Observations of Wall-less Protoplasm in White- and Brown-Rot Fungi

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Contents

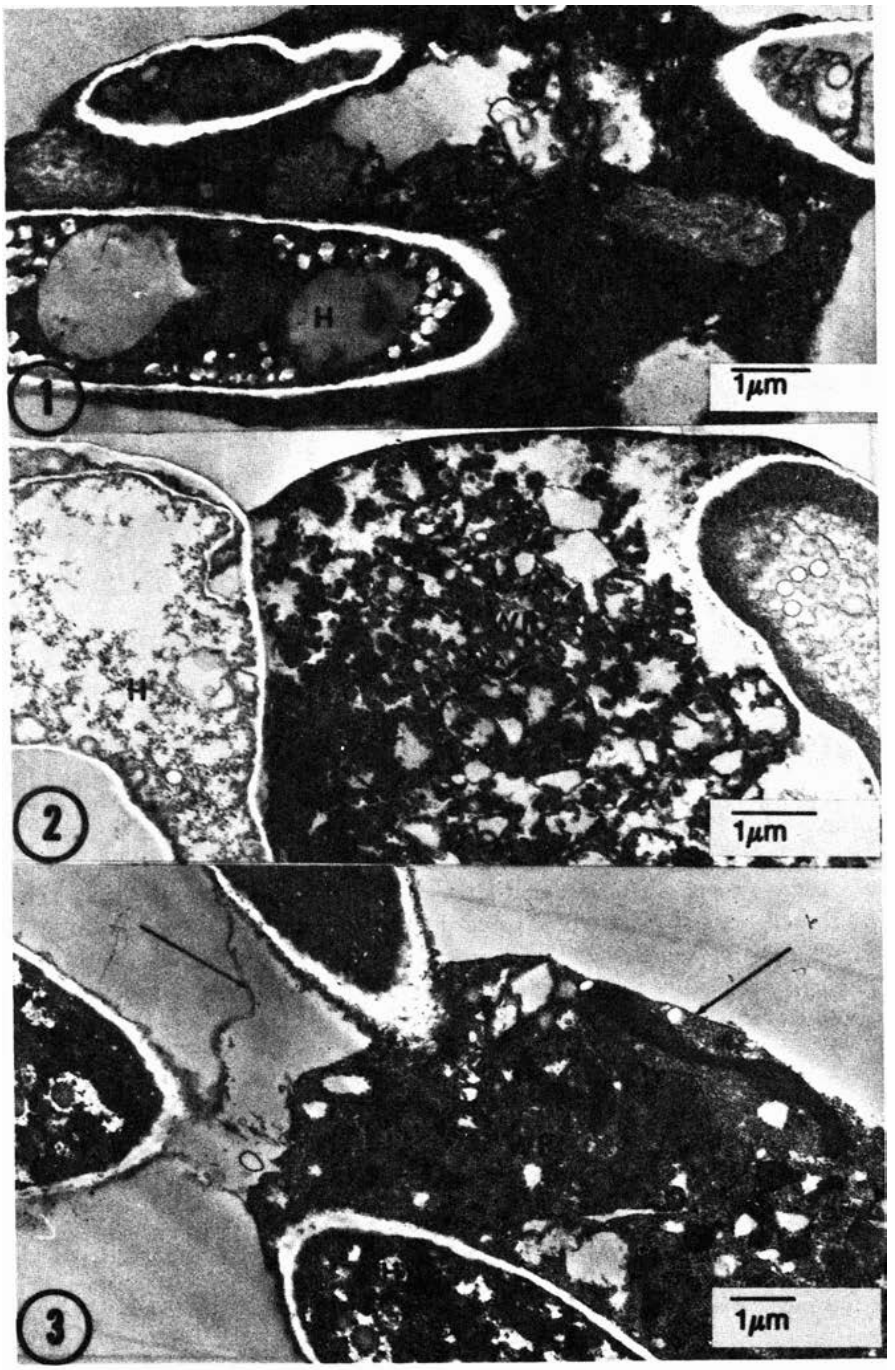
1. Introduction

While studying the occurrence of hyphal sheaths in several brown- and white-rot Hymenomycetes on a variety of substrates (T. L. HIGHLEY, L. MURMANIS, and J. G. PALMER, 1983, 1984; L. MURMANIS, T. L. HIGHLEY, and J. G. PALMER, 1984; J. G. PALMER, L. MURMANIS, T. L. HIGHLEY, 1983a),b), we encountered a phenomenon that we have not seen reported in the literature, i.e. wall-less hyphal protoplasmic material in which hyphae with intact walls were embedded. The purpose of this report is to document the phenomenon, to present some information about its origin, and to speculate about its function in the process of wood decay.

2. Materials and Methods

Isolates of two white-rot fungi, *Coriolus versicolor* (L. ex Fr.) Quél., MAD-697, and *Ganoderma applanatum* (Pers. ex S. F. Gray) Pat., MAD-708, and three brown-rot fungi, *Coniophora puteana* (Schum. ex. Fr.) Karst., MAD-515; *Laetiporus sulphureus* (Bull. ex Fr.) Bond. et Sing., Sh-27-R; and Poria placenta (Fr.)Cke., MAD-698, were obtained from the Center for Forest Mycology Research at the Forest Products Laboratory. Mycelia were cultured on 2% malt agar in glass Petri plates. Sterile

* Maintained at Madison, Wis., in cooperation with the University of Wisconsin.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cellophane disks were placed on some agar surfaces, and inoculum was placed near a disk or partially on the edge of the disk. Cultures containing cotton (Hercules fibers, Type A-600) or wood (sawdust of Western Hemlock, Tsuga heterophylla (Raf.) Sarg.) were prepared, inoculated, and grown as previously described (T. L. HIGHLEY, J. G. PALMER, and L. MURMANIS, 1983; L. MURMANIS, T. L. HIGHLEY, and J. G. PALMER, 1984). For light microscopy, samples were fixed in formaldehyde-glutaraldehyde mixture (M. J. KARNOVSKY, 1965), washed in 50 % ethanol and distilled water, stained in aqueous 0.125% Cotton Blue 4 CB, acidified with lactic acid, washed with distilled water, and mounted in a 1 : 1 (v/v) solution of distilled water and glycerine. For transmission electron microscopy, samples were prepared and examined as previously described (T. L. HIGHLEY, J. G. PALMER, and L. MURMANIS, 1983; L. MURMANIS, T. L. HIGHLEY, and J. G. PALMER, 1984).

3. Results and Discussion

Wall-less protoplasm containing embedded hyphae with intact walls was observed in both brown- and white-rot fungi grown on four substrates: agar (Figs. 1 and 2), cellophane (Fig. 3), wood (Figs. 4 and 5), and cotton (Fig. 6). It was found at different developmental stages ranging from very young (Figs. 3 and 4) to senescent-appearing (Figs. 1, 6, and 7). When young, the wall-less protoplasm contained all cell organelles and often appeared to be surrounded by a membrane (arrows) that stretched over embedded hyphae (Figs. 3 und 4). Such coverage suggests that the membrane could not be a plasmalemma but might be a condensed outer border of hyphal sheath. This observation was supported by the occasional occurrence of an entire complex of wall-less protoplasm and embedded hyphae surrounded by hyphal sheath (Fig. 6). However, a sheath was not always visible, and the wall-less protoplasm seemed to be restrained by the condensed outer edge of the protoplasm itself (Fig. 7).

