MYCOSES 34, 67–73 (1991) ACCEPTED: DECEMBER 20, 1990

# Glycosidic activities of *Candida albicans* after action of vegetable latex saps (natural antifungals) and isoconazole (synthetic antifungal)

## Glykosid-Aktivitäten an *Candida albicans* nach Einwirkung natürlicher Antimyzetika pflanzlicher Milchsäfte und des synthetischen Antimyzetikums Isoconazol

R. Giordani[1], J. Moulin-Traffort[2] and P. Régli[2]

**Key words.** *Candida albicans, Lactuca sativa, Asclepias curassavica,* latex sap, antifungals, glycosidases, isoconazole, fungal cell wall.

**Schlüsselwörter.** *Candida albicans, Lactuca sativa, Asclepias curassavica,* Milchsaft, Antimyzetika, Glykosidasen, Isoconazol, Pilzzellwand.

**Summary.** Glycosidic activities have been examined in *Candida albicans* grown on medium culture containing latex sap (natural antifungal) or isoconazole (synthetic antifungal). The different types of utilized latex sap were those of *Lactuca sativa* (latex exuded from articulated laticifers) and *Asclepias curassavica* (latex flowing from non-articulated laticifers). The same enzyme assays were performed on *C. albicans* grown without antifungal compounds. Except for α-arabinosidase, all glycosidase activities were increased when *C. albicans* was grown in medium supplemented with *L. sativa* latex sap. The most stimulated activities were those of β-fucosidase, α- galactosidase, α- and β-glucosidase, α- and β-mannosidase, acetyl-β-glucosaminidase. The presence of *A. curassavica* latex sap in culture medium produced similar results: the most stimulated activities were those of α-mannosidase, α-galactosidase, acetyl-β-glucosaminidase and β-fucosidase. Electron microscope observations suggested a correlation between this stimulation of glycosidic activities and the fungal cell wall breakdown. For comparison the presence of isoconazole in culture medium yields no increase in glycosidic activities and no ultrastructural modification of fungal cell wall. The mode of action of latex saps in cell wall breakdown is discussed.

**Zusammenfassung.** Es wurden die Glykosid-Aktivitäten an *Candida albicans* unter der Einwirkung natürlicher Antimyzetika pflanzlicher Milchsäfte vergleichend mit der Wirkung des synthetischen Antimyzetikums Isoconazol untersucht. Die eingesetzten Milchsäfte stammten von *Lactuca sativa* (Pflanze mit gegliederten Milchsaftgefäßen) und *Asclepias curassavica* (Pflanze mit ungegliederten Milchsaftgefäßen). Die gleichen Enzymansätze wurden zur Kontrolle an *C. albicans* ohne Antimyzetika durchgeführt. Außer für α-Arabinosidase waren alle Glykosidase-Aktivitäten bei Zusatz von *L. sativa*-Milchsaft zum Kulturmedium erhöht. Die höchsten Aktivitäten wurden bei β-Fucosidase, α-Galactosidase, α- und β-Glucosidase, α- und β-Mannosidase und Acetyl-β-Glucosaminidase beobachtet. Der Zusatz von *A. curassavica*-Milchsaft zum Kulturmedium bewirkte ähnliche Resultate. Hier waren die am stärksten stimulierten Enzyme α-Mannosidase, α-Galactosidase, Acetyl-β-Glucosaminidase und β-Fucosidase. Elektronenmikroskopische Untersuchungen zeigten, daß eine Korrelation zwischen der Glykosidasen-Stimulation und der Zellwandzerstörung besteht. Zum Vergleich wurde der Einfluß von Isoconazol auf *C. albicans*

[1] Centre de Biochimie et de Biologie Moléculaire, CNRS, Marseille, and [2] Laboratoire de Botanique et Cryptogamie, Faculté de Pharmacie, Université d'Aix-Marseille II, Marseille, France

Correspondence: Dr Roger Giordani, Centre de Biochimie et de Biologie Moléculaire, CNRS, B.P. 71, F-13402 Marseille Cedex 09, France.

untersucht. Dies führte nicht zu einem Anstieg der Glykosidasen-Aktivitäten und zu keiner ultrastrukturellen Veränderung der Zellwand. Der Wirkungsmechanismus der Milchsäfte bei der Pilzzellwand-Zerstörung wird diskutiert.

