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# Ciclohexadespipeptide beauvericin degradation by different strains of *Saccharomyces cerevisiae*

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ABSTRACT

The interaction between the mycotoxin beauvericin (BEA) and 9 yeast strains of *Saccharomyces cerevisiae* named LO9, YE-2, YE5, YE-6, YE-4, A34, A17, A42 and A08 was studied.

The biological degradations were carried out under aerobic conditions in the liquid medium of Potato Dextrose Broth (PDB) at 25 °C for 48 h and in a food/feed system composed of corn flour at 37 °C for 3 days, respectively.

BEA present in fermented medium and corn flour was determined using liquid chromatography coupled to the mass spectrometry detector in tandem (LC–MS/MS) and the BEA degradation products produced during the fermentations were determined using the technique of the liquid chromatography coupled to a linear ion trap (LIT).

Results showed that the *S. cerevisiae* strains reduced meanly the concentration of the BEA present in PDB by 86.2% and in a food system by 71.1%.

All the *S. cerevisiae* strains used in this study showed a significant BEA reduction during the fermentation process employed.



## 1. Introduction

Mycotoxins are secondary metabolites produced under appropriate environmental conditions by filamentous fungi, mainly *Fusarium* spp., *Penicillium* spp., and *Aspergillus* spp. in various commodities. Beauvericin (Fig. 1) is a cyclic hexadepsipeptide consisting of alternating D-a-hydroxy-isovaleryl and aromatic Nmethyl-phenylalanine (Meca et al., 2012a). This toxin is produced by various *Fusarium* species such as *F. avenaceum*, *F. poae*, *F. oxysporum* and *F. proliferatum*, and naturally occurs on maize, wheat, barley, rice and oat (Logrieco et al., 1998; Uhlig et al., 2006; Jestoi, 2008; Sorensen et al., 2008; Kokkonen et al., 2010; Waskiewicz et al., 2010). BEA has been detected in grains throughout the world under different climates (South Africa, Poland, Norway, Spain, Croatia.), with concentrations ranging from trace level up to 520 mg/kg in maize in Italy (Ritieni et al., 1997). Meca et al. (2010) have shown that BEA was present in cereals (barley, corn and rice) purchased on Spanish markets, with levels ranging from 0.51 to 11.78 mg/kg.

The toxicity of BEA has been demonstrated in vitro in several cell line models, including human leukemia cells CCRF-CEM, human monocytic lymphoma cells U-937 and promyelocytic leukemia cells HL-60, monkey kidney epithelial cells Vero, Chinese hamster ovary cells CHO-K1 and murine macrophage J774 (Calo et al., 2004; Jow et al., 2004; Ruiz et al., 2011a,b).

Related to the methodologies used to reduce the presence of BEA in food only a US patent (Duvick and Rod, 1998) on the biological detoxification of BEA has been published. In particular the authors, using the bacterium *Nocardia glubera*, reduced BEA contamination in wheat kernels by 50% when the initial level of the toxin was at 1000 mg/L.

The biological detoxification strategies for the reduction of the other *Fusarium* mycotoxins were studied by many authors. In particular Young et al. (2007) evaluated the detoxification of 12 trichothecenes by bacteria isolated from chicken gut, observing a complete conversion to the deepoxy metabolites of the non-acylated trichothecenes and a deacetylation of the monoacetyl trichothecenes.

Also Guan et al. (2009), evaluated the biotransformation of different trichothecenes by microorganism isolated from fish digesta, observing the transformation of the mycotoxin deoxynivalenol (DON) to deepoxy DON at 15 °C in a complex medium after 96 h incubation.

The degradation of the mycotoxin DON was also studied by Islam et al. (2011), using aerobic and anaerobic bacteria isolated from agricultural soil. The obtained bacteria transformed DON into the deepoxy DON after 60 h of incubation.