Wall-less protoplasm with included walled hyphae often surrounded wood and cotton fibers and appeared to be associated with degradation of wood (Figs. 8 and 9) and cellulose (Fig. 10). This suggests that the wall-less protoplasm might contain cellulose and lignin-degrading enzymes. The function of wall-less protoplasm with embedded hyphae was less obvious when it was not in contact with either wood or cotton. Could wall-less protoplasm serve as a source of nutrients for embedded hyphae? In previous work we frequently

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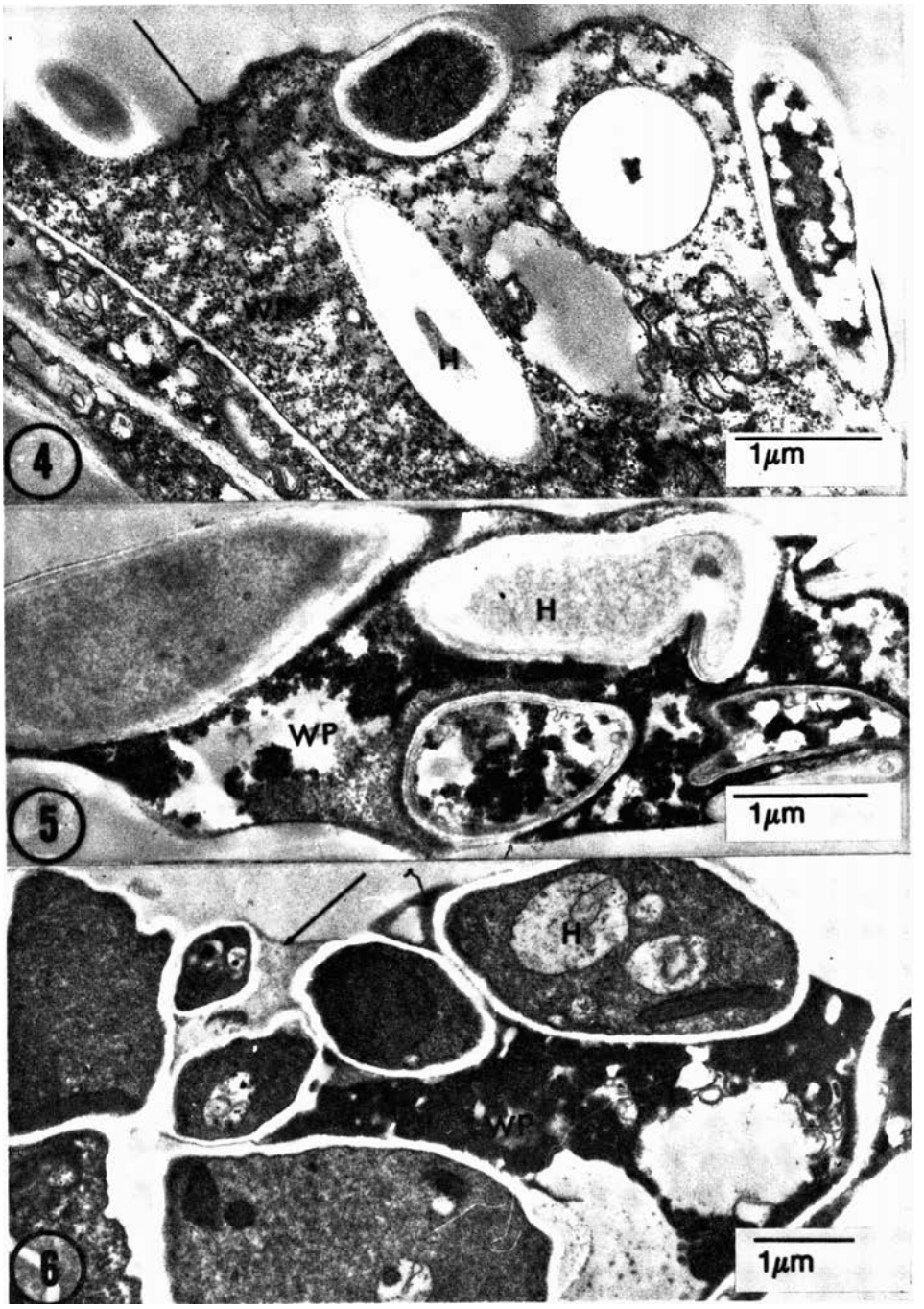
Fig. 1. *Coriolus versicolor*. Wall-less hyphal protoplasm (WP) with embedded walled hyphae (H). 10,000 ×.

