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A ^{13}C-NMR study of exopolysaccharide synthesis in *Rhizobium meliloti* Su47 strain

P. Tavernier[1,*], J.-C. Portais[1], I. Besson[1], J. Courtois[2], B. Courtois[2] and J.-N. Barbotin[1]

[1] *Laboratoire de Génie Cellulaire, UPRES-A 6022 du CNRS, Faculté des Sciences, Université de Picardie Jules Verne, 33 rue Saint-Leu, 80039 Amiens cedex, France*
[2] *Laboratoire de Biotechnologie Microbienne, UPRES-A 6022 du CNRS, IUT, Université de Picardie Jules Verne, Département de Biologie Appliquée, avenue des Facultés, Le Bailly, 80025 Amiens cedex, France*

* *Correspondence and reprints.*

RÉSUMÉ

Les voies métaboliques impliquées dans la synthèse du succinoglycane produit par la souche Su47 de *R. meliloti* ont été évaluées par la spectroscopie de RMN du carbone 13 après incubation des cellules avec du [1-^{13}C] ou [2-^{13}C]glucose. La biosynthèse de ce polymère à partir du glucose se produit par polymérisation directe du glucose et par la voie des pentoses phosphate.

Mots-clés: *Rhizobium meliloti*, exopolysaccharide, biosynthèse, RMN du ^{13}C

ABSTRACT

Metabolic pathways implied in the synthesis of succinoglycan produced by the Su47 strain of *R. meliloti* were evaluated by ^{13}C-NMR spectroscopy after incubation with [1-^{13}C] or [2-^{13}C]glucose. The biosynthesis of this polymer by *R. meliloti* from glucose occurred by a direct polymerisation of the introduced glucose and by the pentose phosphate pathway.

Key words: *Rhizobium meliloti*, exopolysaccharide, biosynthesis, ^{13}C-NMR

INTRODUCTION

Rhizobium meliloti cells are able to accumulate a variety of polymers including glycogen, periplasmic cyclic β-(1,2) glucans and polyhydroxybutyrate [1]. These bacteria excrete a high-molecular weight anionic polysaccharide into the medium [2]: the succinoglycan, composed of repeating subunits containing each glucose, galactose,

acetate, succinate and pyruvate in the ratio 7, 1, 1, 1, 1 [3]. This polymer can be commercially useful for producing gels and modifying the rheological properties of aqueous systems. The pathways involved in the biosynthesis of this succinoglycan are not well known in these cells that possess the enzymatic potential for the Embden-Meyerhof, the Entner-Doudoroff and the pentose phosphate pathways. Here, ^{13}C-NMR was used to characterize the metabolic pathways implied in the synthesis of the glycosyl moieties of the succinoglycan, using glucose as substrate.

EXPERIMENTAL

High-density (5.10^{10} cells/mL) cell suspensions of mid-exponential phase harvested cells of R. *meliloti* Su47 strain (grown in yeast extract medium, as previously described [4]) were incubated aerobically with 30 mM [1-^{13}C] or [2-^{13}C]glucose. After 20 h of incubation, the cell suspension was centrifugated and the supernatant polysaccharides precipitated with propan-2-ol. These polysaccharides were then NMR analyzed at 80°C or hydrolysed with 4.25M trifluoroacetic acid (TFA). The lyophilized hydrolysate was suspended into 0.5 mL D_2O and analyzed by NMR.

^{13}C-NMR measurements were carried out at 30°C on a Bruker AM-300, WB spectrometer with a 5-mm ^{13}C-^{1}H dual probe, using a spectral width of 15 KHz (16 K memory size), a 60° pulse angle, a 1.2 s interpulse delay and a bilevel proton decoupling. The FIDs were exponentially multiplied by a line broadening of 6 Hz prior to Fourier transformation. Chemical shifts were expressed as ppm relative to the resonance of benzene (129.2 ppm).

RESULTS AND DISCUSSION

Experiments were conducted with [1-^{13}C] and [2-^{13}C] glucose to characterize the metabolic pathways involved in succinoglycan synthesis. The same strategy has already been applied for alginate synthesis [5]. The ^{13}C-NMR spectra of native succinoglycan were obtained at 80°C (because of supernatant vicosity) but the complex structure of this compound led to difficulties in interpreting its enrichment pattern. The isotopic content of the glycosyl residues was thus obtained after TFA hydrolysis of the EPS.

The spectra from the hydrolysis products (Figure 1) evidenced ^{13}C enrichment only in the C1 position of glucose (93.5 and 97 ppm) and galactose (97.6 ppm). These

results showed that the labeling of the polymer was found mainly in the original position indicating direct polymerisation of introduced glucose.

