

WORONIN BODIES IN *PENICILLIUM JANCZEWSKII* ZALESKI

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ABSTRACT

Penicillium janczewskii Zaleski is an efficient microorganism for the production of extracellular inulinases and grows rapidly on medium containing sucrose or inulin as carbon source. Maintenance of this filamentous fungus on inulin medium induces secretion of large amounts of inulinases, but the resulting mycelium has thinner cell walls that easily collapse and break. Woronin bodies in hyphae of *P. janczewskii* grown on sucrose and inulin substrates were observed. No significant differences in the number, location, size and shape of Woronin bodies and level of plugging were observed in cultures of the fungus grown on the two carbon sources. The data indicate that the presence of Woronin bodies in *P. janczewskii* could not be associated with more easily damaged hyphae, although the function of these organelles in pore plugging has been confirmed.

Key words: Woronin bodies, *Penicillium*, inulin, sucrose.

INTRODUCTION

Woronin bodies are cellular organelles of filamentous fungus that were first described in the Ascomycetes by the Russian mycologist Michael Stepanovich Woronin (4). Although the precise function of the Woronin bodies has not yet been determined, recent results suggest that they represent a new category of peroxisomes engaged in the maintenance of cellular integrity (3). In intact hyphae, the Woronin bodies seem to be unaffected by normal intercellular cytoplasmic streaming, being attached to the plasma membrane. However, when hyphae lyse, the release of cellular turgor triggers an instant increase in the rate of cytoplasmic flow, and this change dislodges Woronin bodies from their site of attachment to the plasma membrane. These corpuscles then rapidly occlude the septal pore after lysis, sealing them and thereby preventing catastrophic cell death (3).

Penicillium janczewskii, a filamentous fungus isolated from the rhizosphere of *Vernonia herbacea* (Vell.) Rusby (Asteraceae) grows rapidly on media containing either sucrose or inulin as the sole carbon source (2). When grown on inulin, this fungus is considered an efficient microorganism for the industrial

production of extracellular inulinases due to the high yield and specific activity of the enzymes secreted into the culture medium (7). These inulinases can be utilised in industry for the preparation of fructose syrups from inulin (6).

Differences in cell walls thickness and in colony pigmentation of *P. janczewskii* have been found depending on whether this fungus is cultivated in media containing sucrose or inulin. Maintenance of the fungus on inulin medium results in thinner mycelia that easily collapse and break, as observed during preparation of fungal material for scanning-electron microscopy (Fig. 1A).

In the present work we looked for the presence of Woronin bodies in hyphae of the *Penicillium janczewskii* grown on sucrose and inulin substrates.

MATERIALS AND METHODS

Microorganism

Penicillium janczewskii Zaleski (URM 3511 – Universidade Federal de Pernambuco- Recife, Brazil) was isolated from the rhizosphere of *Vernonia herbacea* (Vell.) Rusby,

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Asteraceae, growing naturally in a cerrado area near Moji-Guaçu (SP, Brazil) (2).

Culturing

The fungus was grown on solid medium containing the following components (g.l⁻¹): NaNO₃ (3), KH₂PO₄ (1), KCl (0.5), MgSO₄·7H₂O (0.5), FeSO₄·7H₂O (0.01), agar (1.5%, w/v) and either inulin (1%, w/v) from *Helianthus tuberosus* (Sigma) or sucrose (1%, w/v) as sole carbon sources. Cultures were incubated at 28°C for 5-8 days in darkness.

Transmission electron microscopy (TEM)

A 1 mm³ plug was cut from the actively growing margin of the colony of each fungal culture using a razor blade and initially fixed at room temperature in a solution containing 2.5% (v/v) glutaraldehyde, 2.5% (v/v) paraformaldehyde and 5 mM CaCl₂ in 0.05 M cacodylate buffer pH 7.2. After 1 h, the material was washed with three changes (10 min each) of the same buffer and postfixed for 1 h in 0.05 M cacodylate buffer supplemented with 5 mM CaCl₂ and containing 1% (w/v) OsO₄. Subsequently, the fixed material was transferred to 1% (w/v) uranyl acetate and was left in this solution overnight. Samples were dehydrated with a graded aqueous acetone series (30, 50, 70, 90, 100, 100, 100% [v/v]; 10 min each step). The dehydrated material was first infiltrated with Spurr's resin in 1% (w/v) uranyl acetate (1:1) for 5 h, and then in pure Spurr's resin overnight. The infiltrated tissue was transferred to templates containing 100% Spurr's resin. Polymerisation was carried out at 60°C for 24 h.