## Introduction

Recently the study of the action of latex saps of *Asclepias curassavica* and *Lactuca sativa* has indicated their fungitoxicity shown by fungal cell wall degradation. This was latter revealed by a limited area cell wall perforation and a release of cell wall lamellar material into the culture medium [1]. Cell wall degradation reflecting a fungal cell wall disorganization seems to be the result of hydrolysis of glycosidic linkages by specific glycosidases. Indeed the cell wall of *Candida albicans* has a multilayered structure and contains mainly glucan and mannan polysaccharides with lesser amounts of chitin, protein and lipid [2, 3]. The inner cell wall layers are mainly formed by chitin and β-1,3 glucan [4]. Cell wall glucan from *C. albicans* was found to contain β(1–6)-linked glucopyranose residues with β(1–3)-branching points [5]. *C. albicans* mannans contain mainly α(1–6)- and α(1–2)-linked mannopyranose residues [6] with α(1–3) linkages between the mannose units [7, 8]. The polysaccharide composition of the yeast cell wall includes rhamnose, glucosamine, mannose, fucose, galactose, glucose, arabinose, xylose and chitin [2, 9–12].

Latex sap of articulated laticifers (i.e. *L. sativa*) is cytoplasm rich in secretion granules and globular vacuoles containing lysosomal hydrolases) [13]; latex sap of non-articulated laticifers (i.e. *A. curassavica*) is a vacuolar sap rich in secretion granules and lysosomal hydrolases [14].

The chemical composition of latex and its physicochemical properties varies according to the cytological latex pattern. Latex of *A. curassavica* has a moisture content of about 88% [15] and contains at least 50% cardenolides (cardiac glycosides) [16], a very small amount of α- and β-amyrin acetate, and rubber [17]. Latex of *A. curassavica* was shown to contain 40–60 mg $ml^{-1}$ cardenolides, 0.4–0.6 mg $ml^{-1}$ esterified triterpenes [18] and a proteolytic enzyme named asclepain [19–21]. The known cardenolides are: voruscharin, ascharidin, uscharin, calotropin, asclepogenin, calactin, calotoxin, uzarigenin, corotoxigenin, coroglaucigenin, curassarogenin, ascurogenin and clepogenin [16, 22, 23].

Latex sap of *L. sativa* has a moisture content of about 70% and contains a sesquiterpenoid lactose, lactucin (0.7% of crude latex) and lactucopicrin (p-hydroxyphenyl acetic ester) constituting 3.5% of crude latex, triterpene alcohols (taraxasterol, β-amyrol, germanicol), mannitol, asparagin, camphor, inositol, hyoscyamine and rubber [24].

This study was undertaken to examine the glycosidic activities of *C. albicans* after action of two types of latex sap and their putative role in the breakdown of cell wall linkages during the antifungal action. For comparison, the same glycosidic activities have been tested on *C. albicans* after action of the synthetic antifungal isoconazole [25].

## Material and methods

### *Tapping of latex saps*

Latex sap was tapped from stems of *A. curassavica* and stored at −28 °C. After thawing, this latex, corresponding to vacuolar sap [14], was diluted in distilled water (1:17) containing 0.02% sodium azide. Latex sap of *L. sativa* was tapped from prefloral stems and diluted in an ice-cold medium containing 1.5 mol sorbitol, 1 mmol EDTA, 10 mmol Tris-HCl buffer pH 7.6 and 19% metrizamide to prevent bursting of vacuoles containing the lysosomal hydrolases [26]. The dilution rate was about 1:30.

### *Yeast cultures*

*C. albicans* yeast cells (Institut Pasteur 3153 A) were preincubated in Sabouraud chloramphenicol medium (Diagnostic Pasteur, Marnes-la-Coquette, France) for 24 h at 37 °C. Then 1 ml of suspension was placed into 80 ml of Sabouraud chloramphenicol medium containing 10 ml of diluted latex (sample culture) and cultured under constant stirring (50 rev/min) at 37 °C. In the control culture latex was omitted. Isoconazole (Fournier Laboratories, Dijon, France) was dissolved in 50% dimethylsulfoxide and then added to the culture medium to obtain the required concentration (10 or 50 μg $ml^{-1}$). After incubation for 6 h, when the growth inhibition is maximum [27], the yeast suspensions were centrifuged (5 min at 700 g). The supernatant was discarded. After several washings with culture medium, the pellet was mixed with 6 ml of culture medium and processed for enzymatic analysis.

### *Protein determination*

The proteic contents of the samples were estimated according to Bradford [28] with bovine serum albumin as a standard. Measures were performed in triplicate.

### *Glycosidases assays*

The standard incubation medium (0.6 ml, 37 °C) for the investigation of enzymatic activities con-

tained 0.1 mol citrate phosphate buffer (pH 5.5), 0.02% sodium azide and 2.5 mmol p-nitrophenyl substrate. After incubation for 120 min the reaction was stopped by addition of 0.25 ml sodium carbonate 0.2 mol and the absorbance of the p-nitrophenol formed was read at 400 nm against a control tube containing only substrate. The glycosidic activities present in the latex saps were measured in control flask where *C. albicans* are omitted. These activities were deducted from those obtained from *C. albicans* cultured in the presence of latex; the final result was inscribed in the sample culture column of the Tables. Assays were performed in triplicate. The histograms (Figs 1, 2 and 3) show the distribution of relative activities, the relative activity being defined as the ratio between the glycosidic activity of the sample culture (*C. albicans* grown in culture medium with latex) and those of the control culture (*C. albicans* grown in culture medium without latex).

