

Verticillium fungicola Cell Wall Glucogactomannan-binding of the Lectin from the *Pleurotus* *ostreatus* Fruit bodies

D. Bernardo; A. Pérez Cabo; C. García Mendoza

*Centro de Investigaciones Biológicas, CSIC,
Ramiro de Maeztu 9, 28040 Madrid (Spain)*

The *Verticillium fungicola* mycoparasitism on *Agaricus bisporus* fruit bodies appears to be a complex process made up of successive steps in which the recognition and binding between complementary molecules, the *A. bisporus* fruit body lectin and the *V. fungicola* cell wall glucogalactomannan, have recently been demonstrated. *P. ostreatus* fruit bodies have been described as containing a lectin and also presenting the “dry bubble” or the Verticillium disease. The aim of the present work is to purify and characterize the *P. ostreatus* lectin and compare the properties of both lectins in an attempt to confirm if the specific glucogalactomannan-lectin recognition and binding is the necessary step for the *V. fungicola* mycoparasitism process in *P. ostreatus*.

The characteristics and properties of the purified *P. ostreatus* lectin together with those also previously described by us on *A. bisporus* lectin show that, although both lectins present different chemical structures, they behave very similarly in relation to their glucogalactomannan-binding, thus confirming the existence of the specific recognition and binding step in the Verticillium disease on *P. ostreatus* fruit bodies.

1. Introduction

“Dry bubble” or Verticillium disease, the most serious fungal disease of the commercially grown strains of the white mushroom *Agaricus bisporus*, is

caused by *Verticillium fungicola*. The losses in yield of *A. bisporus* fruit bodies in Europe produced by *V. fungicola* are estimated at millions of euros annually. This mycopathogen infects not only *A. bisporus* but also other cultivated mushrooms such as *Pleurotus ostreatus* (Marlowe and Romaine, 1982). The only fungicide that is now used to control the disease, prochloraz, will probably be banned in the near future from commercial mushroom growing because *V. fungicola* has developed a resistance towards it (Gea et al. 1996). So, to elucidate the interaction between the mycopathogen and its host it will be necessary to know the molecular mechanisms of the infection.

Bernardo et al. (2004) described that an *A. bisporus* fruit body purified lectin recognized and binded the isolated glucogalactomannan from cell walls of *V. fungicola*, suggesting the specific interaction between both organisms, prior to the secretion of *V. fungicola* extracellular hydrolytic enzymes conducive to the development of the disease and the further *A. bisporus* fruit bodies necrosis.

This paper describes the characteristics and properties of a *P. ostreatus* fruit body lectin comparing them with those of *A. bisporus* fruit body lectin, in an attempt to confirm that the same molecular mechanisms of the infection occur in both mushrooms.

2. Materials and Methods

2.1. *Organisms and culture conditions*

Pleurotus ostreatus fruit bodies (commercial strain Amycel 3000) were grown in the CIES (Centro de Investigación, Experimentación y Servicios del Champiñón, Quintanar del Rey, Cuenca, Spain).

2.2. *Purification and characterization of P. ostreatus lectin*

Purification of the lectin was carried out by ammonium sulfate precipitation and ion-exchange chromatography as described previously (Bernardo et al. 2004). All procedures for characterization of the lectin (SDS-PAGE, MALDI-TOF mass spectrometry, chemical analysis and hemagglutination assays) have also been described before (Bernardo et al. 2004).

3. Results

The purification of the *P. ostreatus* lectin carried out following ammonium sulfate precipitation and ion-exchange chromatography is shown in Table 1. Preliminary experiments with ammonium sulfate fractionation showed that the hemagglutinating activity was distributed mainly in the 30%-100% saturated fraction. In the first anion-exchange chromatography all the coloured materials were absorbed by the column and the protein was eluted with the NaCl continuous gradient. In the cation-exchange chromatography the protein with the hemagglutinating activity was bound to the column, and it was eluted by means of the corresponding NaCl continuous gradient. The hemagglutinating activity evaluated at each step of purification is also shown in Table 1.

SDS-PAGE analysis of the purified lectin showed that the single band obtained was a pure protein, of an apparent molecular mass of 40 ± 4 kDa (Fig. 1). On the basis of gel filtration the native molecular mass obtained was around 80 kDa. The molecular weight of the lectin was confirmed by MALDI-TOF mass spectrometry, obtaining a peak of 44270 m/z (Fig 2). The sugar composition analysis showed that this protein contained a 8.15% of carbohydrate content, so it was concluded that this lectin is a dimeric glycoprotein.

The sugar binding specificity of the lectin examined by hemagglutination inhibition assay is shown in Table 2. Some neutral sugars had no antagonizing activity against hemagglutination, however lactose and galactose showed some effect (50 and 25 mmol L⁻¹ respectively), and N-acetylgalactosamine and glucogalactomannan from *V. fungicola* cell walls treated and not treated with the fungicide prochloraz (Bernardo et al. 2002) behaved as the best inhibitors (3.12, 6.25 and 12.5 mmol L⁻¹ respectively).

4. Discussion

The chemical characteristics of the *P. ostreatus* lectin purified through this work are in good agreement with those described by Kawagishi et al. (2000) in a different strain of *P. ostreatus*. The specificity of this protein towards sugars has been established, and the results show that it deals with a galactose-binding lectin.