Thin sections (<100nm) were cut with an MT 6000-XL ultramicrotome and collected on Formvar-coated, gilded copper slots grids and placed on Formvar bridges to dry (9). The sections were poststained for 30 s with 2% (w/v) lead citrate (8). The sections were examined at 80 kV with a Zeiss EM 902A electron microscope.

Statistics

Measurements of the outside diameter of the Woronin bodies were performed only on those organelles in which the outer membrane was clearly visible, in order to insure that the plane section caught them through or near the centre. At least 80 measurements of Woronin bodies were recorded for fungal cultures grown on each substrate. The means were compared by Student's test at 95% confidence level.

RESULTS AND DISCUSSION

Woronin bodies were shown to be present in hyphae of *Penicillium janczewskii* grown for 5-8 days on both sucrose (Figs. 2A, B) and inulin (Fig. 2C). No substantial differences were observed in the location, number, size and shape of the Woronin bodies observed in hyphae grown on media containing the two

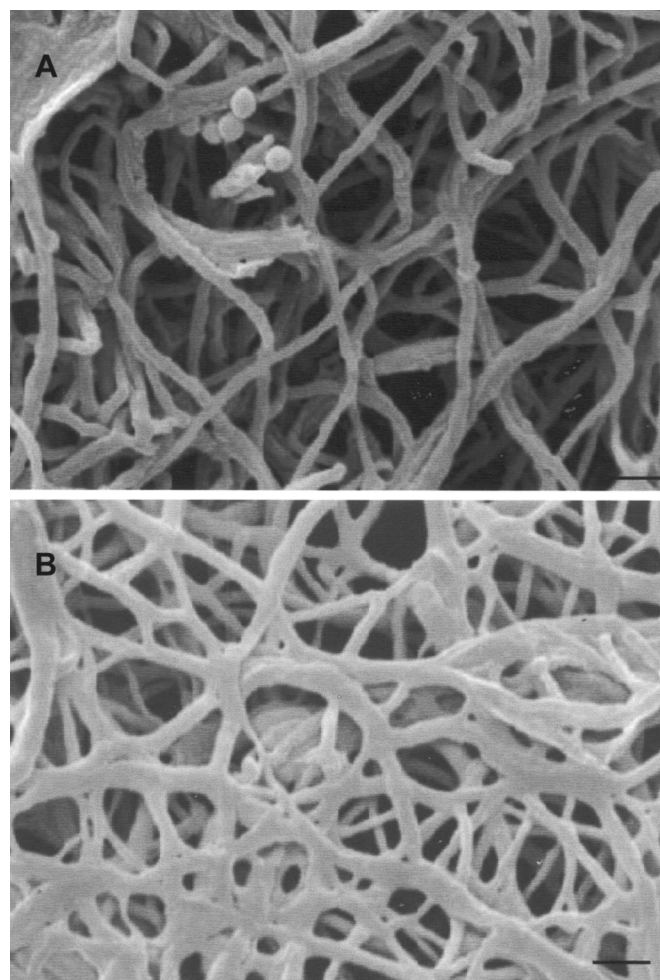


Figure 1. Scanning electron microscopy of *Penicillium janczewskii* hyphae grown on inulin (A) (X 6380) and sucrose (B) (X 5560), bar = 3 µm.