*Reagents and chemicals*

Synthetic p-nitrophenyl substrates were purchased from Sigma Chemical Co; all other reagent grade chemicals were purchased from Merck, Darmstadt, Germany.

*Electron microscopy*

Fragments of pellets of *C. albicans* cultured under the above described conditions were pre-fixed in a mixture of 6% (v/v) glutaraldehyde and 2% (w/v) paraformaldehyde in 0.1 mol phosphate buffer, pH 7.2, for 17 h at 4 °C, then carefully rinsed with the same buffer and postfixed with 2% (w/v) osmium tetroxide in distilled water for 1 h at 4 °C.

For sample processing for transmission electron microscopy (TEM) the yeasts cells were dehydrated in an acetone series, and embedded in araldite. Ultrathin sections were cut on a Porter Blum MT2 type ultra-microtome and stained by Thiéry's method [29] to characterize polysaccharide compounds. Adequate controls (hydrogen peroxided sections stained with thiocarbohydrazide and silver proteinate, periodic acid oxidized sections stained with silver proteinate, non-oxidized sections stained with thiocarbohydrazide and silver proteinate, non-oxidized sections stained with silver proteinate) were used for Thiéry's test. Specimens were analyzed with a Philips EM400 electron microscope at 60 KV.

## Results

Except for α-arabinosidase all glycosidase activities increased when *C. albicans* was grown in medium with *L. sativa* latex sap (Table 1). The relative activities (ratio between sample activity and control activity) indicate that the most stimulated activities were those of β-fucosidase, α-galactosidase, α- and β-glucosidase, α- and β-mannosidase, acetyl-β-glucosaminidase (Figs 1 and 2): β-flucosidase indicates a relative activity of about 37 while the other glycosidases are stimulated to 16–1 100%.

The presence of *A. curassavica* latex sap in culture medium gave similar results: except for α-ar-

**Table 1.** Substrate specificity for glycosidases activities at pH 5.0 in *C. albicans* cultured in standard medium (control culture) or in medium supplemented with *L. sativa* latex sap (sample culture)

| Substrate | Control culture | Sample culture |
|---|---|---|
| α-D-mannoside | 1.38 | 4.76 |
| β-D-mannoside | 1.26 | 3.53 |
| α-D-galactoside | 1.31 | 15.79 |
| β-D-galactoside | 1.40 | 2.0 |
| α-D-glucoside | 1.49 | 7.53 |
| β-D-glucoside | 1.47 | 5.82 |
| β-D-xyloside | 1.43 | 3.11 |
| α-L-arabinoside | 1.83 | 1.82 |
| N-acetyl-β-D-glucosaminide | 1.95 | 6.03 |
| α-L-fucoside | 1.35 | 1.57 |
| β-D-fucoside | 1.20 | 44.45 |

Using p-nitrophenyl-substituted glycosides as substrates, the enzyme activity was expressed in μmol min $ml^{-1}$ based on p-nitrophenol standard. The activities indicated in the sample culture column represent the difference between those measured when yeasts are cultured in presence of latex and those measured in latex sap itself. The enzyme activities were normalised for a defined *C. albicans* concentration and were means of at least three measurements made on independent cultures. The values for replicate assays differed from the mean by about 18%. The diluted crude latex contained 760 μg proteins $ml^{-1}$.

