IDENTIFICATION OF THE CELL WALL RECEPTOR

da Silva S.(1), Aguiar, C. (1), Veríssimo, P. (2), Pires, E. (2) and Lucas, C.(1)

(1) CB-Departamento de Biologia, Universidade do Minho, Braga, Portugal.
(2) CNC- Laboratório de Biologia Molecular e Biotecnologia, Univers. Coimbra, Coimbra, Portugal.

The production of antimycotically active toxins, so-called killer (K) toxins or zymocins, is a widespread phenomenon in various yeast genera, although the most intensively studied killer systems are still those of *Saccharomyces cerevisiae* and *Kluyveromyces lactis* (for reviews see 1-2). During the last two decades, killer toxins and killer yeasts were found to have several potential applications; for instance in the food and fermentation industries, in the bio-typing of medically important microorganisms, in the development of novel antimycotic agents for the treatment of fungal infections and in the field of recombinant DNA technology. This increasingly interest in killer toxins requires undoubtedly a detailed knowledge and understanding of the biology of killer yeasts, which will provide important insights relevant for its use as antimicrobial agents.

In a previous survey, we studied several halotolerant yeasts which killer activity was expressed, even stimulated, under heavy salt-stress conditions (3). From this research, the halotolerant yeast *Candida nodaensis* was identified as one of the strongest salt-stimulated K phenotypes, being selected for further studies. Results obtained so far, in what concerns *C. nodaensis* zymocin activity/stability under temperature, pH and ionic strength, showed that this is in fact a very stable zymocin. Presently, several strategies are under way to achieve the isolation (A.) and purification (B.) of this zymocin, in order to enable further evaluation of its biotechnological potentialities, namely in the high-salt food products preservation from spoilage by other yeasts.

A. Isolation of *C. nodaensis* killer toxin

Growth at 25°C, 160 rpm
Early stationary phase

↓ Centrifugation

↓ 2', 7000 rpm, 4°C

Filtration of culture supernatant

↓ 0.45 µm

Precipitation of culture filtrate

↓ Adition of 3/4 vol. ethanol

Centrifugation

↓ 10', 7000 rpm, 0°C

Liophilization

↓

Ressuspension in
10 mM CPB, 200mM NaCl pH 4.0

B. Partial purification of K toxin
Molecular Exclusion Chromatography

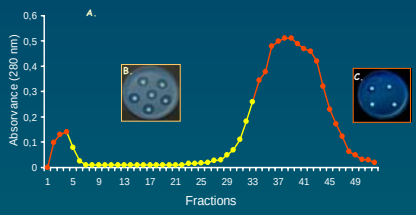

Fig.1. A- Elution profile of K toxin through a Superdex 200 column (Pharmacia) equilibrated and eluted with CPB 200 Mm, pH 4.0 (● fractions with K activity; ● fractions without K activity. B and C -assay for killer activity detection).

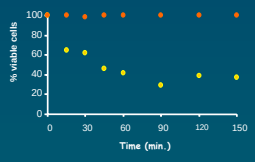

Fig.2. Assay for killer activity quantification (Influence of partially purified K toxin on *P. guilliermondii* viability (●fractions with K activity; ● fractions without K activity).

The killing process mediated by K toxins involves a first step, which corresponds to the adsorption of the toxin to the cell wall of sensitive cells (4). Here we describe the work performed towards the identification of the cell wall receptor for the zymocin produced by the extremely halotolerant yeast *Candida nodaensis* . For this purpose, the main cell wall components of the sensitive yeast *Pichia guilliermondii* were extracted (C.). These cell wall components (D., F., G.) and other commercial polymers (E.,F.) were tested for its binding capacity to partially purified (B.) *C. nodaensis* K toxin.

C. Fractionation of *P. guilliermondii* Cell wall

Growth at 30°C, 150 rpm
Stationary phase

↓ Centrifugation

↓ 5', 5000 rpm

Cell breakage by homogenizer

↓ 5` , 0°C

Centrifugation

↓ 20', 1000 rpm

Cell wall

Cell wall fractionation

Glucans extraction — Chitin extraction

↓ ↓

Fractions

P-1 | P-2 | S-1 | S-2 — Chitin

D. Binding of K toxin to cell wall fractions of sensitive cells

Table 1: Fractionation of *P. guilliermondii* cell walls. Glucans were extracted according to (5) and Chitin were extracted according to (6).

Fraction	Wt (mg)	%
P-1[4]	208,6	4,2
P-2[4]	1625	32,5
S-1[4]	638,7	12,8
S-2[4]	840,3	17
Chitin[5]	80	2,98

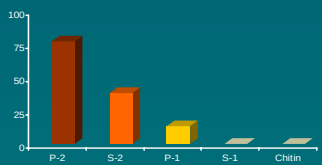

Fig.3. Killer activity after adsorption to *P. guilliermondii* cell wall components.

E. Binding of K toxin to commercial polysaccharides

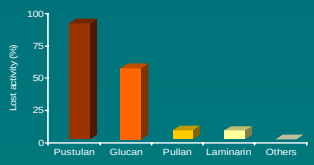

Fig. 4. Killer activity after adsorption to different commercial carbohydrates. (others: Amylopectin, Amylose, Chitin, Polygalacturonic acid and Xylan).

Table 2. Different commercial carbohydrates.

Carbohydrate	*Main glucosidic linkage*
Amylopectin	(1-4)-α
Amylose	(1-4)-α
Chitin	(1-4)-β
Laminarin	(1-3)-β
Polygalacturonic acid	(1-4)-α
Pullan	(1-6)- α / (1-4)-α
Xylan	(1-4)-β
Pustulan	(1-6)-β
Glucan by yeast	_

F. Killer toxin adsorption to P-2 fraction and Pustulan

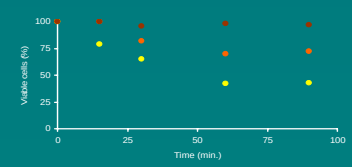

Fig.5. Toxin mediated cell death after pre-incubation with (●) P-2 fraction and (○) Pustulan or (●) without incubation with any polyssaccharyde .

G. Time course of K toxin adsorption to P- 2 fraction

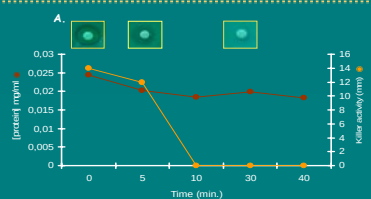

Fig. 6. Killer toxin adsorption to the cell wall P-2 fraction during 40 minutes . (A)- Assay for killer activity detection.

Concluding remarks:

- Glucan seems to be the primary receptor for the zymocin under study (Fig.3).
- Killer toxin is quickly adsorbed to *P. guilliermondii* cell wall P-2 fraction (Fig. 6).
- *C. nodaensis* K toxin binds primarily to β-(1-6)-glucan (pustulan) (Fig. 4 and 5).

These results can be explored for the development of purification strategies by affinity chromatography.

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