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Common mechanism of recognition and binding of the complementary molecules, carbohydrate-lectin, in the verticillium disease of *Agaricus bisporus* and *Pleurotus ostreatus* cultivated mushrooms

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ABSTRACT

The carbohydrate-lectin interaction between the isolated glucogalactomannan of *Verticillium fungicola* cell walls, and lectins, either from *Agaricus bisporus* or *Pleurotus ostreatus* fruit bodies, was compared in order to establish the molecular mechanism of the «dry bubble» or verticillium disease exhibited in the commercial cultures of both edible mushrooms. This interaction between complementary molecules, «target molecules», appears to be due to the terminal galactose linked at (1-4) to the (1-6) mannose bone of the *V. fungicola* glucogalactomannan molecule.

Key words: «Dry bubble».—Verticillium disease.—*Verticillium fungicola* glucogalactomannan.—*Agaricus bisporus* lectin.—*Pleurotus ostreatus* lectin.

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RESUMEN

Mecanismo común de reconocimiento y unión de las moléculas complementarias, carbohidrato-lectina, en la verticiliosis de las setas cultivadas *Agaricus bisporus* y *Pleurotus ostreatus*

Se compara la interacción carbohidrato-lectina entre el glucogalactomanano aislado de las paredes celulares de *Verticillium fungicola* y las lectinas de los carpóforos de *Agaricus bisporus* o de *Pleurotus ostreatus,* para establecer el mecanismo molecular de la «mole seca» o verticiliosis de los cultivos comerciales de ambas setas comestibles. La interacción entre las moléculas complementarias, «moléculas diana», parece ser debida a la galactosa terminal unida en (1-4) al esqueleto de manosa unido en (1-6) de la molécula del glucogalactomanano de *V. fungicola*.

Palabras clave: «Mole seca».—Verticiliosis.—Glucogalactomanano de *Verticillium fungicola*.—Lectina de *Agaricus bisporus*.—Lectina de *Pleurotus ostreatus*.

INTRODUCTION

Verticillium fungicola (Preuss) Hassebrauk var. *fungicola* is a mycoparasite that causes dry bubbles, stipe blow-out and necrotic lesions («dry bubble» or verticillium disease) in the commercial cultures of white button mushroom *Agaricus bisporus* (Lange) Imbach (1, 2), the losses in the yield having been estimated at several hundred million dollars. Control of the disease has been partially achieved by the use of fungicides as well as cultural and sanitary practices, but resistance to the most effective chemical (prochloraz) is increasing (3). Therefore, to develop alternative strategies for its control, it has been necessary to elucidate the nature of the interaction between the mycoparasite and its host. Recently studies carried out in this laboratory have demonstrated by different approaches: i) agglutination of *V. fungicola* germinated spores by an *A. bisporus* extract from fruit body cell walls; ii) inmunofluorescence microscopy of *A. bisporus* hyphae from fruit bodies and vegetative mycelia pretreated with purified *V. fungicola* cell wall glucogalactomannan (4), and iii) by hemagglutination experiments carried out with an *A. bisporus* fruit body glycoprotein lectin in the presence and absence of the same *V. fungicola* glucogalactomannan, that a specific recognition step is involved in this mycosis (5) before the development of the external symptoms of the disease. This mechanism of specific

recognition between complementary molecules, lectin-carbohydrate, which are present on both the host and the mycoparasite, is similar to those described in many other parasitic and symbiotic fungi (6-9).

On the other hand, *P. ostreatus* (Jacq.: Fries) Kumm fruit bodies (oyster mushrooms) have also been described presenting the «dry bubble» disease in their commercial cultures (10). For this reason, parallel studies were carried out on in order to purify and characterize another similar lectin in this fungus (11, 12). So the aim of the present work is to confirm that the same mechanism of recognition and binding of carbohydrate-lectin is the previous step to the mycosis symptoms development in both mushrooms, and to establish a common molecular basis between the complementary molecules, glucogalactomannan-lectin, in this verticillium disease.

MATERIALS AND METHODS

Organisms and culture conditions

Fruit bodies of *Agaricus bisporus* (commercial strain Fungisem H 25) and *Pleurotus ostreatus* (commercial strain Amycel 3000) were grown in the CIES (Centro de Investigación, Experimentación y Servicios del Champiñón, Quintanar del Rey, Cuenca, Spain). *Verticillium fungicola* var. *fungicola* (CBS 992.69) was grown on Raper medium (13).

Preparation of *V. fungicola* cell wall glucogalactomannan

The preparation of *V. fungicola* cell walls and the fractionation and purification procedure for obtaining the glucogalactomannan were performed as reported in earlier studies (14).