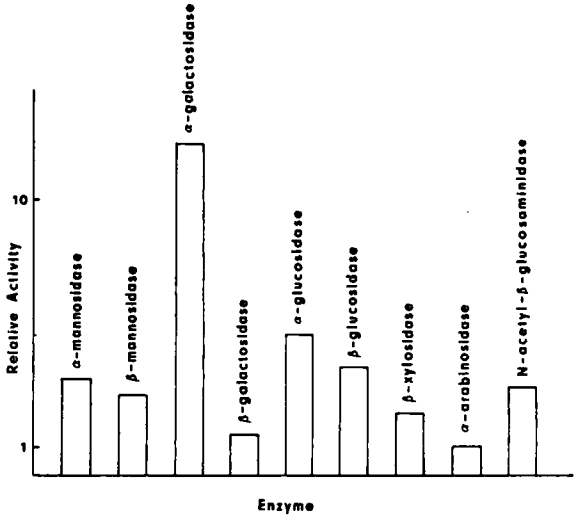


**Figure 1.** Glycosidase activities in *C. albicans* cultured in medium containing *L. sativa* latex sap.

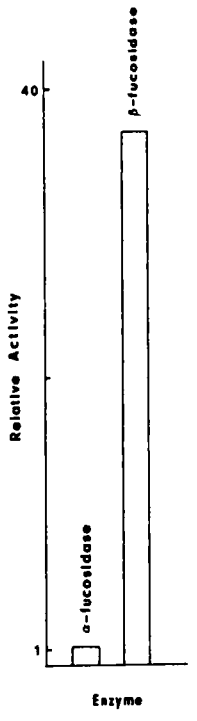


**Figure 2.** Glycosidase activities in *C. albicans* cultured in medium containing *L. sativa* latex sap.

**Table 2.** Substrate specificity for glycosidases activities at pH 5.0 in *C. albicans* cultured in standard medium (control culture) or in medium supplemented with *A. curassavica* latex sap (sample culture)

| Substrate | Control culture | Sample culture |
|---|---|---|
| α-D-mannoside | 1.2 | 16.4 |
| β-D-mannoside | 1.13 | 2.0 |
| α-D-galactoside | 1.13 | 8.8 |
| β-D-galactoside | 1.16 | 2.97 |
| α-D-glucoside | 1.31 | 2.62 |
| β-D-glucoside | 1.37 | 4.55 |
| β-D-xyloside | 1.27 | 2.55 |
| α-L-arabinoside | 1.69 | 1.54 |
| N-acetyl-β-D-glucosaminide | 1.70 | 12.3 |
| α-L-fucoside | 1.18 | 1.68 |
| β-D-fucoside | 1.18 | 6.26 |

Using p-nitrophenyl-substituted glycosides as substrates, the enzyme activity was expressed in μmol min $ml^{-1}$ based on p-nitrophenol standard. The activities indicated in the sample culture column represent the difference between those measured when yeasts are cultured in presence of latex and those measured in latex sap itself. The enzyme activities were normalised for a defined *C. albicans* concentration and were means of at least three measurements made on independent cultures. The values for replicate assays differed from the mean by about 20%. The diluted crude latex contained 1,1 mg proteins $ml^{-1}$.

abinosidase, all other glycosidic activities tested increased (Table 2); the most stimulated were α-mannosidase, α-galactosidase, acetyl-β-glucosaminidase and β-fucosidase (Fig. 3).

For comparison no significant increase (0–31%) of the same glycosidic activities was obtained when

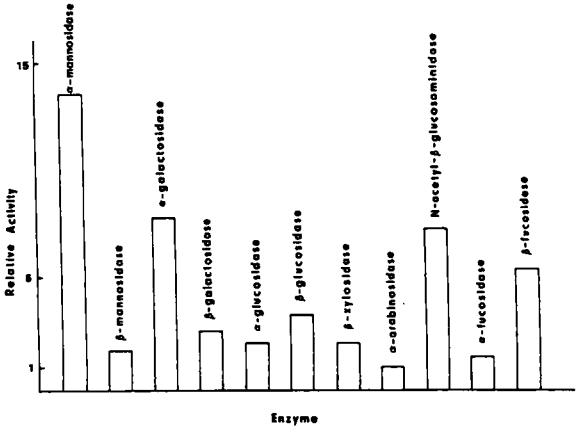


**Figure 3.** Glycosidase activities in *C. albicans* cultured in medium containing *A. curassavica* latex sap.

*C. albicans* was grown in medium with 10 μg $ml^{-1}$ isoconazole (Table 3).

A decrease of glycolytic activities was observed (39–64%) on *C. albicans* cultured in medium containing 50 μg $ml^{-1}$ isoconazole (Table 3).

The fungistatic effect of latex saps was confirmed by transmission electron microscopy. Thus, *C. albicans* cultured in medium containing *L. sativa* latex presented a partial cell wall disruption while the cytoplasm appeared degenerated (Fig. 4b). *C. albicans* cultured in medium supplemented with *A. curassavica* latex showed discontinuous walls when the Thiéry reaction was applied which indicates a modification of its polysaccharides content (Fig. 4c). The control sections indicated the same contrast in the cell wall as in unstained sections (data not

