SHORT COMMUNICATION

Isolation and Characterization of Chitin from the Cell Walls of Achlya radiosa

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An insoluble fraction obtained from the hyphal wall of the Oomycete Achlya radiosa was characterized as chitin by the products of acid and enzymic hydrolysis and by its infrared and X-ray diffraction spectra. This material showed the characteristic random microfibrillar structure of chitin under light and electron microscopes.

INTRODUCTION

The presence of chitin in the cell walls of Oomycetes belonging to the orders Saprolegniales and Peronosporales has been suggested on the basis of the following observations: (a) the liberation of N-acetylglucosamine by enzymic hydrolysis with chitinase-enriched snail digestive juice (Dietrich, 1975); (b) the inhibition of incorporation of N-acetyl[14C]glucosamine into the wall by polyoxin D, a specific inhibitor of chitin synthase (Dietrich & Campos, 1978). Since the most unequivocal characterization of chitin is based on its physico-chemical properties, an attempt was made to characterize chitin from the walls of the Oomycete Achlya radiosa in this manner. The results are reported here.

METHODS

Achlya radiosa Maurizio, strain SPC30, from the collection of the Instituto de Botânica of São Paulo, was maintained and grown as previously described (Dietrich & Campos, 1978). Cell walls were obtained from mycelium grown for 6 d at 22 °C in the dark in 20 l of liquid medium (Dietrich, 1975). A 1 g portion of cell walls was fractionated using the procedure described by Sangar & Dugan (1973), followed by treatment of the residue with the Schweizer reagent and subsequent washing with 1 M-acetic acid and then water. Each fraction was hydrolysed with 6 M-HCl for 10 h or with chitinase (EC 3.2.1.14; from Streptomyces griseus; Sigma) for 96 h. The products were analysed for reducing sugars, hexosamines and N-acetylglucosamine, and characterized by paper chromatography and thin-layer chromatography as previously described (Dietrich & Campos, 1978).

The infrared spectrum of the insoluble wall fraction was recorded, in KBr discs, in a Perkin-Elmer Infracord spectrometer (model 137). The X-ray diffraction pattern of this fraction was obtained in a Debye-Scherrer chamber of 57-3 mm diameter (North American Philips Co.) using Cu Kα radiation, at 40 kV, 20 mA for 5 h. A 22 nm thick platinum carbon replica of this fraction was photographed in a Zeiss 9S-2 electron microscope. Authentic chitin, obtained from lobster according to the method of Foster & Webber (1960), was analysed by the same procedures.

RESULTS AND DISCUSSION

From 1 g of cell wall from A. radiosa, 40 mg of an insoluble fraction was obtained. This fraction produced glucosamine as the main product of acid hydrolysis and N-
Short communication

Wavenumber (cm⁻¹)

4000 3000 2000 1500 1200 1000 900 800

(a)

(b)

Transmittance (%)

3 4 5 6 7 8 9 10 11 12

Wavelength (µm)

Fig. 1. Infrared spectra of authentic chitin from lobster (a) and chitin from A. radiosa (b).

acetylglucosamine as the main product of hydrolysis with chitinase: hydrolysis with 6 M-HCl for 10 h yielded 36.9% glucosamine from the insoluble A. radiosa fraction, as against 50.1% from authentic chitin, while hydrolysis with chitinase for 96 h yielded 30.6% N-acetylglucosamine, as against 34.9% from authentic chitin. No N-acetylglucosamine was obtained from the hydrolysis of the other fractions with the chitinase preparation, although the alkali-soluble fraction yielded glucosamine on acid hydrolysis.

The infrared spectrum of the insoluble fraction (Fig. 1b) showed peaks which coincided with those shown by the authentic chitin (Fig. 1a) and chitin from other sources including
fungi (Marchessault et al., 1960; Mitchell & Scurfield, 1970). The X-ray diffraction pattern of the insoluble fraction from A. radiosa was identical with that of authentic chitin (Fig. 2). The variations in band spacing of the A. radiosa component compared with authentic chitin are within the range of variation found among different samples of chitin (Kreger, 1954; Kreger & Kopecká, 1976; Aronson & Preston, 1960; Aronson & Lin, 1978).

The component extracted from A. radiosa appeared as thin fibrous fragments under the optical microscope and no organelles were observed in the preparation. Under the electron microscope the material showed the characteristic random microfibrillar structure of pure chitin obtained from other fungi (Wang & Bartnicki-Garcia, 1970; Muzzarelli, 1977).

From these results, we conclude that chitin is, indeed, present in the wall of the Oomycete Achlya radiosa and corresponds to about 4% of the total wall.

The presence of chitin in Oomycetes has been unequivocally established in Apodachlya sp. (Lin & Aronson, 1970) and in Leptomitus lacteus (Aronson & Lin, 1978), both belonging to the family Leptomitaceae. It was suggested (Lin et al., 1976; Aronson & Lin, 1978) that chitin-cellulose walls might be characteristic of species in the family Leptomitaceae and possibly a useful addition to conventional taxonomic criteria. Also, the possible taxonomic significance of differences in relative amounts of hexosamine in species from different orders of Oomycetes has been stressed (Dietrich, 1975; Vaziri-Tehrani & Dick, 1980). The present finding of chitin in a species of Oomycete in the family Saprolegniaceae (order Saprolegniales) may also prove to be useful in taxonomy as well as an aid in understanding the phylogenetic relationships within Oomycetes.

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