded by *C. podzolicus* Y3 in NYDB compared with PDB. There was a sharp decrease in CIT level, and 0.94 μg/ml CIT was detected at 28 h. Thereafter, the level of CIT remained below 1 μg/ml throughout the duration of the experiment. However, it was observed that the CIT level in PDB medium remained relatively unchanged. These findings indicated that *C. podzolicus* Y3 cannot degrade CIT in PDB.

Effect of the Concentration of *C. podzolicus* Y3 on the Degradation of CIT

The concentrations of *C. podzolicus* Y3 showed significant impact on CIT degradation (Fig. 6). The results revealed that CIT was degraded by *C. podzolicus* Y3 at all tested concentrations. However, at concentration of 10^4 and 10^5 cells/ml, it was observed that the level of CIT was relatively constant until 24 h, and then decreased to 2.7 μg/ml at 42 h. At concentrations of 10^6 and 10^7 cells/ml, CIT was degraded quickly from 21 to 35 h, and the degradation rate was 86% and 91% at 42 h, respectively. With regard to the CIT level in the medium treated with 10^8 cells/ml, it was found that there was a significant decrease from 14 to 28 h and 94% CIT was degraded at 42 h. Generally, there was a significant effect of the different concentrations of *C. podzolicus* Y3 on CIT degradation, with 10^8 cells/ml showing the best efficacy.

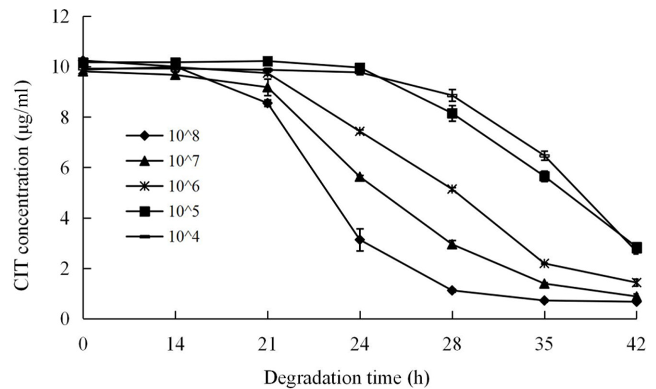

Fig. 6. Effect of the concentration of *Cryptococcus podzolicus* Y3 on the degradation of citrinin (CIT).
10^8, 10^7, 10^6, 10^5, 10^4: *C. podzolicus* Y3 added at the concentration of 1×10^8, 1×10^7, 1×10^6, 1×10^5, and 1×10^4 cells/ml, respectively. Values are the means of three independent experiments. Error bars represent the standard errors of the means.

Effect of Culture Temperature of *C. podzolicus* Y3 on the Degradation of CIT

As shown in Fig. 7, the different temperatures had an

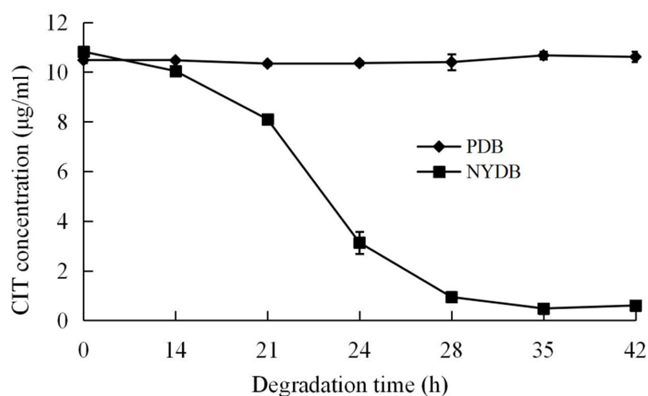

Fig. 5. Effects of different culture media of *Cryptococcus podzolicus* Y3 on the degradation of citrinin (CIT).
Values are the means of three independent experiments. Error bars represent the standard errors of the means.