**Table 3.** Substrate specificity for glycosidases activities at pH 5.0 in *C. albicans* cultured in standard medium (control culture) or in medium supplemented with 10 μg $ml^{-1}$ and 50 μg $ml^{-1}$ isoconazole (sample culture)

| Substrate | Control culture | Sample culture 10 μg $ml^{-1}$ | Sample culture 50 μg $ml^{-1}$ |
|---|---|---|---|
| α-D-mannoside | 1.28 | 1.49 | 0,75 |
| β-D-mannoside | 1.17 | 1.35 | 0.47 |
| α-D-galactoside | 1.22 | 1.46 | 0.51 |
| β-D-galactoside | 1.30 | 1.35 | 0.46 |
| α-D-glucoside | 1.38 | 1.49 | 0.68 |
| β-D-glucoside | 1.36 | 1.64 | 0.73 |
| β-D-xyloside | 1.33 | 1.46 | 0.53 |
| α-L-arabinoside | 1.68 | 2.21 | 1.03 |
| N-acetyl-β-D-glucosaminide | 1.76 | 1.73 | 0.84 |
| α-L-fucoside | 1.25 | 1.40 | 0.50 |
| β-D-fucoside | 1.12 | 1.26 | 0.43 |

Using p-nitrophenyl-substituted glycosides as substrates, the enzyme activity was expressed in μmol min $ml^{-1}$ based on p-nitrophenol standard. The enzyme activities were normalised for a defined *C. albicans* concentration and were means of at least three measurements made on independent cultures. The values for replicate assays differed from the mean by about 17%.

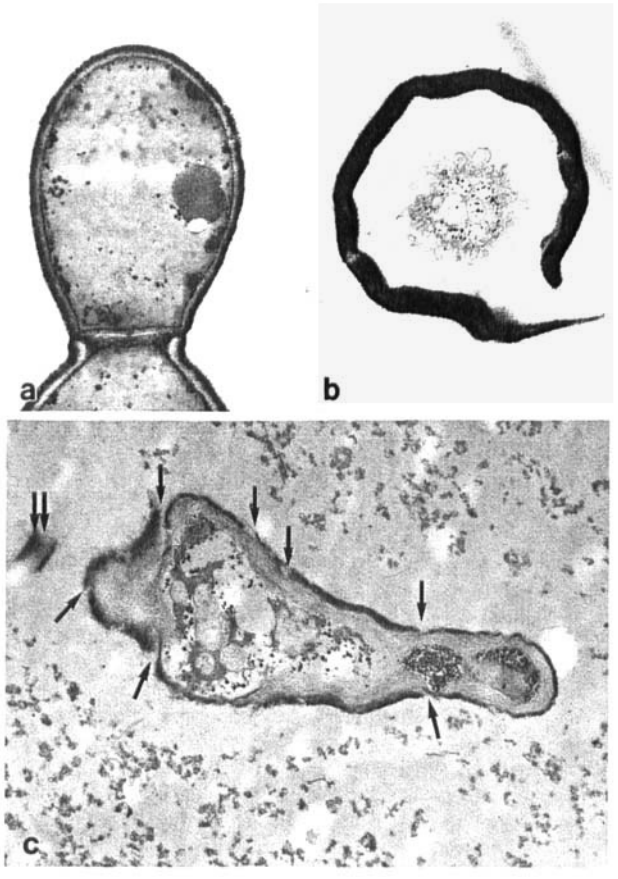


**Figure 4.** (a) Transmission electronmicrograph of *Candida albicans* from a control culture after incubation for 6 h: No cell wall perforation is visible (G × 13 600). (b) Transmission electronmicrograph of *C. albicans* grown in medium containing *L. sativa* latex sap after 6 h of culture: a partial cell wall disruption was observed (G × 12 800). (c) Transmission electronmicrograph of *C. albicans* grown in medium containing *A. curassavica* latex sap after 6 h of culture: discontinuous staining of the cell wall by Thiéry's method (arrows) indicating a limited cell wall degradation in specific sites. A release of parietal fragments (double arrow) in culture medium is recognizable (G × 10 600).