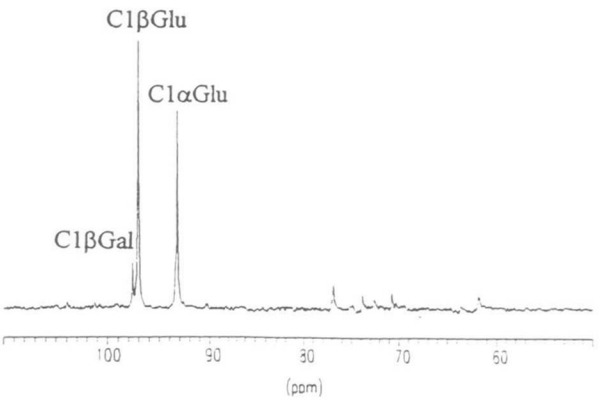

Figure 1: Proton-decoupled ^{13}C-NMR spectra at 30°C from the EPS hydrolysate produced after 20h of incubation with [1-^{13}C]glucose.

The Embden-Meyerhof pathway and the Entner-Doudoroff pathway were not evidenced in exopolysaccharide synthesis because of the lack of transfer from C1 to C6 carbon and from C1 to C3 and C4 carbons respectively. The pentose phosphate pathway cannot be evidenced because of the decarboxylation of C1 during the first step of this pathway. To determine an involvement of the pentose phosphate pathway, experiments were performed with [2-^{13}C]glucose. When entering this pathway the C2 of glucose give rise to enrichment at C1 and C3 (in the ratio 2:1) of the fructose-6-phosphate which can be converted into glucose (Figure 2).

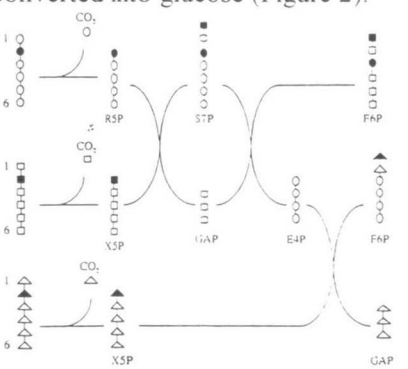

Figure 2: Fate of [2-^{13}C]glucose in the pentose phosphate pathway (F6P: fructose-6-phosphate; R5P: ribulose-6-phosphate; X5P: xylulose-5-phosphate; S7P: sedoheptulose-7-phosphate; GAP: glyceraldehyde-3-phosphate; E4P: erythrose-4-phosphate).

On the spectrum of the hydrolysis compounds (Figure 3), three enrichments can be distinguished: the C2 signals of β-glucose (75.3 ppm), α-glucose (72.6 ppm) and β-galactose (73 ppm); the C1 signals of β-glucose (97 ppm) and α-glucose (93.5 ppm) and the C3 signals of β-glucose (76.9 ppm) and α-glucose (73.8 ppm).

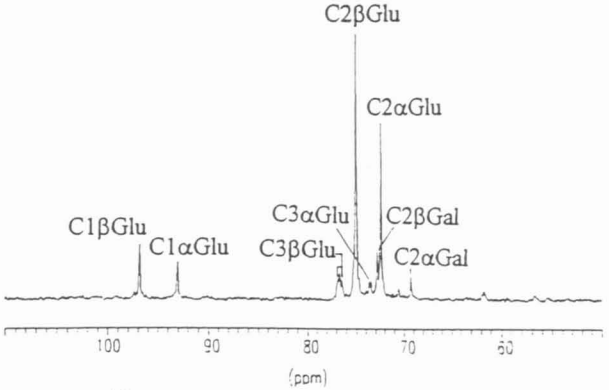

Figure 3: Proton-decoupled ^{13}C-NMR spectra at 30°C from the EPS hydrolysate produced after 20h of incubation with [2-^{13}C]glucose.

As with the [1-^{13}C]glucose, the labeling was found mainly in the original position, indicating direct polymerisation of introduced glucose. The lack of transfer from C2 to C5 carbon in exopolysaccharide confirms that the Embden-Meyerhof pathway and the Entner-Doudoroff pathway were not involved in EPS synthesis. On the other hand, the transfer of labeling from C2 to C1 and C3 carbons indicated that the polysaccharide synthesis occurred via the pentose phosphate pathway.

CONCLUSION

It is evidenced in the present work that in addition to direct polymerisation, the pentose phosphate pathway was involved in the synthesis of succinoglycan from glucose in *R. meliloti*. A quantification of this involvement will be performed.

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