Fig. 2. *Coniophora puteana*. Wall-less hyphal protoplasm (WP) with embedded walled hyphae (H). 11,070 ×.

Fig. 3. *Laetiporus sulphureus*. Wall-less, young hyphal protoplasm (WP) with embedded walled hyphae contains all cell organelles. A membrane (arrows) surrounds the wall-less protoplasm and embedded hyphae. 8,200 ×.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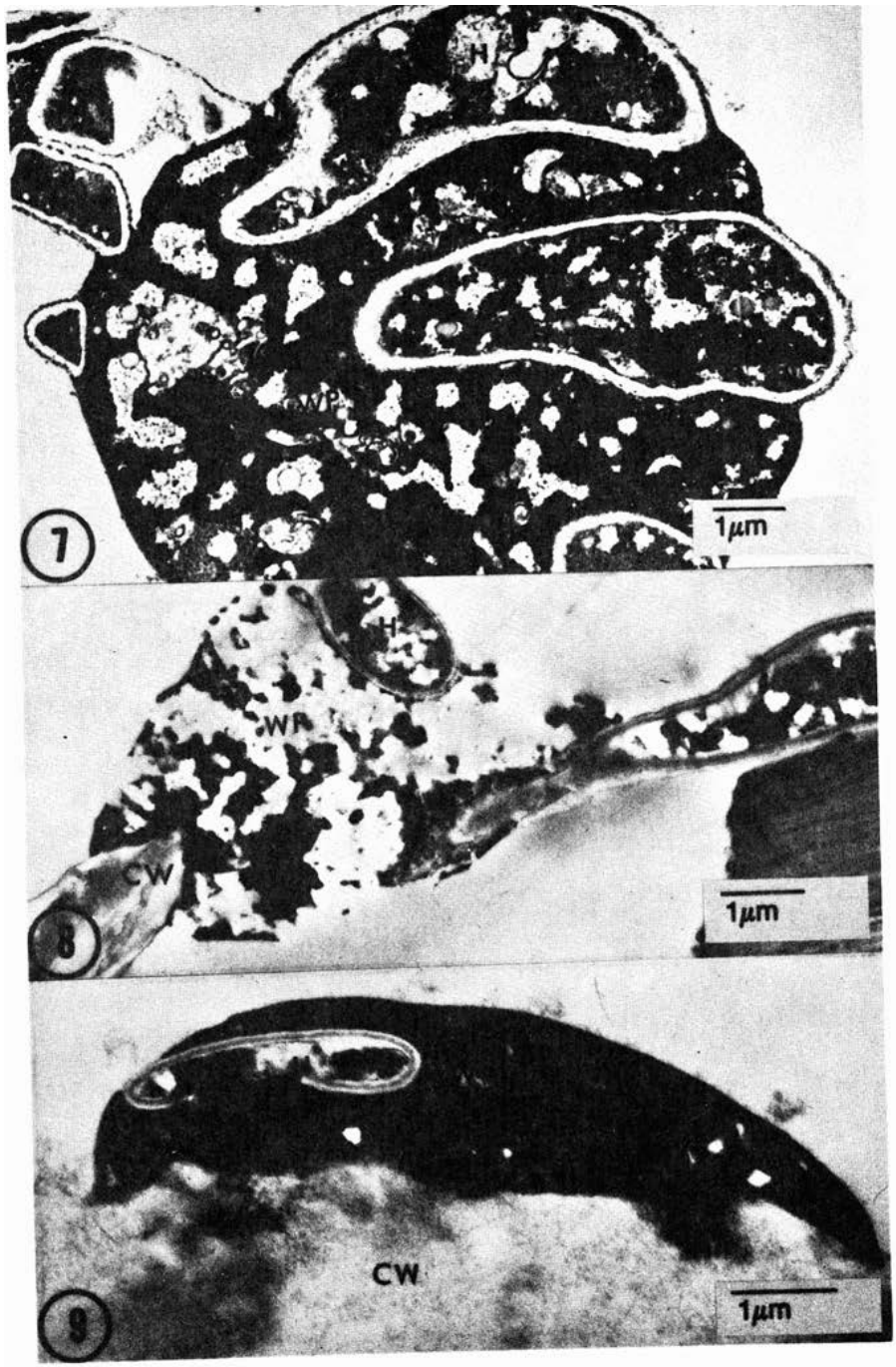
observed autolysis of hyphae in brown- and white-rot fungi and presumed that the released protoplasmic material might serve for nutrition of adjacent hyphae (J. G. PALMER, L. MURMANIS, and T. L. HIGHLEY, 1983 a, b). Autolysis appeared to take place within the hyphal sheath, and the released protoplasm was always in a diffuse state in contrast to the compact wall-less protoplasm. That wall-less protoplasm did not originate from protoplasmic contents released during autolysis is demonstrated in Fig. 11. In this instance the hyphae undergoing autolysis released its contents into wall-less protoplasm which probably had surrounded the hypha and been in existence before autolysis began.