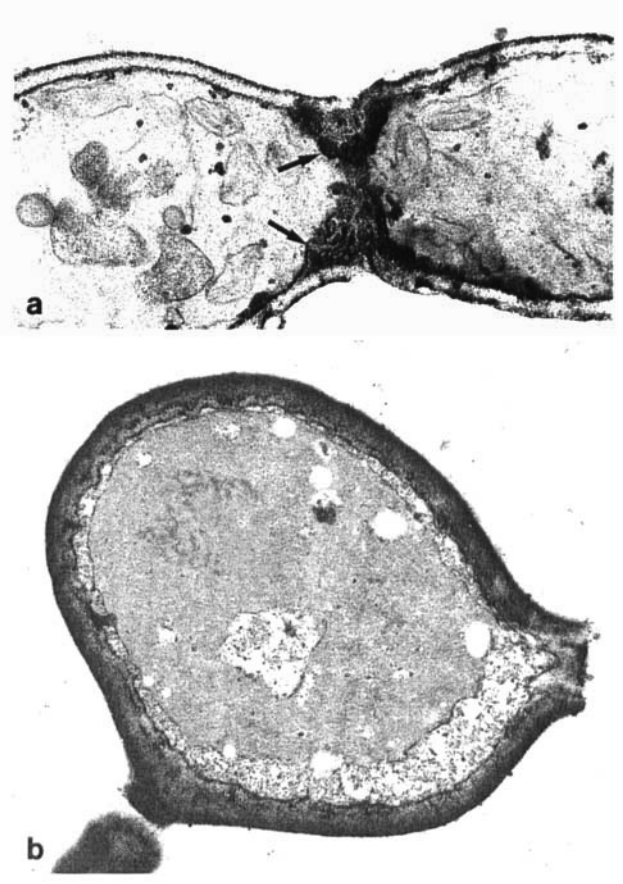


**Figure 5.** (a) Transmission electronmicrograph of *C. albicans* grown in culture medium supplemented with 10 μg ml$^{-1}$ isoconazole. No cell wall breakdown is visible. In contrast an accumulation of cell wall material can be observed in the segmentation zone (arrows) (G × 28 000). (b) Transmission electronmicrograph of *C. albicans* grown in culture medium supplemented with 50 μg ml$^{-1}$ isoconazole. An enlargement of the periplasmic space and accumulation of granules in this area are visible; no cell wall breakdown occurs (G × 8 400).

shown). Cell wall fragments were released into the culture medium and the cytoplasm appeared granular or was not present (Fig. 4c). No ultrastructural cell wall disruption was observed in *C. albicans* grown in medium supplemented with isoconazole (Figs 5a and b). Accumulation of abnormal cell wall material was observed in areas between mother and daughter blastospores (Fig. 5a) as well as an enlarging of the periplasmic space and accumulation of granules in this area (Fig. 5b). Controls of *C. albicans* grown in medium devoid of latex saps or isoconazole did not show any alteration of the fungal cell wall (Fig. 4a).

## Discussion

In cells of *C. albicans* grown in the presence of latex saps cell wall breakdown is shown to be concomitant with a substantial increase of glycosidic activities indicating a relationship between these two processes. This increase of glycosidic activities cannot be explained by selective adsorption of latex glycosidases to the fungal cell surface since the latex sap glycosidic activities present in the latex inoculum have been deducted from those measured in sample cultures. Glycosidase activities characterized in latex saps of *L. sativa* and *A. curassavica* [13] hydrolyse the fungal glycolytic linkages of hemicelluloses permitting thus access of cell wall glycosidases to artificial substrates as p-nitrophenyl glycosides. One can find intraparietal glycosidases which are activated by the disruption of glycosidic linkages between enzyme and hemicelluloses (proenzyme-enzyme process). This is particularly probable since it is known that the plant cell wall can be disrupted *in vitro* by latex saps (Giordani, unpublished data) specially by their glycosidases content such as β-fucosidase [30]. The stimulation of glycolytic activities due to the action of latex saps is the reflection

of additional activation of enzyme molecules located in the fungal cell wall since artificial substrates cannot go across the cell wall without specific carriers. It is therefore unlikely that fungal cytoplasmic glycosidases might interfere in enzymatic assays with those of the cell wall. It is possible that cell wall glycosidases are activated by a non-enzymatic substance located in latex saps. Otherwise, *de novo* synthesis of glycosidases after exposition to elicitors present in the latex sap may be possible. The parietal localization of glycosidase activities measured here is all the more likely as the fungal cell wall contains β-glucanases [31] while other hydrolases such as acid phosphatase, melibiase and β-fructofuranosidase are located in the periplasmic space [32–35].

The cell wall breakdown of *C. albicans* after the action of latex saps may be compared with autolytic processes. During autolysis the porosity of the cell wall is increased by endogenous β-glucanase action which acts specifically on β(1–6)-linked and β(1–3)-linked glucans [36]. An increase in fungal cell wall porosity may be the result of the latex sap action effectuating thus the influx of latex constituents such as terpenes and cardiac glycosides with well-known toxicity [22, 37] into the *C. albicans* cytoplasm [17, 18, 24].

Hydrolytic activity stimulated in cell walls of *C. albicans* after the action of latex saps can be compared with the induction of hydrolases in plants after having been attacked by pathogenic microorganisms. Thus, chitinase and β-1,3-glucanase purified from pea pods acted synergistically in the degradation of fungal cell walls causing the inhibition of fungal growth [38].

The absence of modifying glycolytic activities of *C. albicans* exposed to isoconazole 10 μg $ml^{-1}$ may be due to mode of action of this antifungal compound. Iconazole, an imidazole derivative, modifies the permeability of the plasmalemma by inhibiting the ergosterol synthesis [39, 40]. The strong decrease of glycosidases activities after exposition to the higher dose of 50 μg $ml^{-1}$ isoconazole may reflect a secondary disorganization of parietal structures the morphological modifications of which have been studied [25, 41].