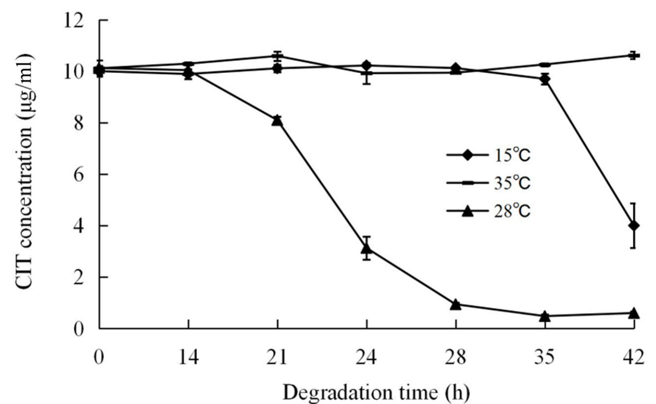

Fig. 7. Effect of culture temperature of *Cryptococcus podzolicus* Y3 on the degradation of citrinin (CIT).
Values are the means of three independent experiments. Error bars represent the standard errors of the means.

effect on degradation of CIT by *C. podzolicus* Y3. The results showed that Y3 cultured with CIT at 35°C did not have any effect on CIT degradation throughout the duration of the experiment. However, in the sample incubated at 15°C, CIT level was constant at the initial stage and then decreased sharply after 35 h. It was also noticed that there was a significant reduction of CIT in samples that were incubated at 28°C. The degradation rate of CIT in the sample incubated at 28°C was the highest (94%).

Effect of Culture pH of *C. podzolicus* Y3 on the Degradation of CIT

The results about the effect of pH on CIT degradation by *C. podzolicus* Y3 showed that there was indeed a significant pH effect. In Fig. 8, the CIT level of both samples at pH 4.0 and 6.0 was significantly decreased compared with those at pH 8.0. After 35 h, the amount of CIT in samples incubated at pH 4.0 and 6.0 was 0.1 μg/ml and 0.6 μg/ml, respectively.

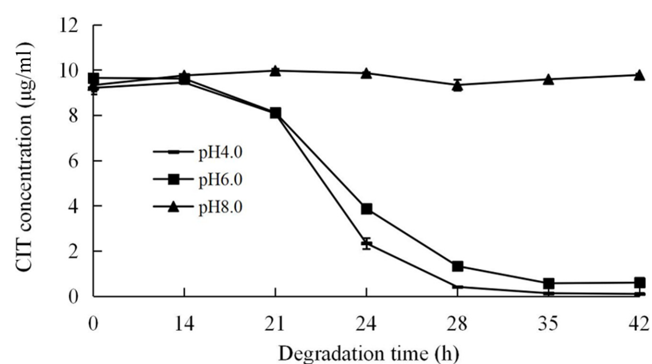

Fig. 8. Effect of culture pH of *Cryptococcus podzolicus* Y3 on the degradation of citrinin (CIT).

Values are the means of three independent experiments. Error bars represent the standard errors of the means.

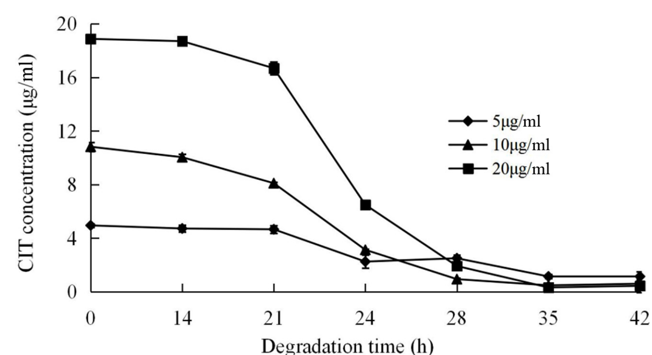

Fig. 9. Effect of citrinin (CIT) concentration on the degradation of CIT by *Cryptococcus podzolicus* Y3.

Values are the means of three independent experiments. Error bars represent the standard errors of the means.

However, the CIT concentration in the sample incubated at pH 8.0 was relatively constant during the experiment. This indicated that an acidic environment was favorable for CIT degradation by *C. podzolicus* Y3.