different carbon sources (Figs. 2B, C). As shown in Fig. 2, Woronin bodies of *P. janczewskii* are spherical, have irregular edges, are often within a well-defined membrane (Fig. 2B) and are variable in size. Two to four Woronin bodies could generally be observed in the zones close to the septum (Fig. 2A). Woronin bodies were generally observed to be located on both sides of the septa (Figs. 2A, D), and sometimes crossing the central pore (Figs. 2D, E). They ranged in diameter from 102 to 129 nm, the average being 116 ± 9 (SD), and were larger than the average diameter of septal pores (ca. 70 nm). Collinge and Markham (1) reported the presence of Woronin bodies of 167 ± 8 nm diameter in mycelia of *Penicillium chrysogenum*, larger than those observed here in *P. janczewskii*. Woronin bodies have also been observed in many other fungal species, including those belonging to the genus *Arthrinium* (4) and in *Disciotis venosa* (10), where they appear spherical and hexagonal in shape, respectively.

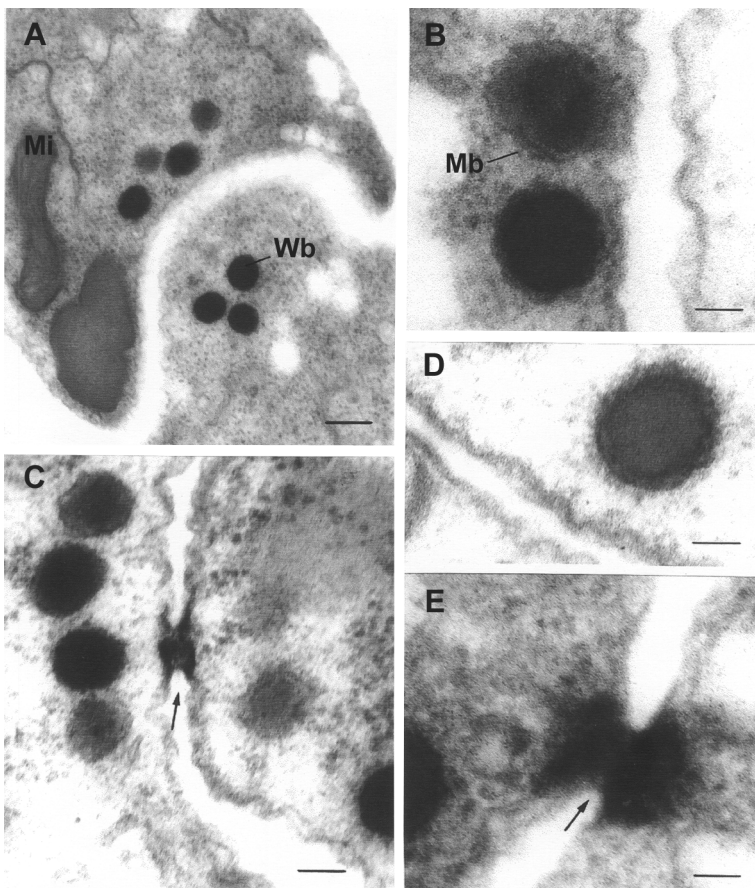


Figure 2. Woronin bodies (Wb) in *Penicillium janczewskii* hyphae grown on sucrose (A, B, D, E) and on inulin (C). (A) – Woronin bodies in both sides of the septum (X 22000), bar = 0.3 μ m, Mi = mitochondria. (B) and (D) – Spherical Woronin bodies showing the irregular edge and the surrounding membrane (Mb) (X 90000), bar = 0.08 μ m. (C) View of a pore plugged by the Woronin body (arrow) (X 52000), bar = 0.14 μ m. (E) Detail of the pore plugging (X 90000), bar = 0.08 μ m.

The plugging of septal pores by Woronin bodies has consistently been observed at very high levels in damaged mycelia when compared to undamaged actively growing hyphae, suggesting that a major function of this organelle is the emergency plugging of septal pores in response to severe hyphal damage that breaches the hyphal wall (5). For example, pore plugging by Woronin bodies in *P. chrysogenum* was demonstrated to be a rapid and specific response to the induced severing of hyphae (1).

