Composition of Lipopolysaccharides from Various Strains of *Rhodomicrobium vannielii*

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Dedicated to Professor Hans Grisebach on the occasion of his 60th birthday

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The compositions of lipopolysaccharides from the photoheterotrophic budding *Rhodomicrobium vannielii* strains DSM 162, Rm5, E3 and 2/1 are reported. Common constituents of these lipopolysaccharides are glucose, mannose, glucosamine, glucuronic and galacturonic acids, 2-keto-3-deoxyoctonate (KDO) and the fatty acids 3-OH-C_{16}:0, 3-OH-C_{14}:0, C_{14}:0, Δ^{14}-C_{22}:1, aside from strain specific differences.

Two different, medium-dependent growth forms of strain DSM 162 are described. The vitamin/yeast extract-concentration in the medium and/or the growth temperature were found as factors triggering the different growth forms. Lipopolysaccharides of the two growth forms had only quantitative differences in composition. Lipopolysaccharide from swarmer cells of strain Rm5 showed a chemical composition comparable to that of chain cells and from simple cycle cells of the same strain.

Introduction

Rhodomicrobium vannielii is a phototrophic budding bacterium with a complex cell cycle. During its life cycle, motile swarmer cells, non-motile filamentous cells (chain cells) and exospores are produced [1]. The complex cell cycle may change to a simple cell cycle under certain growth conditions such as high CO_2 tension and low light intensity [2]. The simple cycle consists of only single cells and filamentous two-cell forms.

The cell wall of *Rm. vannielii* is of the Gram-negative type and contains lipopolysaccharide whose composition is known, as is the lipid A structure [3, 4] for the type strain (ATCC 17100) obtained from the American Type Culture Collection. Interestingly, lipid A contains mannose and lacks phosphate. It was found to exhibit little toxicity [4].

Two different, culture-medium dependent growth forms of *Rm. vannielii* DSM 162 (= ATCC 17100, from the Deutsche Sammlung von Mikroorganismen) were now studied and shown to be qualitatively unchanged in lipopolysaccharide composition. This paper also gives data on the lipopolysaccharides of *Rm. vannielii* strains E3 and 2/1 as well of the different cell types of strain Rm5 [1].

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Materials and Methods

Rhodomicrobium vannielii strains were obtained from the following sources: strain ATCC 17100 from the American Type Culture Collection, Rockville, USA; strain DSM 162 (= ATCC 17100) from the Deutsche Sammlung von Mikroorganismen (DSM), Göttingen, FRG; strain Rm5 from the culture collection of the Department of Biological Sciences, University of Warwick, U.K.; and strains E3 and 2/1 from the culture collection of the Institut für Biologie II, Mikrobiologie, der Universität Freiburg i. Br.

Bacteria were grown phototrophically as batch cultures in 1-l-flasks without stirring in R8ÄH medium [5]. Strain DSM 162 was grown on either R8ÄH or Rhodospirillaceae medium [6], the latter containing per liter: 0.2 g yeast extract, 1 g disodium succinate, 5 ml ferric citrate solution (0.1%), 0.5 g KH_2PO_4, 0.4 g $MgSO_4 \cdot 7\,H_2O$, 0.4 g NaCl, 0.4 g NH_4Cl, 0.05 g $CaCl_2 \cdot 2\,H_2O$, 1 ml Sl-6 trace element solution [6].

The pH was adjusted to 6.9. The inoculum was always 5% (v/v).

Preparation of swarmer and chain cells of strain Rm5 was achieved by column filtration [1]. Lipopolysaccharide was extracted by phenol–chloroform–petroleum ether (PCP) (2:5:8; by vol [7]). Separation of lipopolysaccharide and ornithine-containing lipid was performed as described [8]. Methods of identification and quantitative determination of fatty acids, neutral sugars, 2-keto-3-deoxyoctonate (KDO), uronic acids, amino sugars and phosphate were as given in ref. [3].

Results

Growth forms and lipopolysaccharide composition of Rhodomicrobium vannielii DSM 162

After six days of growth (late log phase), batch cultures of *Rm. vannielii* DSM 162 in R8ÄH medium showed complete sedimentation in which all cell types except exospores were observed. After several passages in the same medium, this growth form (called "sedimenting (s-form)") changed to one which showed less sedimentation and thus was called "less sedimenting (ls-form)". All cell types, again with the exception of exospores, were present. Investigation by light or electron microscopy showed no apparent differences between the cell types of the s- and ls-forms (J. R. Golecki, personal communication).

We modified the composition of both the R8ÄH and the *Rhodospirillaceae* medium to study the expression of the two different growth forms. The results of a long series of experiments [9] can be briefly summarized as follows: (a) alteration of the pH or using succinate or malate alternatively as carbon sources did not influence the expression of s- and ls-forms. Growth was better at pH 6.9 than at pH 5.7. (b) the concentration of yeast extract appeared to influence the growth form: in the yeast-extract-rich R8ÄH medium, the ls-form was expressed and stable, in the Rhodospirillaceae medium containing little yeast extract and no additional vitamins, the s-form was expressed and stable.

Change from the s-form to the ls-form and *vice versa* was possible by switching media. For example, starting with the ls-form (in R8ÄH), after eight passages in Rhodospirillaceae medium, the s-form was obtained which in turn switched back to the ls-form after five passages in R8ÄH, and back to s-form after three further cultures in Rhodospirillaceae medium.

Lipopolysaccharide was obtained from mass cultures of both the s- and ls-forms of *Rm. vannielii* DSM 162 by using the PCP method. The yields were 0.7–1.5% of cell dry weight in both cases. The composition of both was found to be qualitatively similar (Table I), although Δ^{14}-C_{22}:1 (Δ^{14}-docosenoic acid [4]) was mostly replaced by a C_{24}:1 fatty acid in the ls-form.

