Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 67 (2006) 2189–2196
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Structural characterization of a polysaccharide and a β-glucan isolated from the edible mushroom *Flammulina velutipes*

Fhernanda R. Smiderle, Elaine R. Carbonero, Caroline G. Mellinger, Guilherme L. Sassaki, Philip A.J. Gorin, Marcello Iacomini *

Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná, CP-19046, CEP-81531-990 Curitiba, PR, Brazil

Received 19 February 2006; received in revised form 9 May 2006
Available online 1 August 2006

Abstract

Two polysaccharides were isolated from the basidiomycete *Flammulina velutipes*, via successive hot extraction with water, 2% and 25% aq. KOH, and then submitted to freeze-drying. The precipitate formed by repeated freeze-thawing from the 2% aq. KOH extraction PK2 was analyzed by determination of its monosaccharide composition, as well as by methylation analyses using GC–MS, mono- (^{13}C, ^{1}H NMR) and bidimensional (^{1}H (obs.), ^{13}C HMQC) spectroscopy, and controlled Smith degradations. It was established to be a branched β-glucan, with a main chain of (1 → 3)-linked-Glc*p* residues, substituted at O-6 by single-unit β-Glc*p* side chains. The precipitate formed by repeated freeze-thawing from the 25% KOH extraction PK25 contained Xyl, Man, and Glc and was heterogeneous by HSPEC and extraction with DMSO gave a soluble xylomannan (XM). It was homogeneous with a molar mass 30.8×10^4 g/mol ($dn/dc = 0.186$). Using the above chemical analyses, it was a xylomannan with Man and Xyl in a 3:2 molar ratio. Its main chain consisted of (1 → 3)-linked α-Man*p* units, mainly substituted at O-4 by β-Xyl*p* units or with some β-Xyl*p*-(1 → 3)-β-Xyl*p* groups.

Keywords: *Flammulina velutipes*; Basidiomycete; Branched β-glucan; Xylomannan

1. Introduction

Edible mushrooms have, since ancient times, been consumed with the goals of maintaining health and promotion of longevity (Manzi and Pizzoferrato, 2000). As well as for their medicinal or nutritional properties, they were especially appreciated for their texture and flavor. Nowadays, following nutritional investigations, they are well known as a very rich food supplement, due to their favorable protein, carbohydrate, and dietary fiber contents (Manzi and Pizzoferrato, 2000; Leung et al., 1997; Mallavadhani et al., 2006; Manzi et al., 2004).

The biological importance of these basidiomycetes arises from their chemical components, especially various biologically active polysaccharides. Among these, the β-glucans from different organisms have been the most studied. Edible fungi from the genera *Ganoderma*, *Agaricus* and *Lentinus* have been widely investigated (Wasser, 2002; Kües and Liu, 2000), and have also demonstrated some interesting biological properties, such as immunomodulatory and antitumor activity (Guterrez et al., 2004; Zheng et al., 2005; Peng et al., 2005).

Also studied has been another genus of edible mushrooms, that of *Flammulina*, the main one being *Flammulina velutipes* (Curt. ex Fr.) Sing. This is popularly known by its Japanese name "enokitake" which, however, was first cultivated in China during the 8th century. Its consumption is now worldwide and ranks in fourth place in the production and consumption of edible mushrooms (Leifa et al., 2001).

Studies on *F. velutipes* polysaccharides have demonstrated strong immunomodulatory and antitumoral activities for its glucans and heteropolysaccharides (Yoshioka et al., 1973; Ikekawa et al., 1982; Otagiri et al., 1983). Ikekawa et al. (1982) reported a β-(1 → 3)-glucan and two heteropolysaccharides to have antitumoral activity. This was

* Corresponding author. Tel.: +55 41 33611655; fax: +55 41 32662042.
E-mail address: iacomini@ufpr.br (M. Iacomini).

0031-9422/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2006.06.022

followed by a report by Otagiri et al. (1983), who found an intensification of antitumor-immunity by a protein-bound polysaccharide containing glucose, galactose, mannose, xylose, and arabinose.

Apart from these studies, which focused on biological properties, little is known about the detailed structure of these polysaccharides. A study carried out by Mukumoto and Yamaguchi (1997) revealed the structure of a mannofucogalactan from the fruiting bodies of *F. velutipes*. This polysaccharide was obtained by cold water extraction that was precipitated at a concentration 50% of acetone in water and it consisted of a main chain of (1 → 6)-linked-α-Gal*p* units, every third of which are substituted by 3-*O*-α-D-Man*p*-L-Fuc*p* or L-Fuc*p* residues.

