

IDENTIFICATION OF THE CELL WALL RECEPTOR FOR *CANDIDA NODAENSIS* KILLER TOXIN

da Silva S. (1), Aguiar, C. (1), Verissimo, 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 nodaeensis* was identified as one of the strongest salt-stimulated K phenotypes, being selected for further studies. Results obtained so far, in what concerns *C. nodaeensis* 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.

B. Partial purification of K toxin

Molecular Exclusion Chromatography

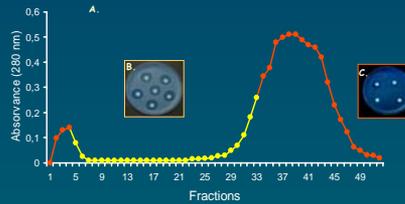


Fig. 1. A- Elution profile of K toxin through a Superdex 200 column (Pharmacia) equilibrated and eluted with CPB 200 mM, pH 4.0 (● fractions with K activity; ○ fractions without K activity). B and C- assay for killer activity detection.

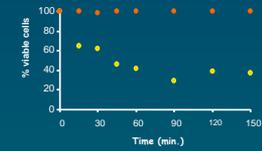
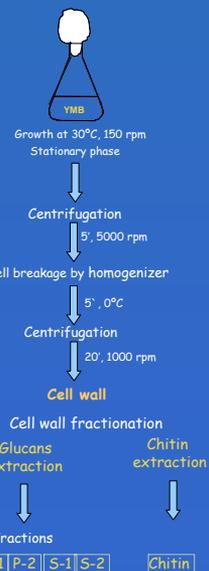


Fig. 2. Assay for killer activity quantification (Influence of partially purified K toxin on *P. guilliermondii* viability (● fractions with K activity; ○ fractions without K activity)).

A. Isolation of *C. nodaeensis* killer toxin



C. Fractionation of *P. guilliermondii* Cell wall



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 nodaeensis*. 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. nodaeensis* K toxin.

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 ^a	208,6	4,2
P-2 ^a	1625	32,5
S-1 ^a	638,7	12,8
S-2 ^a	840,3	17
Chitin ^b	80	2,98

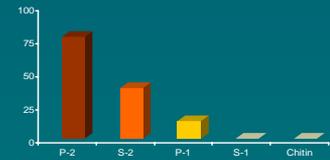


Fig. 3. Killer activity after adsorption to *P. guilliermondii* cell wall components.

E. Binding of K toxin to commercial polysaccharides

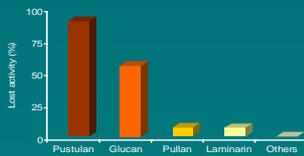


Fig. 4. Killer activity after adsorption to different commercial carbohydrates. (Others: Amylopectin, Amylose, Chitin, Polygalacturonic acid and Xylan).

Table 2: Different commercial carbohydrates.

Carbohydrate	Main glucoisidic 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

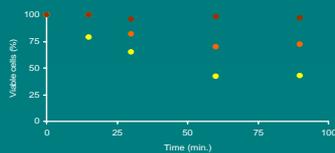


Fig. 5. Toxin mediated cell death after pre-incubation with (●) P-2 fraction and (●) Pustulan or (●) without incubation with any polysaccharide.

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

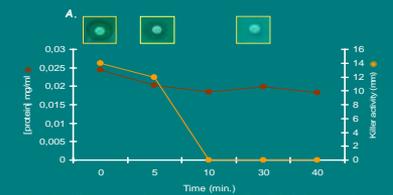


Fig. 6. Killer toxin adsorption to the cell wall P-2 fraction during 40 minutes. (A)- Assay for killer activity detection.

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. nodaeensis* 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.

- (1) Magliani, W. et al. (1997) Yeast killer systems. *Clin. Microbiol. Rev.*, 10 (3): 369-400.
- (2) Schmitt, M. And Breinig, F. (2002) The viral killer system in yeast: from molecular biology to application. *FEMS Microbiol. Rev.* 26: 257-276.
- (3) Aguiar, C. and Lucas, C. (2000) Killer/sensitivity phenotypes and halotolerance. *Food Technol. and Biotechnol.*, 38: 39-46.
- (4) A. Santos et al (2002). Letters in Appl. Microbiol. 34, 95-99.
- (5) Manners, D.J. et al. (1973) *Biochem. J.* 135, 19-30.
- (6) Fleet, G. H. (1991). Cell walls, p. 200-277. In A. H. Rose and J. S. Harrison (ed.). *The yeast vol. 4. Yeast organelles.*