Purification, characterization and carbohydrate specificity of *A. bisporus* and *P. ostreatus* lectins

The purification of *A. bisporus* and *P. ostreatus* lectins from their corresponding mushrooms were carried out by ammonium sulfate

precipitation and ion-exchange chromatography and their respective characterizations performed as previously described (5, 12). Hemagglutination inhibition procedure was used for comparative carbohydrate specificity determination of both lectins (5).

RESULTS AND DISCUSSION

A. bisporus and *P. ostreatus* lectins purified and characterized as previously reported (5, 12) have shown that both molecules are structurally distinct glycoproteins, the former constituting a tetramer and the latter being dimeric in nature, accounting their respective monomer molecular masses for 16014 m/z and 44270 m/z. Comparison of the sugar binding specificities of the two lectins studied by hemagglutination inhibition assays is exhibited in Table I. Most of the neutral sugars tested had no effect, except for the galactose at different concentration in each case. N-acetylgalactosamine also showed a distinct degree of the hemagglutination inhibition, being more significant with the *P. ostreatus* lectin. However purified *V. fungicola* glucogalactomannan behaved rather similarly against both lectins, and was even a little more effective when the same glucogalactomannan was isolated from cell walls of phrocloraz-Mn pretreated *V. fungicola* mycelium. This effect can be explained by the increase of the terminal galactose residues of the molecule caused by the fungicide (14).

The carbohydrate-protein interaction appears to be due to the terminal galactose linked at (1-4) to the (1-6) mannose bone of the *V. fungicola* glucogalactomannan molecule (5) in which, the importance of the similar position of the axial C-3 and C-4 hydroxyl groups of the monosaccharide linked to the galactose molecule was previously demonstrated by conformational analysis (15). The fact that this glucogalactomannan behaves as a specific complementary molecule for both lectins could explain the same mechanism of recognition and binding of such specific molecules —«target molecules»— in this disease, as well as the absence of verticillium disease on the *A. bisporus* vegetative mycelial phase due to the lack of lectin (5).

Table I. *Comparative inhibition of hemagglutinating activity of A. bisporus and P. ostreatus lectins by specific carbohydrates*

		Carbohydrate concentration (mmol/L)								
	Carbohydrates	1,56	3,12	6,25	12,5	25	50	100	200	PBS
A. bisporus lectin	Galactose	+	+	+	+	+	+	+	±	+
«	N-acetyl galactosamine	+	+	+	+	+	–	–	–	+
«	Glucogalactomannan*	+	+	–	–	–	–	–	–	+
«	Glucogalactomannan+F**	+	–	–	–	–	–	–	–	+
P. ostreatus lectin	Galactose	+	+	+	+	–	–	–	–	+
«	N-acetyl galactosamine	+	–	–	–	–	–	–	–	+
«	Glucogalactomannan*	+	+	+	–	–	–	–	–	+
«	Glucogalactomannan+F**	+	+	–	–	–	–	–	–	+

*glucogalactomannan of V. fungicola; **glucogalactomannan of V. fungicola treated with the fungicide prochloraz-Mn; +, hemagglutination positive; –, hemagglutination negative; ±, 200 mmol·L^{-1}.

All these results suggest possible alternative strategies to overcome the verticillium disease without the routinary use of fungicides as could be, the production of *A. bisporus* and *P. ostreatus* genetically lectin transformants, or the use of analogous molecules to the *V. fungicola* glucogalactomannan ligand which can be recognized by the carbohydrate-binding site(s) of each lectin.

This report may be the first establishing a close relationship between the verticillium disease in *A. bisporus* and *P. ostreatus* fruit bodies, demonstrating that the glucogalactomannan of *V. fungicola* cell walls and a lectin from each mushroom behave as the «target molecules» initiators of the subsequent molecular signaling which, successively, will produce the respective «dry bubble» disease with the necrosis of the fruit bodies. The present studies suggest that the same glucogalactomannan-lectin interaction may occur in the *V. fungicola* mycoparasitism described in the «hot mushroom» *Agaricus bitorquis* also commercially grown (16) and, although up to now it has not been described the presence of any lectin in these fruit bodies, it presumably may exist.

Moreover, this article also suggests that other important verticillium wilt diseases described in several crop plants (olive, cotton etc.) could exhibit a rather similar mechanism of recognition and binding between determined cell wall polysaccharides from *V. albo-atrum*, *V. dahliae*, etc. (17) and their specific complementary plant lectins, as the necessary step for the development of their corresponding mycosis processes. Further studies are required to prove whether this is a common phenomenon in the strategy of *Verticillium* infections or if it occurred only in the cases previously reported.

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