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Chemical structure of a partially 3-*O*-methylated mannofucogalactan from edible mushroom *Grifola frondosa*

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ABSTRACT

An unusual heteropolysaccharide was isolated from the fruiting bodies of the medicinal mushroom *Grifola frondosa*, via successive cold aqueous extraction, followed by fractionation through freeze-thawing, precipitation with Fehling solution and dialysis using a membrane with a size exclusion cut-off of 500 kDa. Its chemical structure was determined based on total acid hydrolysis, methylation analysis and NMR studies. The mannofucogalactan had a molar mass of 15.9×10^3 g mol^{-1}, which was determinate by HPSEC-MALLS. This heteropolymer showed to have a main chain of (1 → 6)-linked α-D-Gal*p* partially substituted at O-2 by 3-*O*-α-D-mannopyranosyl-α-L-fucopyranosyl groups and in a minor proportion with α-L-Fuc*p* single-unit side chains. Moreover, the presence of 3-*O*-Me-Gal*p* units could also be observed in the main chain of the *G. frondosa* mannofucogalactan.

1. Introduction

Mushrooms have been valued as edible and medicinal resources. *Grifola frondosa* (*Maitake*), a basidiomycete belonging to the Polyporaceae family, may be one of the most versatile and promising medicinal mushroom used as a dietary supplement (Wu et al., 2006). It have been widely used in Japan, China and Korea as a traditional food additive (Gu et al., 2007) and is one of the most valuable and expensive mushrooms (Mayell, 2001).

Since the beginning of its cultivation in 1981, the study of its medicinal applications has been ongoing, and the activity of its purified polysaccharides has been highlighted (Mayell, 2001). Over the past three decades, many polysaccharides have been isolated from the fruiting bodies of *G. frondosa* and showed antitumor activity (Masuda et al., 2009), besides of antihypertensive (Konno, 2007; Talpur et al., 2002) anti-diabetic (Gu et al., 2007), and anti-hyperliposis effects (He et al., 2017; Minamino, Nagasawa, & Othtsuru, 2008).

Most of the polysaccharides from *G. frondosa* fruiting bodies were characterized as D-glucans with different linkage types, such as β-(1 → 3), β-(1 → 6) and α-(1 → 4) (He et al., 2017; Wasser, 2002). Grifolan (GRN) is the best known and most potent substances with antitumor and immunomodulating properties (Borchers, Keen, & Gershwin, 2004) isolated from the cultured fruiting bodies (Ohno et al., 1984), matted mycelia (Ohno et al., 1985) and liquid culture supernatant (Ohno et al., 1986) of *G. frondosa* (Fang et al., 2012). Grifolans are characterized as β-D-glucans (1 → 3)-linked in the backbone with a single (1 → 6)-linked β-D-glucosyl side branching unit on every third residue.

In addition to β-D-glucans, some heteropolysaccharides showing different compositions, most of them biologically active, have been obtained from *G. frondosa* (Cui et al., 2007; Masuda et al., 2009; Masuda, Ito, Konishi, & Nanba, 2010; Mizuno,Ohsawa, Hagiwara, & Kuboyama, 1986; Xu, Liu, Shen, Fei, & Chen, 2010; Wang et al., 2014). With the exception of the acid heteropolysaccharide, named GFPS1b, obtained from cultured mycelia of *G. frondosa* (Cui et al., 2007), and the water-soluble polysaccharide named GFPW from the fruiting bodies of this mushroom (Wang et al., 2014), the primary structures of the heteropolymers have not been unambiguously elucidated. GFPS1b showed to have a backbone consisting of (1 → 4)-linked α-D-Gal*p* and (1 → 3)-linked α-D-Glc*p* residues, the latter being partially substituted at O-6 by 4-*O*-α-L-arabinofuranosyl-α-D-glucopyranosyl groups, which showed to be effective in the inhibition of proliferation of mammary tumor MCF-7 cells in vitro (Cui et al., 2007). The other heteropolysaccharide chemically elucidate was the fraction GFPW, which had a main chain of (1 → 6)-linked α-D-Gal*p* residues, with branches of (1 → 3)-linked