Unfortunately we were unable to obtain any definite clues that pointed toward the origin of protoplasm lacking cell wall or toward inclusion of embedded hyphae by this protoplasm. Using techniques available to date, light microscopy only confirmed the observations obtained by transmission electron microscopy. Consequently we were restricted to speculation regarding the origin and function of the wall-less protoplasm. Some hyphae might never develop walls in the first place. We observed some young hyphae without walls while all other hyphae around them possessed walls. We also observed that some hyphae appeared to lose their walls by dissolution (Fig. 12), and adjacent hyphae with walls appeared to invade them. Dissolution of the hyphal walls could be a response induced by internal or external nutrient imbalances. Perhaps both - no formation of walls and dissolution of walls - are in fact the ways in which the wall-less protoplasm arises. Momentary or local conditions of the culture and the nutritional needs of the hyphae may dictate the pathway by which the wall-less protoplasm is formed. More work would be required to elucidate this phenomenon and to substantiate these speculations.

Fig. 4. *Coriolus versicolor*. Wall-less, young hyphal protoplasm (WP) with embedded walled hyphae (H). A membrane (arrow) surrounds the wall-less, young hyphal protoplasm. 18,700 ×.

Fig. 5. *Ganoderma applanatum*. Wall-less hyphal protoplasm (WP) with embedded walled hyphae (H). 16,800 ×.

Fig. 6. *Coniophora puteana*. Wall-less hyphal protoplasm (WP) with embedded walled hyphae (H) are within hyphal sheath (arrow). 12,300 ×.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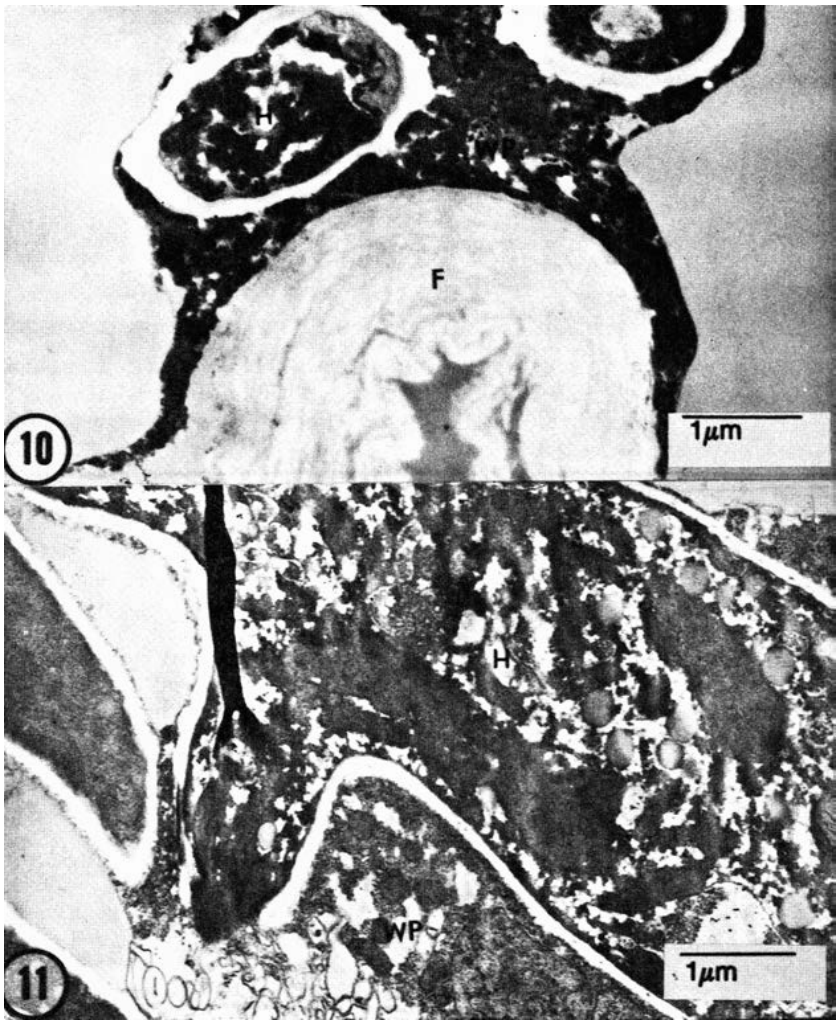

