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Note

The structure of the O-specific polysaccharide of the lipopolysaccharide from *Burkholderia gladioli* pv. *agaricicola*

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Abstract—A neutral O-specific polysaccharide containing D-mannose, D-rhamnose and D-galactose was obtained by mild acid hydrolysis of the lipopolysaccharide of the plant pathogenic bacterium *Burkholderia gladioli* pv. *agaricicola*. By means of compositional analyses and NMR spectroscopy, the chemical repeating unit of the polymer was identified as a linear trisaccharide of the structure shown below, in which the mannose residue was quantitatively acetylated at C-2.

Ac
↓
2
→3)-α-D-Man*p*-(1→2)-α-D-Rha*p*-(1→4)-β-D-Gal*p*-(1→

Keywords: Mushroom disease; *Agaricus bitorquis*; *Burkholderia gladioli* pv. *agaricicola*; Lipopolysaccharide; O-Specific polysaccharide; NMR spectroscopy

Many pectolytic bacteria including pseudomonads are found in soil, on root or leaf surfaces, and are known to cause soft rots of vegetables, however none of such bacterial species has so far been implicated in the soft rot of mushrooms.[1] Several potentially devastating cases of wet or soft rot of mushrooms have been observed in the UK. Bacteria pathogenic for cultivated mushrooms are often members of the genus *Pseudomonas*. *Pseudomonas tolaasii* and *P. gingeri* are the cause of brown blotch[2] and ginger blotch,[3] respectively, of *Agaricus bisporus*. *Burkholderia gladioli* pv. *agaricicola* infects sporophores of the edible mushroom *A. bitorquis* causing soft rot disease. The disease is manifested by a rapid development of deep oozing lesions on the pileal which renders the mushroom unmarketable.[4,5] Due to the practical and economical importance of the disease, studies on the isolation and chemical and biological characterization of toxic metabolites are in progress. Preliminary results suggest that these metabolites belong to the class of lipodepsipeptides. Other studies were initiated on the toxicity and structures of the

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lipopolysaccharide (LPS) and the exopolysaccharide (EPS) in order to clarify their role in the plant-pathogen interaction and the disease. A putative exopolysaccharide containing D-rhamnose was recently isolated and its repeating unit was determined by chemical and spectroscopic methods.[6]

In this paper, the isolation and the structural determination of the O-specific polysaccharide of the LPS from *B. gladioli* pv. *agaricicola* are reported.

After cultivation and harvest, the bacterial cell mass was lyophilized (yield: 4.57 g bacterial dry mass) and then extracted utilizing the hot phenol/water method.[7] The aqueous phase of the phenol/water treatment was dialyzed, then freeze dried (335 mg, 7.3% of bacterial dry mass), dissolved in water, and the LPS was precipitated by ultracentrifugation and lyophilized (52.2 mg, 18.3% of the dry water phase). The EPS was isolated from the supernatant.[6]

Mild acid hydrolysis of the LPS and centrifugation gave a lipid as sediment (lipid A) and a water soluble carbohydrate fraction. This carbohydrate fraction was purified by gel-permeation chromatography (GPC) on Sephadex G-200, yielding two main fractions, the first of which contained the EPS and the second, the O-specific polysaccharide (OPS) with some EPS. Further GPC separation experiments on Sephadex G-200, Sephadex G-50, Sephacryl S-400, and TSK 55 (S) resins using 0.01 M, or 0.025 M ammonium hydrogencarbonate (pH 8.65) or 0.1 M pyridinium acetate (pH 4.20) did not succeed in a pure OPS preparation. Furthermore, a partial de-O-acetylation was observed during GPC under alkaline conditions, as indicated by an additional anomeric proton signal at δ 5.155 (compare: **a**1 in Table 1). Finally, the second fraction obtained from GPC on Sephadex G-200 was used for further investigations.

Sugar analysis of the OPS fraction identified rhamnose, mannose and galactose in an approximate molar ratio of 5:2:2. Methylation analysis gave mainly 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methyl-rhamnitol, 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-hexitol and 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-hexitol in an approximate molar ratio of 1:1:1, which revealed the OPS to be composed of 2-substituted rhamnopyranose and two 3- and 4-substituted hexopyranose residues. Also, 1,3,5-tri-*O*-acetyl-2,4-di-*O*-methyl-rhamnitol and 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-rhamnitol (approximate molar ratio of 0.75:0.25) were identified which originated from the EPS contamination.[6] The absolute configurations of the sugars were identified as D-mannose, D-galactose and D-rhamnose.[8,9]

The ^{1}H NMR spectrum (Fig. 1, Table 1) of the OPS preparation contained four main signals in the anomeric region, that is, at δ 5.433 (proton **A**2), δ 5.170 (**A**1), δ 4.955 (**B**1) and δ 4.568 (**C**1), a signal characteristic for a methyl group of rhamnose at δ 1.275, and one signal at δ 2.171, characteristic for an *O*-acetyl group. Other resonances in the anomeric region and characteristic

Table 1. ^{1}H and ^{13}C NMR data of the OPS (residues **A**, **B** and **C**) and de-O-acetylated OPS (residues **a**, **b** and **c**) from the LPS of *Burkholderia gladioli* pv. *agaricicola*

Residue	^{1}H, ^{13}C Chemical shifts [δ] ($^3J_{H-1,H-2}/^1J_{C-1,H-1}$ (Hz))								
	1	2	3	4	5	6a	6b	CH_3CO	*CO*
→3)-α-D-Man*p*-	5.170	5.433	4.259	3.910	3.770	3.874	3.839	2.171	—
A	(<1.5)	*70.01*	*76.70*	66.48	74.25	61.79		21.38	174.22
	100.47								
	(175)								
a	5.155	4.289	4.067	3.849	3.731	3.813	3.877		
	(<1.5)	68.64	*79.39*	66.27	74.31	62.04			
	103.03								
	(173)								
→2)-α-D-Rha*p*-	4.955	4.074	3.920	3.491	4.111	1.275	—	—	—
B	(<1.5)	*79.60*	70.40	73.22	70.29	17.63	—	—	—
	100.94								
	(172)								
b	4.975	4.087	3.934	3.494	4.128	1.288	—		
	(<1.5)	*79.36*	71.20	73.30	70.36	17.67	—		
	101.19								
	(172)								
→4)-β-D-Gal*p*-	4.568	3.518	3.733	4.001	3.765	3.761	3.761	—	—
C	(7.6)	72.10	73.15	*78.44*	76.25	61.56		—	—
	101.51								
	(162)								
c	4.613	3.617	3.774	4.024	3.786	3.784	3.784		
	(7.7)	71.92	73.15	*78.53*	76.28	61.67			
	102.06								
	(163)								

Spectra were recorded at 50 °C in ^2H_2O relative to internal acetone (δ_H 2.225; δ_C 31.45). Italicized chemical shifts indicate substituted positions.