In *P. janczewskii*, hyphae from fungus grown on inulin-containing medium were much thinner than those cultivated on sucrose (Figs. 1A, B), and presenting significant differences in the cell wall thickness, being more easily damaged. Transmission electron microscopy of 5-day-old cultures indicated that the

cell wall thickness is around 384 ± 90 (mean \pm SD) and 800 ± 20 nm for hyphae grown on inulin and sucrose media, respectively. More Woronin bodies or higher level of pore plugging thereby would be expected to be present in hyphae grown on inulin. However, no significant differences in the number of Woronin and level of plugging were observed in cultures of *P. janczewskii* grown on the two carbon sources. Our data indicate that, at least in *P. janczewskii*, the presence of Woronin bodies could not be associated with more easily damaged hyphae, although the function of these organelles in pore plugging has been confirmed. These findings indicate that more work is necessary in order to bring conclusive evidence of the exact correlation between the level of pore plugging by Woronin bodies and damage control during fungal growth.

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RESUMO

Corpúsculos de woronin em *Penicillium janczewskii* Zaleski

Penicillium janczewskii Zaleski é um microrganismo eficiente para a produção de inulissases extracelulares e cresce rapidamente em meio contendo sacarose ou inulina como fonte de carbono. A manutenção desse fungo filamentososo em meio com inulina induz a secreção de grandes quantidades de inulissases, mas resulta na presença de um micélio com parede celular mais fina, que facilmente colapsa e se danifica. A presença de corpúsculos de Woronin foi analisada nas hifas de *P. janczewskii* crescido em sacarose e inulina. Nenhuma diferença significativa foi observada no número, localização, tamanho e forma dos corpúsculos de Woronin e na obstrução dos poros dos septos nas culturas do fungo crescido nas duas diferentes fontes de carbono. Os resultados obtidos indicam que a presença dos corpúsculos de Woronin em *P. janczewskii* não pode ser associada com a presença de hifas mais facilmente danificadas, embora a função dessas organelas na obstrução dos poros dos septos tenha sido confirmada.

Palavras-chave: corpúsculos de Woronin, *Penicillium*, inulina, sacarose.

REFERENCES

1. Collinge, A.J.; Markham, P. Ultrastructure of hyphal tip bursting in *Penicillium chrysogenum*. *FEMS Microbiol. Lett.*, 91: 49-54, 1992.
2. Cordeiro Neto, F.; Pessoni, R.A.B.; Figueiredo-Ribeiro, R.C.L. Fungos produtores de inulinases isolados da rizosfera de asteráceas herbáceas do cerrado. *Rev. Bras. Ci. Solo*, 21: 149-153, 1997.
3. Jedd, G.; Chua, N. A new self-assembled peroxisomal vesicle required for efficient resealing of the plasma membrane. *Nat. Cell. Biol.*, 2: 226-231, 2000.
4. Larrondo, J.V.; Calvo, M.A. Woronin bodies in *Arthrinium* genus. *Mycopathologia*, 119: 157-160, 1992.
5. Markham, P. Occlusions of septal pores in filamentous fungi. *Mycol. Res.*, 98: 1089-1106, 1994.
6. Pandey, A.; Soccol, C.R.; Selvakumar, P.; Soccol, V.T.; Krieger, N.; Fontana, J.D. Recent developments in microbial inulinases. *App. Biochem. Biotechnol.*, 81: 35-52, 1999.
7. Pessoni, R.A.B.; Figueiredo-Ribeiro, R.C.L.; Braga, M.R. Extracellular inulinases from *Penicillium janczewskii*, a fungus isolated from the rhizosphere of *Vernonia herbacea* (Asteraceae). *J. App. Microbiol.*, 86: 141-147, 1999.
8. Reynolds, E.S. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell. Biol.*, 17: 208-212, 1963.
9. Rowley, J.C. III; Moran, D.T. A simple procedure for mounting wrinkle-free sections on Formvar-coated slot grids. *Ultramicroscopy.*, 1: 151-155, 1975.
10. Turnau, K.; Kottke, I.; Oberwinkler, F. Comparative study of elongated and globose Woronin bodies using electron energy loss spectroscopy (EELS) and imaging (ESI). *Mycol. Res.*, 97: 1499-1504, 1993.