According to our results the modes of action of latex saps and isoconazole are different. Isoconazole acts principally on the plasmalemma and hardly on cell walls, while latex saps seem to act specifically on fungal cell walls by activating glycosidases activities. Studies are underway to investigate enzymes purified from latex saps in their action on fungal cell walls.

## References


1 Giordani, R., Moulin-Traffort, J. & Régli, P. (1990) Fungal cell wall degradation after action of latex. 30ieme Colloque de la Société Française de Microscopie Electronique, Toulouse, France (19–22/6). *Biol. Cell* **69**, 37a.

2 Chattaway, F. W., Holmes, M. R. & Barlow, A. J. E. (1968) Cell wall composition of the mycelial blastospore forms of *Candida albicans*. *J. Gen. Microbiol.* **51**, 367–376.

3 Kessler, G. & Nikerson, W. J. (1959) Glucomannan-protein complexes from cell walls of yeasts. *J. Biol. Chem.* **234**, 2281–2285.

4 Poulain, D., Tronchin, G., Dubremetz, J. F. & Biguet, J. (1978) Ultrastructure of the cell wall of *Candida albicans* blastospores: study of its constitutive layers by the use of a cytochemical technique revealing polysaccharides. *Ann. Microbiol.* (Institut Pasteur) **129**, 141–153.

5 Bishop, C. T., Blank, F. & Gardner, P. E. (1960) The cell wall polysaccharides of *Candida albicans*: glucan, mannan and chitin. *Can. J. Chem.* **38**, 869–881.

6 Odds, F. C. (1979) *Structure, physiology and biochemistry of Candida and candidosis*. Leicester: University Press.

7 Kockoureck, J. & Ballou, C. E. (1969) Method for fingerprinting yeast cell wall mannans. *J. Bacteriol.* **100**, 1175–1181.

8 Suzuki, S. & Usuyama, M. (1971) Effect of *Arthrobacter* α-mannosidase digestion on the immunochemical properties of the mannans of two serotypes of *Candida* yeasts. *Tohuku Yakka Daigaku Kenkyu Nempo* **18**, 55–62.

9 Chiew, Y. Y., Shepherd, M. G. & Sullivan, P. A. (1980) Regulation of chitin synthesis during germ-tube formation in *Candida albicans*. *Arch. Microbiol.* **125**, 97–104.

10 Do Carmo-Sousa, L. & Barroso Lopes, C. (1970) A comparative study of the extracellular and cell wall polysaccharides of some *Candida* species. *Antonie van Leeuwenhoek* **36**, 209–216.

11 Ray, T. L., Hanson, A., Ray, L. F. & Wuepper, K. D. (1979) Purification of a mannan from *Candida albicans* which activates serum complement. *J. Invest. Dermatol.* **73**, 269–274.

12 Vega, R. & Dominguez, A. (1986) Cell wall composition of the yeast and mycelial forms of *Yarrowia lipolytica*. *Arch. Microbiol.* **144**, 124–130.

13 Giordani, R., Noat, G. & Marty, F. (1987) Compartmentation of glycosidases in a light vacuole fraction from the latex of *Lactuca sativa* L. In: Marin, B. (ed) *Plant vacuoles*. Their importance in solute compartmentation in cells and their applications in plant biotechnology. NATO ASI Series 134, 383–391. New-York – London: Plenum Press.

14 Giordani, R., Blasco, F. & Bertrand, J. C. (1982) Confirmation biochimique de la nature vacuolaire et lysosomale du latex des laticifères non-articulés d'*Asclepias curassavica*. *C. R. Acad. Sci.*, (Paris) **295**, 641–646.

15 Van Emon, J. & Seiber, J. (1985) Chemical constituents and energy content of two milkweeds, *Asclepias speciosa* and *A. curassavica*. *Economic Botany* **39**, 47–55.

16 Seiber, J. N., Nelson, C. J. & Mark Lee, S. (1982) Cardenolides in the latex and leaves of seven *Asclepias* species and *Calotropis procera*. *Phytochemistry* **21**, 2343–2348.

17 Nielsen, P. E., Nishimura, H., Otvos, J. & Calvin, M. (1977) Plant crops as a source of fuel and hydrocarbon-like materials. *Science* **198**, 942–944.

18 Groeneveld, H. W. & van der Made, L. A. (1982) Cardenolide and triterpene synthesis in the laticifers of *Asclepias curassavica* L. *Acta Bot. Neerl.* **35**, 5–10.

