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Note

Phosphorus-containing glycopolymers of *Clavibacter michiganense* cell walls*

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It is known that information on the monosaccharide composition of the cell-wall polysaccharides of coryneform bacteria[1] can be used to classify them as to genus and species. Data of this kind demonstrated the heterogeneity of the genus *Corynebacterium* and made it possible for Vidaver *et al.*[2] to propose a new genus *Clavibacter* to include some phytopathogenic corynebacterial species. In this Note we report the isolation of cell-wall polysaccharides from two strains of *Clavibacter michiganense* and the characterization of the teichoic acid component.

The cell-wall polysaccharides of two type strains, *Clavibacter michiganense* subsp. *michiganense* NCPPB 2979 and *Clavibacter michiganense* subsp. *insidiosum* NCPPB 1109, were extracted by mild alkaline hydrolysis of the cells. Purification of the glycopolymers by ion-exchange chromatography (see Fig. 1) gave three fractions, the first (I) neutral and comprised of glucans, the next two acidic.

Fraction II was made up of heteropolysaccharides of galactose, glucose, mannose, fucose, and rhamnose. The acidic nature of these substances is due to pyruvic acid residues.

Fraction III from each strain gave on hydrolysis only one neutral monosaccharide, glucose. The ^{13}C-n.m.r. spectra (Fig. 2) of the polysaccharides were typical of regular polymers and consisted of nine lines of about equal intensity. Six narrow lines represented the complete set of signals of a β-D-glucopyranosyl unit (see Table I for chemical shifts) and three lines corresponded to the three carbon atoms of a chirally substituted glycerol residue. In the ^{31}P-n.m.r. spectra the sole signal was a broad singlet at δ 0.7, characteristic of teichoic acids. The widening of three ^{13}C-signals (δ 78.85, 66.40, 66.10) was attributed to the attachment of phosphoric acid residues to C-1 and C-3 of glycerol. The weak polar chemical shift of C-2 is conditioned by the presence of the

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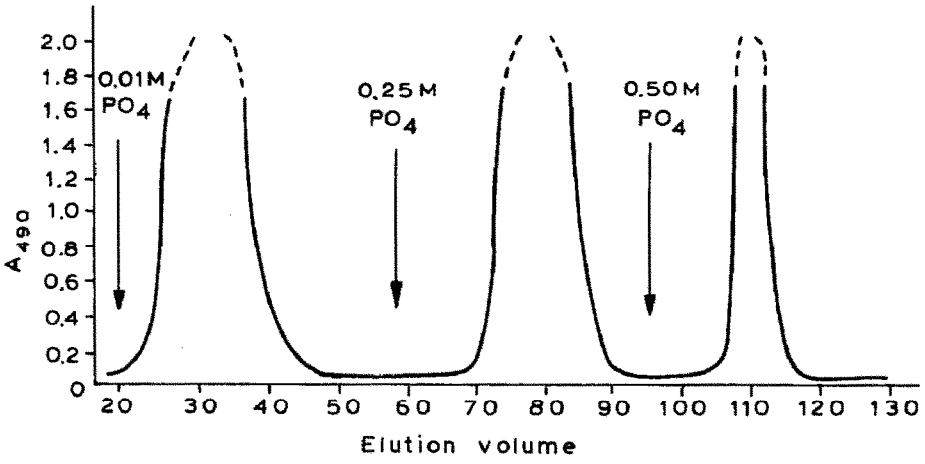

Fig. 1. Elution profile of *C. michiganense* polysaccharides on Toyopearl DEAE-TSK gel.

β-D-glucopyranose residue at this position. Thus, the polymers are glycerol teichoic acids composed of repeating units having the following structure:

$$(\text{1-Gro-3-P-})_n$$
$$\begin{matrix} 2 \\ \uparrow \\ 1 \end{matrix}$$
β-D-Glc*p*

Naumova *et al.*[3] isolated from *Arthrobacter crystallopoietes* a glycerol teichoic acid having an analogous structure. This may be seen in Table I, which presents comparative data on the chemical shifts of the carbon atoms in the teichoic acids of the two species. Until now teichoic acids had not been detected in phytopathogenic corynebacteria. Our results are the first to show the presence of glycerol teichoic acids in two type strains of *Clavibacter michiganense*.