Since there is a lack of information on the detailed structure of the polysaccharides that are present in *F. velutipes*, we now have fractionated extracts of its fruiting bodies and characterized a previously known branched (1 → 3), (1 → 6)-linked β-glucan (Ikekawa et al., 1982) and a poorly characterized xylomannan.

2. Results and discussion

A dry sample of *F. velutipes* was submitted to successive aq. and 2% aq. KOH, and 25% aq. KOH extraction at 100 °C. The fractions obtained from 2% and 25% aq. KOH, named K2 and K25, respectively, were submitted to several freeze-thawing procedures until no more precipitates were formed (Fig. 1).

After centrifugation of the fractions, soluble SK2 (1% yield) and SK25 (0.9% yield) and insoluble PK2 (3.7% yield) and PK25 (2.6% yield) subfractions were isolated (Fig. 1).

PK2 contained mainly glucose (Table 1) consistent with a predominant glucan. ^{13}C NMR and ^{1}H (obs.), ^{13}C HMQC spectra (Fig. 2a and b, respectively) (Table 3) had signals corresponding to all carbons from the polysaccharide: C-1/H-1 at δ 103.1/4.53 corresponding to 3-*O*-substituted units (A) (Fig. 2c, while those at δ 103.1/4.23 are from 3,6-di-*O*-substituted units (B). The β-configuration was shown by H-1 signals at high field and C-1 signals at low field. The resonances at δ 86.7 and 86.3 arise from substitutions at O-3 in units A, while those at δ 86.0 and 76.7 are from similar substitutions in units B and free O-3 from non-reducing end units of β-Glc*p* (C), respectively. Signals at δ 76.4; 76.2 and 74.9 arise from C-5 of units A, A

Table 1
Monosaccharide composition of the fractions obtained from *F. velutipes*

Fractions	Monosaccharides (%)[a]		
	Xyl	Man	Glc
PK2	–	–	100
PK25	18	32	50
XM	40	60	–
SM1	9	91	–
SM2	3	97	–

[a] Alditol acetates obtained on successive hydrolysis, $NaBH_4$ reduction, and acetylation, analyzed by GC–MS.

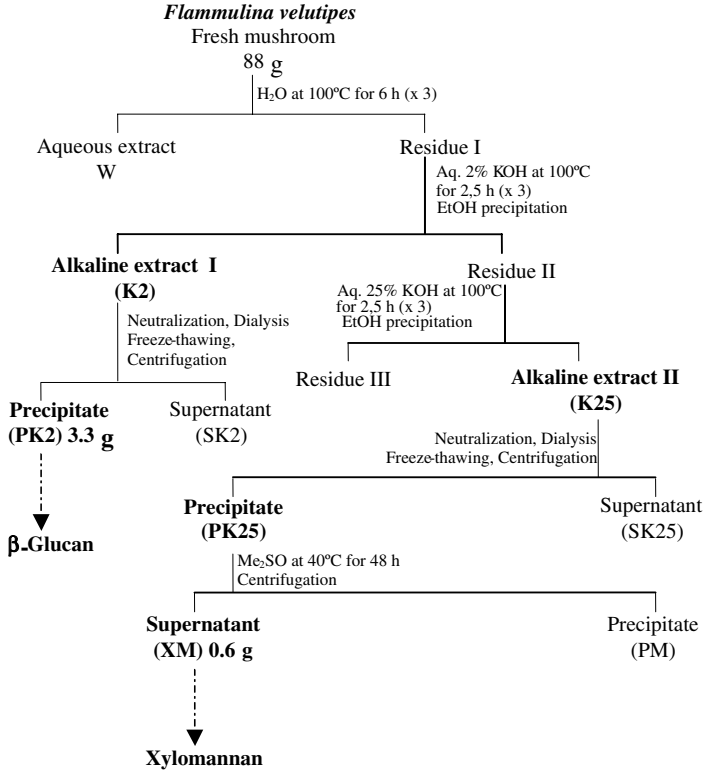

Fig. 1. Extraction and purification of glucan (PK2) and xylomannan (XM).