Effect of CIT Concentration on the Degradation of CIT by *C. podzolicus* Y3

As shown in Fig. 9, all the tested concentrations of CIT (5, 10, and 20 μg/ml) added in the samples were degraded by *C. podzolicus* Y3 and the degradation rates increased with increasing concentrations of CIT. The degradation rates of CIT at the concentration of 5, 10, and 20 μg/ml were 77%, 94% and 98%, respectively.

Toxicity of CIT and Degradation Product(s) to HEK293

The survival rates of HEK293 decreased obviously when the concentration of CIT increased (Fig. 10). Compared with HEK293 treated with CIT, the survival rates of HEK293 treated with CIT degradation product(s) were much higher, and all were more than 95.68% when the concentrations of CIT degradation product(s) were lower than 10 μg/ml. Furthermore, the survival rate of HEK293 treated with 20 μg/ml of CIT degradation product(s) was 86.72%, but only 59.04% for HEK293 treated with CIT at the same concentration. A significant difference in survival rate was observed between HEK293 treated with CIT and with its degradation product(s) when the concentrations were higher than 2.5 μg/ml.

Discussion

The results in this study showed that *C. podzolicus* Y3 has

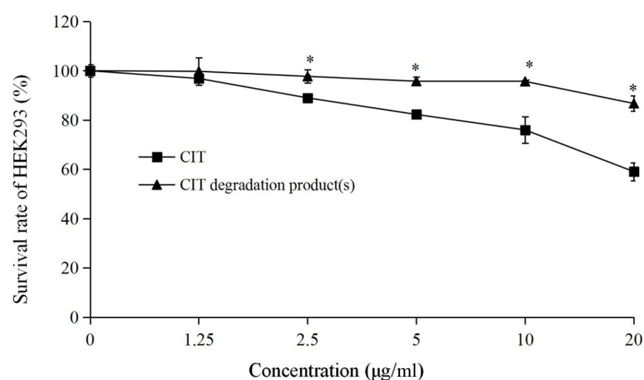

Fig. 10. Cell survival rate of HEK293 treated with citrinin (CIT) and the degradation product(s).

Values are the means of three independent experiments. Error bars represent the standard errors of the means. *Represents significant difference ($p < 0.05$) according to the *t*-test.

the ability to eliminate CIT even at the concentration of 20 μg/ml, which is 10 times the limits recommended by the European Union (EU; 2 ppm). By now, several microorganisms selected have the ability to eliminate CIT. *Rhizobium borbori* PS45 degraded 60−65% CIT at initial concentration of 5 ppm at 96 h [29]. The degradation rate of CIT by *R. borbori* PS45 was lower than that by *C. podzolicus* Y3, but the degradation time was longer. It has been reported that *Saccharomyces cerevisiae* can degrade CIT from 1.1−35.5 to 0.8−30 ppb in flour at 48 h [32]. This showed that the initial concentration of CIT was much lower than the limits recommended by the EU. A study by Chen *et al*. [28] showed that *Klebsiella pneumoniae* could degrade CIT effectively when cultured with 10 ppm CIT as the sole carbon source. As human pathogen, *K. pneumoniae* is unsafe for use in food. A safety test conducted by our research team showed that *C. podzolicus* Y3 is non-toxic for *Mus musculus albus* (data not shown). Thus, *C. podzolicus* Y3 is safe for use in food. Moreover, the results about toxicity showed that the toxicity of CIT degradation product(s) by *C. podzolicus* Y3 to HEK293 was much lower than that of CIT.

The mechanisms of mycotoxin elimination by microorganisms include adsorption and degradation [18, 20, 21, 25]. The results showed that neither heat-inactivated *C. podzolicus* Y3 nor viable *C. podzolicus* Y3 absorbed CIT, and there was no uptake of CIT by *C. podzolicus* Y3. This suggested that CIT elimination by *C. podzolicus* Y3 was attributed to the degradation by enzyme(s). However, the extracellular metabolites (including extracellular enzymes) of *C. podzolicus* Y3 (stimulated by CIT or not) was not involved in the CIT degradation (Fig. 4). Therefore, CIT elimination by *C. podzolicus* Y3 was attributed to the degradation by intracellular enzyme(s).