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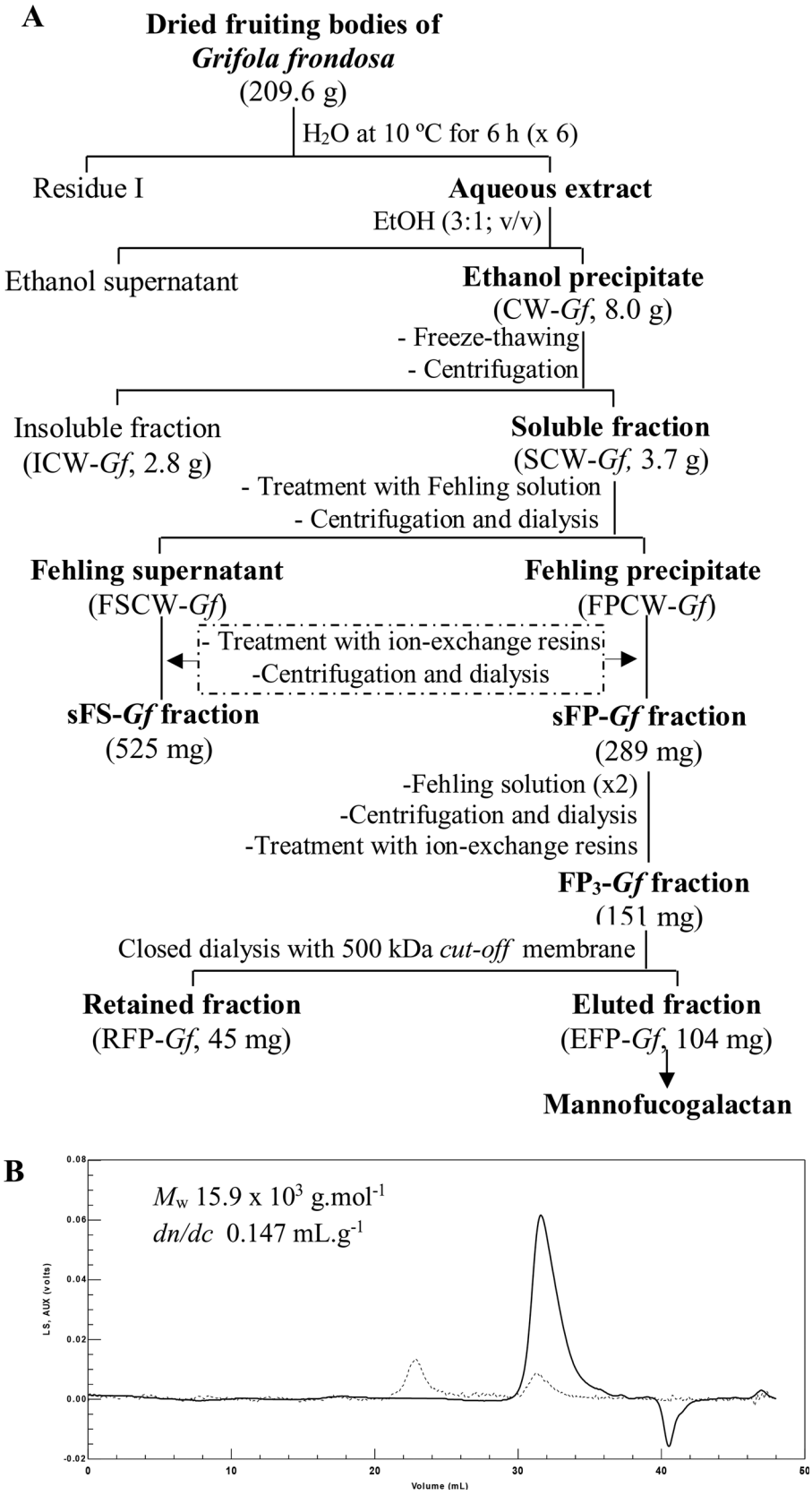

Fig. 1. (A) Scheme of extraction and purification of the heterogalactan from fruiting bodies of *G. frondosa*. (B) Elution profile of fraction EFP-*Gf* determined by HPSEC-MALLS using light scattering (—) and refractive index detectors (—).

fucose residues and α-terminal mannose substituting the O-2 position (Wang et al., 2014).

Novel polysaccharides from *G. frondosa* have been frequently isolated, purified and evaluated. As most of them have been shown to be bioactive, it is important to know the fine chemical structure of those compounds in an attempt to determine the structure-activity relationship. Thus, at the present study the isolation and structural characterization of a different heteropolysaccharide, a partially methylated