Fig. 7. *Laetiporus sulphureus.* Wall-less hyphal protoplasm (WP) with embedded walled hyphae (H). 9,240 ×.

Fig. 8. *Ganoderma applanatum.* Wall-less hyphal protoplasm (WP) with embedded walled hyphae (H) surrounds the wood cell wall (CW). 11,070 ×.

Fig. 9. *Ganoderma applanatum.* Wall-less hyphal protoplasm (WP) with embedded walled hyphae (H) lies within a degraded wood cell wall (CW). 13,530 ×.

Fig. 10. *Poria placenta.* Wall-less hyphal protoplasm (WP) with embedded hyphae (H) surrounds a cotton fiber (F). 16,800 ×.

Fig. 11. *Laetiporus sulphureus.* Hypha (H), with the wall releases cell contents into a wall-less protoplasm (WP). 15,600 ×.

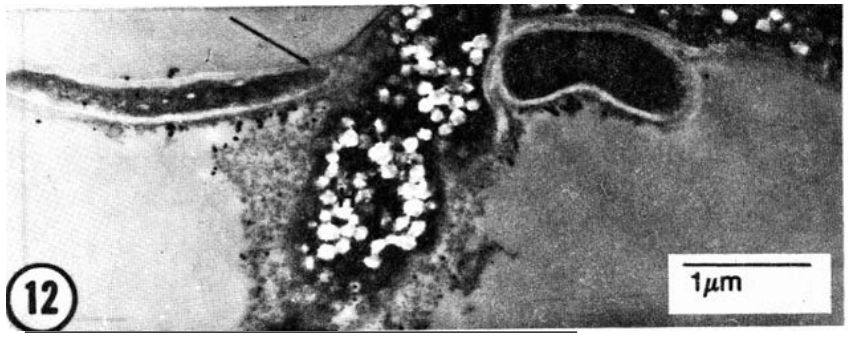

Fig. 12. *Coriolus versicolor*. In hypha (H) at left, the hyphal wall is dissolving; hypha at top (arrow) is entering the wall-losing hypha. 17,000 ×.

4. Summary

Wall-less hyphal protoplasm in which hyphae with intact cell walls were embedded was found in isolates of two white- and three brown-rot fungi grown on agar, cellophane, cotton, and wood. The contents of wall-less protoplasm ranged from young- to senescent-appearing and was compact and organized in contrast to the diffuse protoplasm released during hyphal autolysis. Sometimes a membrane-like structure surrounded the wall-less protoplasm and its embedded hyphae, and we assumed it to be a condensed outer border of the hyphal sheath. Wall-less protoplasm also surrounded wood and cotton fibers suggesting involvement in degradation of wood and cellulose. When not in contact with a substrate, we hypothesize that such wall-less protoplasm might serve as a source of nutrients for embedded hyphae with intact cell walls. Origination probably occurs either by hyphal development without cell walls or by dissolution of walls around some hyphae.