Lipopolysaccharide of Rhodomicrobium vannielii, strains E3 and 2/1

Lipopolysaccharides from strain E3 (yield: 0.4% of cell dry weight) and strain 2/1 (yield 0.7% of cell dry weight) grown in the R8ÄH medium had the same qualitative composition except for the presence of a 3- or 4-O-methyl-hexose which was not characterized further (Table I). The spectrum was different from that of strain DSM 162 in that xylose and 3-O-methyl-xylose were lacking.

Rhodomicrobium vannielii (Rm5) cell types and their lipopolysaccharides

Swarmer cells were separated from filamentous chain cells using glass-wool according to ref. [1]. The yields of lipopolysaccharide from the two cell types were 0.5 and 1.2% of cell dry weight, respectively. From "simple cycle" cells [1] a respective value of 0.8% was obtained. The sugar composition differed from that of the lipopolysaccharide from strains DSM 162, E3, and 2/1 (Table I), in that xylose was found to be missing, and ribose and rhamnose were identified in swarmer and chain, but not in "simple cycle" cells. No qualitative and essentially no quantitative differences in composition between swarmer and chain cells were observed. The fatty acid spectra, as found with the other lipopolysaccharides investigated, showed no major differences (Table I).

Discussion

Common marker constituents of lipopolysaccharides from all *Rhodomicrobium vannielii* strains investigated were glucose, mannose and the fatty acids β-hydroxy-hexadecanoic, β-hydroxy-tetradecanoic, tetradecanoic and Δ^{14}-docosenoic (Δ^{14}-C_{22}:1) acids. All contained glucosamine, glucuronic and galacturonic acids and 2-keto-3-deoxyoctonate. Taken to-

Table I. Composition of lipopolysaccharide from various strains of *Rhodomicrobium vannielii* (% of lipopolysaccharide dry weight).

	Rm. vannielii strain							
	ATCC 17100[a]	DSM 162 (s-form)	DSM 162 (ls-form)	E3	2/1	Rm5 (swarmer cells)	Rm5 (chain cells)	Rm5 (simple cycle cells)
Glc	6.8	9.5	13.1	13.7	16.0	11.5	10.0	7.3
Rha	1.4	–	–	–	–	2.7	2.8	–
Man	2.5	4.2	6.9	2.6	2.6	2.0	2.2	1.6
Xyl	3.1	5.3	3.1	–	–	–	–	–
Rib	–	–	–	–	–	1.5	1.8	–
O-Methyl-hexose[b]	–	–	–	1.8	–	–	–	–
3-O-Me-Xyl	–	1.0	1.6	–	–	–	–	–
GlcN	11.2	4.2	8.7	9.3	7.2	+	10.7	9.0
GlcUA	+[c]	+	+	+	+	+	+	+
GalUA	+	+	+	+	+	+	+	+
KDO	+	+	+	+	+	+	+	+
Phosphorus	0.1	0.1	0.1	0.1	0.1	0.5	0.5	0.5
C_{14}:0	3.3	2.7	5.0	0.7	5.0	1.1	2.1	1.8
Δ^2-C_{14}:1[d]	1.5	1.6	3.9	0.1	1.7	2.2	2.2	2.5
C_{18}:1	–	–	–	–	–	2.1	1.0	1.8
Δ^{14}-C_{22}:1	4.0	2.2	0.6	0.6	1.1	1.7	2.2	1.8
C_{24}:1	0.7	–	1.2	–	–	–	–	–
β-OH-C_{14}:0	6.3	5.4	7.7	1.2	6.0	5.0	5.4	5.3
β-OH-C_{16}:0	6.1	6.2	11.1	2.0	7.6	8.8	9.4	9.8

[a] Values taken from ref. [7].
[b] 3- Or 4-O-methyl-hexose.
[c] Present but not quantified.
[d] Formed from β-OH-C_{14}:0 by β-elimination of water.

gether with the lack of phosphate, the same lipid A type [4] is suggested to be present in all *Rm. vannielii* lipopolysaccharides. However, different O-antigen chemotypes, as were found with other Rhodospirillaceae [10], are indicated in all of the strains investigated.

Sedimentation of bacteria in batch cultures, as observed with *Rm. vannielii* DSM 162, is known also for *Rhodopseudomonas gelatinosa* [11]. Cells of the latter form mucuous sediments, often at high pH, whereas with *Rm. vannielii* slimy materials were not observed and the pH did not influence the growth forms. In this case sedimentation occurred in medium containing five times less yeast extract and no additional vitamins, in contrast to the R8ÄH medium in which – reversibly – less sedimentation occurred. The chemical reason for triggering the two different growth forms is not known. Recent investigations (data not shown) revealed that the growth temperature also influences growth as ls- or s-form of *Rm. vannielii* ATCC 17100 in the R8ÄH medium. The ls-form is observed at 28 °C and the s-form at 32 °C, both forms were stable at these temperatures but could be interchanged depending of the temperature used.

We found that the lipopolysaccharide composition from *Rm. vannielii* ATCC 17100 obtained from the American Type Culture Collection was different from that of strain DSM 162 of the Deutsche Sammlung von Mikroorganismen. Lipopolysaccharide of strain ATCC 17100 contained rhamnose but no 3-O-methyl-xylose as does strain DSM 162. Consequently they represent two different chemotypes of lipopolysaccharide, although the strain designations are essentially the same (DSM 162 corresponds to ATCC 17100).

The lipopolysaccharide analyses of the different cell types of *Rm. vannielii* Rm5 showed essentially little differences in sugar and fatty acid composition between swarmer and chain cells, except that the lack of rhamnose and ribose in lipopolysaccharide from "simple cycle" cells might indicate differences in sugar composition of O-chains.

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