In this study, the CIT degradation rate increased with increase of the concentration of *C. podzolicus* Y3. Cao *et al*. [18] reported that the degradation rate of patulin has a positive correlation with the concentration of *P. caribbica*. The concentration of *Yarrowia lipolytica* also has a positive effect on the degradation rate of OTA [26]. The ability to eliminate OTA is related to the cell count of the yeasts [33, 34]. Additionally, the initial concentration of CIT influences the degradation rate of CIT by *C. podzolicus* Y3. The degradation rate of CIT by *C. podzolicus* Y3 depends on CIT concentration. This relation was also observed in the degradation of OTA by *Y. lipolytica* [26]. In the studies about patulin degradation, it was assumed that the enzymes responsible for degradation were induced by patulin [35, 36]. This may be the case in CIT degradation, where a high concentration of CIT induces *C. podzolicus* Y3 to degrade CIT more quickly.

It was found that most of CIT was degraded by *K. pneumoniae* and *R. borbori* PS45 during the exponential growth phase [28, 29]. Furthermore, the degradation efficiency was the best when *K. pneumoniae* degraded CIT at the optimal temperature of growth. These results suggested that degradation efficiency is closely related to the growth of strains used for degradation. In this study, *C. podzolicus* Y3 showed the highest degradation efficiency of CIT at 28°C, which is the optimal temperature for the growth of *C. podzolicus* Y3. The effect of temperature on the degradation of mycotoxin was also observed in other toxins, such as OTA [26]. Nutrients have significant effect on the growth of microorganisms. Various nutrient supplements, especially carbon source, nitrogen source, and some important growth factors, could influence the degradation of CIT by affecting the growth of *K. pneumoniae* [28]. NYDB medium is frequently used for culturing yeasts. CIT was effectively degraded by *C. podzolicus* Y3 in NYDB, but not in PDB.

pH also plays an important role in the degradation of mycotoxins. *K. pneumoniae* degraded CIT rapidly and 91.33% of CIT (9.133 ppm) was degraded at 5 h when cultured at pH 7.0 [28]. It seems that acids might have been produced by *K. pneumoniae* and secreted into the broth, which decreased the pH value from 7.0 to 5.03 at 5 h. A similar result was observed in degradation of CIT by *R. borbori* PS45. Sixty-three percent of CIT was degraded by *R. borbori* PS45 and the initial pH decreased from 7.5 to 4.8. Contrarily, this was not observed in CIT degradation by *Enterobacter cloacae* PS21, where only 44% CIT was degraded and the pH remained stable at 7.5 [29]. These results indicate that an acidic environment is favorable for CIT degradation. We found that pH had a significant effect on CIT degradation by *C. podzolicus* Y3. CIT was quickly degraded in an acidic environment, especially at pH 4.0. Yang *et al*. [26] found that an acidic environment accelerated OTA degradation by *Y. lipolytica*. It may suggest that pH influences the charge distribution on the molecular surface of the enzymes involved in OTA biodegradation, thus altering the enzyme activity. Furthermore, the pH could influence the ionization state of OTA and change the charge of the molecule. This change would affect the interaction between the toxin and the enzymes [26, 37, 38]. The pH could also affect the ionization of CIT, considering the availability of free carboxyl and hydroxyl groups of CIT and OTA.

In conclusion, a yeast strain with the ability to degrade CIT was screened and identified as *C. podzolicus.* The results showed that CIT elimination by *C. podzolicus* Y3 was

attributed to the degradation by intracellular enzyme(s). Most of the CIT was degraded by *C. podzolicus* Y3 in NYDB at 42 h but not in PDB medium. The degradation rate of CIT increased as the concentration of *C. podzolicus* Y3 and CIT increased. The highest and most rapid degradation of CIT was observed at 28°C. An acidic environment, especially pH 4.0, is favorable for CIT degradation by *C. podzolicus* Y3. The toxicity of CIT degradation product(s) by *C. podzolicus* Y3 to HEK293 was much lower than that of CIT. These results would contribute to the understanding of the factors that affect the degradation of CIT in agricultural products. Furthermore, the results revealed the potential of *C. podzolicus* Y3 to degrade CIT in vitro. This study therefore serves as a firm basis for further in vivo studies using *C. podzolicus* Y3.

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