Zusammenfassung

Beobachtungen an zellwandfreiem Protoplasma in Weiß- und Braunfäulepilzen

In Kulturen von 2 Weiß- und 3 Braunfäulepilzen, die auf Agar, Zellophan, Baumwolle und Holz angezüchtet waren, wurde zellwandfreies Hyphenprotoplasma gefunden, in dem Hyphen mit intakten Zellwänden eingebettet waren. Der Inhalt des zellwandfreien Protoplasmas reichte - dem Aussehen nach - von jung bis alt, es war kompakt und geordnet im Gegensatz zu dem diffusen Protoplasma, das während der Autolyse der Hyphen entsteht. Manchmal umgab eine membranartige Struktur das zellwandfreie Protoplasma und die eingeschlossenen Hyphen; wir nehmen an, daß es sich um eine verdichtete äußere Oberfläche der Hyphenhülle handelt. Zellwandfreies Protoplasma umgab auch Holz- und Baumwollfasern, was offensichtlich mit dem Abbau des Holzes und der Zellulose in Zusammenhang stand. Wenn solches zellwandfreies Protoplasma nicht in Berührung mit einem Substrat steht, könnte es - so nehmen wir an - als Nährstoffquelle für darin befindliche Hyphen mit intakten Zellwanden dienen. Die Entstehung erfolgt wahrscheinlich durch eine Bildung von Hyphen ohne Zellwände oder durch eine Auflösung der Wände einiger Hyphen.

Résumé

Observations sur le protoplasme sans paroi chez les champignons de pourritures blanche et brune

Le protoplasme sans paroi dans lequel ont été enrobés les hyphes à parois cellulaires intactes a été trouvé dans des souches isolées de deux champignons de pourriture blanche et de trois champignons de pourriture brune sur agar, cellophane, coton et bois. Le contenu du protoplasme sans paroi variait de jeune à senescent, etait compact et organisé, contrastant avec le protoplasme diffus résultant de l'autolyse des hyphes. Parfois, une structure semblable à une membrane entourait le protoplasme sans paroi et ses hyphes enrobés et nous admettons que cette structure était le bord extérieur condensé de la gaine des hyphes. Le protoplasme sans paroi entourait aussi les fibres de bois et de coton suggérant son application dans la degradation du bois et de la cellulose. Lorsqu'il n'y a pas contact avec un substrat, nous supposons que ce protoplasme sans paroi peut servir de source nutritive pour les hyphes enrobes à parois cellulaires intactes. L'origine provient probablement soit du développement d'hyphes sans parois cellulaires, soit de la dissolution des parois de quelques hyphes.

Resumen

Observaciones del protoplasma exento de pared celular en hongos de la pudrición blanca y parda

En cultivos de dos hongos de la pudrición blanca y tres de la pudrición parda sobre agar, celofán, algodón y madera se encontró protoplasma de hifa exento de pared celular, en cuyo interior se hallaban introducidas hifas con paredes celulares intactas. El contenido del protoplasma exento de pared celular variaba, según su aspecto, entre joven y senescente, era compacto y organizado, en contraste con el protoplasma difuso producido durante la autolisis de las hifas. A veces, una estructura tipo membrana rodeaba al protoplasma exento de pared celular y a las hifas en él encerradas, estructura que suponemos se trata de una superficie exterior condensada de la envoltura de hifas. Protoplasma exento de pared celular rodeó también fibras de madera y de algodón, lo que aparentemente estuvo relacionado con la degradación de la madera y de la celulosa. Cuando tal protoplasma exento de pared celular no se halla en contacto con un substrato, partimos de la hipótesis de que podría servir de fuente de nutrición para las hifas con paredes celulares intactas que en el se encuentran. La formación se produce, probablemente, por la creación de hifas sin pared celular o por la descomposición de las paredes de algunas de ellas.

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