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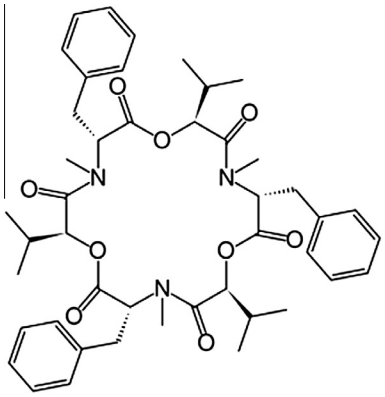

**Fig. 1.** BEA chemical structure.

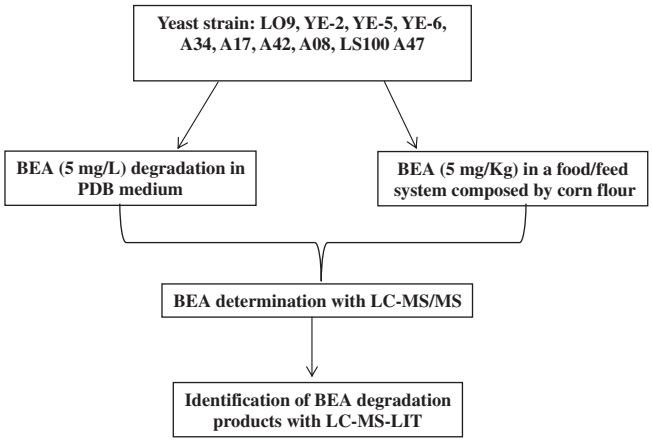


**Fig. 2.** Experimental plan used for the study of the biological degradation of the minor *Fusarium* mycotoxin BEA.

Considering the lack of data related to the biological degradation of BEA, the aims of this study were: (a) to evaluate the reduction of BEA employing different strains of *Saccharomyces cerevisiae* in a model system; (b) to determine the biological degradation of BEA in a food sample represented by the corn flour (Fig. 2).

## 2. Materials and methods

### 2.1. Chemicals

A stock standard solution of BEA (Sigma–Aldrich, St. Luis, USA) was prepared by dissolving 1 mg of BEA standard in 1 mL of pure methanol, obtaining a 1 mg BEA/mL (1000 μg/mL) solution. This stock solution was diluted with methanol in order to obtain the appropriated work solutions (1–10–100 mg/L). All BEA solutions were stored in darkness at 4 °C until the LC–MS/MS analysis. Acetonitrile, methanol, water, ethyl acetate (all of LC grade) and acetic acid were purchased from Merck (Whitehouse Station, NJ, USA).

### 2.2. Strains and methodology

The study was carried out using nine strains of *S. cerevisiae* named LO9, YE-2, YE5, YE-6, YE-4, A34, A17, A42 and A08. The strains were obtained from the personal collection of Dr. Ting Zhou at Guelph Food Research Centre. For longer survival and higher quantitative retrieval, the yeast cultures were stored in sterile 18% glycerol at −80 °C. When needed, recovery of the strains was undertaken by two consecutive subcultures in appropriate media prior to use.

The yeasts were cultured in 50 mL sterile plastic centrifuge tubes with 20 mL Potato Dextrose Broth (PDB) at 25 °C under aerobic conditions for 48 h.

### 2.3. BEA extraction from fermented medium

The fermentation tubes were centrifuged at 4000 rpm (Centrifuge 5810R, Eppendorf, Germany) for 5 min at 4 °C in order to separate the fermented medium from the cells. BEA in the fermented medium was extracted as follows (Jestoi, 2008). Five milliliters of fermented PDB were putted in a 20 mL test tube, and extracted three times with 5 mL of ethyl acetate utilizing a vortex (VWR International, Barcelona, Spain) for 1 min. The resulting extracts were centrifuged (Centrifuge 5810R, Eppendorf, Germany) at 4000 rpm for 10 min at 4 °C. The organic phases were completely evaporated using a rotary evaporator (Buchi, Switzerland) operating at 30 °C and 30 mbar pressure; the remaining was resuspended in 1 mL of methanol, filtered with 0.22 μM filters (Pheomenex, Madrid, Spain) and analyzed by LC–MS/MS (Meca et al., 2010).