In a previous work, Bernardo et al. (2004) showed that the *A. bisporus* fruit body lectin recognized and binded the glucogalactomannan from *V. fungicola* cell walls, suggesting that this specific interaction was essential for the further secretion of *V. fungicola* hydrolytic enzymes and the development of the Verticillium disease on *A. bisporus* fruit bodies. In this report, we present evidence that the *P. ostreatus* fruit body lectin, although showing different chemical structure, behaves very similarly to the *A. bisporus* lectin in relation to its carbohydrate-binding specificity, and particularly towards the *V. fungicola* glucogalactomannan, thus indicating the same molecular mechanism for the *V. fungicola* mycoparasitism process in both *A. bisporus* and *P. ostreatus*.

The strongest inhibition effect shown by the glucogalactomannan isolated from cell walls of prochloraz pretreated *V. fungicola* mycelium can be explained by the increase of the terminal galactose residues of the molecule caused by the fungicide (Bernardo et al. 2002).

Further investigations may be needed to establish if the function related to the Verticillium disease is a general role of these lectins.

5. Acknowledgements

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6. References

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Table 1
Purification of the *Pleurotus ostreatus* fruit body lectin

Fraction	Protein (mg)	Hemagglutinating activity		Recovery (%)
		Total (units)	Specific (units/mg protein)	
(NH ₄) ₂ SO ₄	335	12000	35.8	100.0
Anion-exchange	95.6	8256	86.4	68.8
Cation-exchange	12.3	4480	364.2	37.3

Table 2
Inhibition of hemagglutinating activity of *Pleurotus ostreatus* lectin by several carbohydrates

Carbohydrates	Carbohydrate concentration (mmol/L)									
	0.78	1.56	3.12	6.25	12.5	25	50	100	200	PBS
Glucose	+	+	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	-	-	-	+
Galactose	+	+	+	+	+	-	-	-	-	+
Arabinose	+	+	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+	+	+
Rhamnose	+	+	+	+	+	+	+	+	+	+
N-acetyl-D-glucosamine	+	+	+	+	+	+	+	+	+	+
N-acetyl-D-galactosamine	+	+	-	-	-	-	-	-	-	+
Glucogalactomannan*	+	+	+	+	-	-	-	-	-	+
Glucogalactomannan+F**	+	+	+	-	-	-	-	-	-	+

* glucogalactomannan of *V. fungicola*; ** glucogalactomannan of *V. fungicola* treated with the fungicide Prochloraz-Mn; +, hemagglutination positive; -, hemagglutination negative

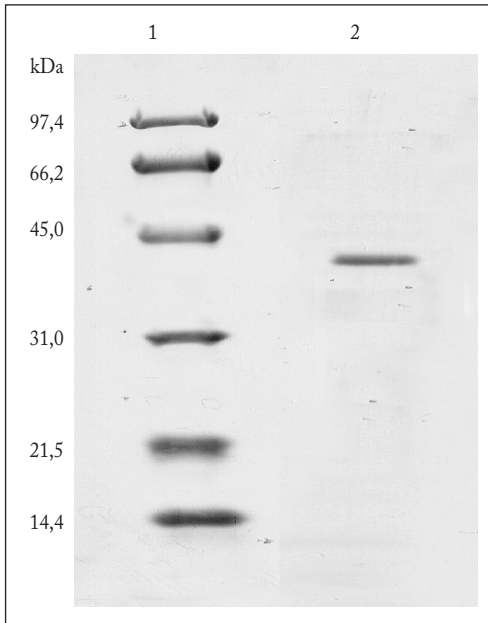


Figure 1. SDS-PAGE of the purified lectin from *Pleurotus ostreatus* fruit bodies: lane 1, molecular weight standards; lane 2, purified lectin.

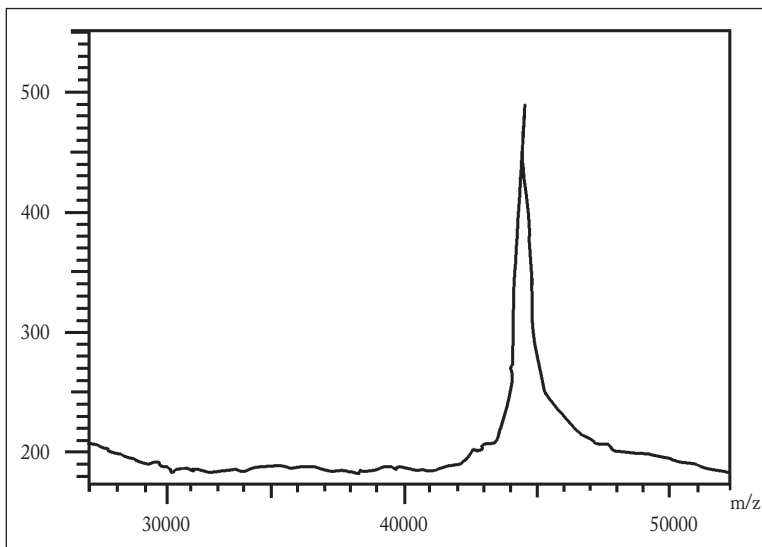


Figure 2. Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) analysis of the purified lectin.