19 Brockbank, W. J. & Lynn, K. R. (1979) Purification and preliminary characterization of two asclepains from the latex of *Asclepias syriaca* L. (milkweed). *B.B.A.* **578**, 13–22.

20 Lynn, K. R., Brockbank, W. J. & Clevette, N. A. (1980) Multiple forms of the asclepains cysteinyl proteases from milkweed. *B.B.A.* **612**, 119–125.

21 Winnick, T., Davis, A. R. & Greenberg, D. M. (1940) Physicochemical properties of the proteolytic enzyme from the latex of the milkweed, *Asclepias speciosa* Torr. Some comparisons with other proteases. *J. Gen. Physiol.* **23**, 275–308.
22 Kupchan, S. M., Knox, J. R. & Kelsey, J. E. (1964) Calotropin, a cytotoxic principle isolated from *Asclepias curassavica* L. *Science* **146**, 1685–1686.
23 Tschesche, R., Forstmann, D. & Rao, V. K. M. (1958) Zur Kenntnis der Cardenolid-Inhaltsstoffe von *Asclepias curassavica* L. *Chem. Berl.* **91**, 1204–1211.
24 Garnier, G., Bezanger-Beauquesne, G. & Debraux, G. (1961) *Ressources médicinales de la flore française.* Paris: Vigot frères éd.
25 Moulin-Traffort, J., Venot, C. & Régli P. (1986) Action de l'isoconazole sur *Candida albicans.* Etude en microscopie électronique à balayage et à transmission. *Pathol. Biol.* **34**, 899–907.
26 Giordani, R., Noat, G. & Marty, F. (1983) Isolement et purification des vacuoles des laticifères articulés de *Lactuca sativa* L. *Ann. Sci. Nat. Bot.* (Paris) **5**, 229–243.
27 Moulin-Traffort, J., Giordani, R. & Régli, P. (1990). Antifungal action of latex saps from *Lactuca sativa* L. and *Asclepias curassavica* L. *mycoses* **33**, 383–392.
28 Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254.
29 Thiéry, J. P. (1967) Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. *J. Microscopie* **6**, 987–1018.
30 Giordani, R. & Noat, R. (1988) Isolation, molecular properties and kinetic studies of a strict β-fucosidase from *Lactuca sativa* latex. Its possible role in the cell wall degradation of articulated laticifers. *Eur. J. Biochem.* **175**, 619–625.
31 Barras, D. R. (1972) A β-glucan endohydrolase from *Schizosaccharomyces pombe* and its role in cell wall growth. *Antonie van Leeuwenhoek* **38**, 65–80.
32 Arnold, W. N. (1969a) Chromatography of a polydisperse enzyme. Yeast β-fructofuranosidase on DEAE-cellulose. *Anal. Lett.* **2**, 547–552.
33 Arnold, W. N. (1969b) Heat inactivation kinetics for yeast β-fructofuranosidase. A polydisperse system. *Biochim. Biophys. Acta* **178**, 347–353.
34 Friis, J. & Ottolenghi, P. (1960) Localization of melibiase in a strain of yeast. *C. R. Trav. Lab.* (Carlsberg) **31**, 272–281.
35 Odds, F. C. & Hierholzer, J. C. (1973) Purification and properties of a glycoprotein acid phosphatase from *Candida albicans. J. Bacteriol.* **114**, 257–266.
36 Arnold, W. N. (1972) The structure of the yeast cell wall. Solubilization of a marker enzyme, β-fructofuranosidase, by the autolytic enzyme system. *J. Biol. Chem.* **247**, 1161–1169.
37 Bhagirath, S. & Rastogi, R. P. (1969) Chemical investigation of *Asclepias curassavica* L. *Ind. J. Chem.* **7**, 1105–1110.
38 Mauch, F., Mauch-Mani, B. & Boller, T. (1988) Antifungal hydrolases in pea tissue. II. Inhibition of fungal growth by combination of chitinase and β-1,3-glucanase. *Plant Physiol.* **88**, 936–942.
39 Michel-Briand, Y. (1981) Mécanisme d'action de deux familles d'antifongiques: les polyénes et les dérivés de l'imidazole. *Bull. Soc. Fr. Mycol. Méd.* **10**, 159–170.
40 Van den Bossche, H., Willemsens, G., Cools, W. & Cornelissen, F. (1979) Inhibition of ergosterol synthesis in *Candida albicans* by ketoconazole. *Arch. Int. Physiol. Biochim.* **87**, 75–77.
41 Bastide, M., Jouvert, S. & Bastide, J. M. (1982) A comparison of the effects of several antifungal imidazole derivates and polyenes on *Candida albicans*: an ultrastructural study by scanning electron microscopy. *Can. J. Microbiol.* **28**, 1119–1126.