On the basis of the results obtained so far we cannot draw conclusions about the taxonomic significance of glycerol teichoic acid for the *Clavibacter* genus or for *C.*

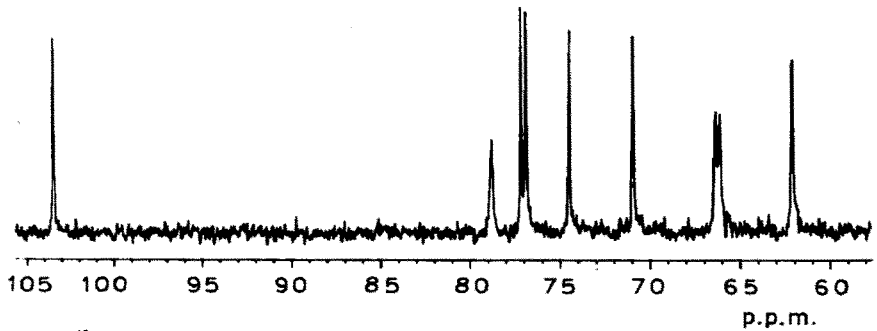

Fig. 2. ^{13}C-N.m.r. spectrum of *C. michiganense* glycerol teichoic acid.

TABLE I

^{13}C-N.m.r. data for teichoic acids (TA) isolated from *C. michiganense* and *A. crystallopoietes*

Carbon atom[a]	δ, C. michiganense-*TA*	*Multiplicity in GD spectrum*[a]	δ, A. crystallopoietes *TA*[b]
C-1′	103,5[c]	d	103.2
C-2′	74.5	d	74.4
C-3′	77.2	d	77.1
C-4′	70.95	d	70.9
C-5′	76.2	d	76.85
C-6′	62.1	t	62.0
C-1	66.4	t	66.25
C-2	78.85	d	78.4
C-3	66.1	t	65.8

[a] Primed numbers are for glucose residue, unprimed for glycerol; GD, gated-decoupled; d, doublet (CH group): t, triplet (CH_2 group). [b] Data from ref. 3. [c] J_{CH} 163.6 Hz (β-configuration of glycosidic linkage).

michiganense, because in addition to *A. crystallopoietes* a teichoic acid with an analogous structure was also detected in *Staphylococcus epidermidis*[4]. Nevertheless, our data on the presence and the structure of these teichoic acids are a contribution to the biochemical characterization of the species *C. michiganense*.

EXPERIMENTAL

General methods. — Neutral sugar analysis was performed by g.l.c. of the polyol acetates[5]. N.m.r. spectra (^{13}C and ^{31}P) were recorded for solutions in D_2O with a Bruker AM-300 instrument operating at 60°, and using methanol (δ 50.15 for ^{13}C) and phosphoric acid (δ 0 for ^{31}P) as external standards. Chemical shifts are expressed in p.p.m. downfield from the standard.

Preparation of the glycopolymers. — Bacteria were grown at 28° on synthetic medium N (ref. 6) shaken at 240 r.p.m. for 50–55 h. Cells, harvested by centrifugation, (10 g/100 mL) were suspended in 5% aqueous KOH and kept for 1.5–2.0 h on a boiling-water bath. Cellular debris was removed by centrifugation, and the supernatant was dialysed against demineralised water. After dialysis the polysaccharides were precipitated from the hydrolysate with 4 volumes of ethanol[6], and dried by successive treatments with acetone and ether.

Samples (500–600 mg) were dissolved in 5 mL of sodium phosphate buffer, pH 6.0, for ion-exchange chromatography on columns (80.0 × 1.5 cm) packed with Toyopearl DEAE-TSK gel. Fractions were eluted by application of a step-gradient (0.01, 0.25, and 0.50M) of the buffer and assayed for carbohydrate with phenol and sulphuric acid[7]. The colour was measured with an SF-26 spectrophotometer at 490 nm.

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