### 2.4. BEA degradation in food system composed of corn flour

Two grams of corn flour free of BEA was introduced in 15 mL plastic tubes, and contaminated with 25 mg/kg of BEA. The corn flour used for the experiments was extracted and analyzed as described below (Sections 2.5 and 2.6) to ensure absence of BEA. Two milliliters of sterile water containing $1 \times 10^8$ of yeast cells were introduced. The tubes were incubated at 37 °C for 3 days.

### 2.5. Extraction of BEA in corn flour

BEA was extracted according to the procedures described by Jestoi (2008). Briefly, 2 g of corn flour samples were extracted with 20 mL methanol using an Ultra Ika T18 basic Ultraturrax (Staufen, Germany) for 3 min. The mixture was centrifuged at 4500*g* for 5 min and then the supernatant was evaporated to dryness with a Büchi Rotavapor R-200 (Postfach, Switzerland). The residue was re-dissolved in 2 mL of extraction solvent. The extract was cleaned up using a Strata C18-E cartridge (6 mL, 1 g). The cartridge was first activated with $2 \times 2$ mL of methanol and conditioned with $2 \times 2$ mL of water before the extract was loaded. The cartridge was then washed with $2 \times 2$ mL of water at a flow rate of 0.5 mL/min till all water was out. BEA was eluted using 1 mL of methanol. Both the methanolic eluate and the water washing were filtered through 0.22 μM nylon filters purchased from Análisis Vínicos (Tomelloso, Spain) before LC and LC–MS analyses.

### 2.6. LC–MS/MS analysis of BEA

LC analysis of BEA was carried out with a triple quadrupole (TQ) mass spectrometer Quattro LC from Micromass (Manchester, UK), equipped with an LC Alliance 2690 system (Waters, Milford, MA) consisted of an autosampler and a quaternary pump, a pneumatically assisted electrospray probe, a Z-spray interface. Mass Lynx NT software 4.1 was used for data acquisition and processing. The autoinjector was programmed to inject 20 μL into the Luna C18 column ($150 \times 4.6$ mm, 5 μm) Phenomenex maintained at 30 °C. The analytical separation for LC–MS/MS was performed using gradient elution with water as mobile phase A, and acetonitrile as mobile phase B, both containing 15 mM of ammonium formate. After an isocratic step of 65% B for 3 min, it was linearly increased to 75% B in 4 min and held constantly for 3 min. Flow rate was maintained at 0.3 mL min. Analysis was performed in positive ion modes. The ESI source values were as follows: capillary voltage, 3.20 kV; source temperature, 125 °C; desolvation temperature, 350 °C; desolvation gas (nitrogen, 99.99% purity) flow, 700 L/h. Identification and quantification, in which protonated molecule $[M+H]^+$ of the analyte (BEA 784,34) was fragmented in the collision cell to the product-ions (BEA 784,34-244,23).

The samples corresponding to the BEA present in the fermented medium were also injected in the modality full scan coupled to the linear ion trap (LIT) with a *m/z* of 200–900, to verify also the presence in the processed mediums of some possible adducts produced by the interaction of BEA with the cell wall of the probiotic bacteria (Meca et al., 2010).

## 3. Results and discussion

### 3.1. BEA degradation by S. cerevisiae strains in PDB medium

All the *S. cerevisiae* strains tested showed a degradation activity toward BEA in PDB medium as indicated in Fig. 3.

The LC–DAD chromatograms in Fig. 5a and b revealed BEA present in PDB before and after the fermentation with *S. cerevisiae* A42, respectively.

The mean degradation evidenced by the reduction of the bioactive compound BEA was of 86.2 ± 2.2%. The highest degradation activity, 98.8 ± 1.2%, was evidenced by the strain of *S. cerevisiae* LO-9, followed with 96.4 ± 2.3% by, whereas the lowest by the strain of LS100 with 39.7 ± 2.9% respectively. A good degradation activity was also evidenced by the strain YE-2 and 94.4 ± 2.5% by YE-5 (Fig. 3). The results suggest that the bioactive compound BEA can be degraded in PDB medium by all the tested strains of *S. cerevisiae* in 48 h.