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Novel acetylated α-cyclosophorotridecaose produced by *Ralstonia solanacearum*

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Abstract—α-Cyclosophorotridecaose (α-C13) produced by *Ralstonia solanacearum* is isolated by trichloroacetic acid treatment and subjected to various chromatographic techniques. Here, we report for the first time that *R. solanacearum* produces acetylated α-C13. Structural analyses of the acetylated α-C13 were performed with 1D or 2D NMR spectroscopy, MALDI-TOF MS and HPLC. The results show that the α-C13 is substituted by mainly one acetyl residue at the C-6 position of the glucose unit.

Keywords: Acetylated α-cyclosophorotridecaose; Periplasmic cyclic glucan; *Ralstonia solanacearum*; Structural analysis

1. Introduction

Ralstonia solanacearum is the causative pathogen of plant wilt. It generally enters a plant through the roots, penetrates the xylem, systemically colonizes the stem, and causes wilt symptoms.[1] It is considered to be a serious disease because the damaged hosts[2] are crop plants such as tomato, potato, tobacco, peanuts, and bananas, as well as many native plant species in the warm temperate and tropical regions of the world, causing great economic losses worldwide.

Generally, the cell-surface carbohydrates of this microorganism are known to be involved in bacterium–plant interactions[3] in pathogenesis, and the carbohydrates produced by *R. solanacearum* are extracellular polysaccharides (EPSs), lipopolysaccharides (LPSs), osmoregulated periplasmic glucans (OPGs), etc. EPSs are acidic heteropolymers involving *N*-acetylgalactosamine, *N*-acetylgalactosaminuronic acid, and rhamnose, and they play an important role in the pathogenicity of *R. solanacearum*.[4,5] LPSs are the major components of the outer membrane of Gram-negative bacteria and have long been demonstrated to be biologically active in the interaction with the host plants.[6,7] α-Cyclosophorotridecaose (α-C13), general constituent of the periplasmic space of Gram-negative bacteria, is a cellular cyclic glucan consisting of 13 glucose residues with one α-(1→6) linkage and 12 β-(1→2) linkages.[8–10]

In some bacterial species, OPGs are modified by non-glucose residues that originate from the membrane phospholipids (phosphoglycerol, phosphoethanolamine, and phosphocholine)[11–13] or from intermediate metabolism (acetyl, succinyl, and methylmalonyl).[14–16] Recently, studies on the presence of the novel glycerophosphorylated α-cyclosophorohexadecaose (α-C16) from *Xanthomonas campestris*[17] and the succinylated cyclic β-(1→2) glucan from *Brucella abortus*[15] have been also reported. Even though the presence of acetylated glucans in OPGs has been known in *Erwinia chrisanthemi*[18] and *Rhodobacter sphaeroides*,[16] no study is reported in *Ralstonia* species as yet.

In this study, we first report that acetylated α-cyclosophorotridecaose (α-C13) is synthesized by the plant pathogenic bacterium, *R. solanacearum*. The exact structure of the novel acetylated α-C13 was elucidated using 1D or 2D NMR spectroscopy, MALDI-TOF MS and HPLC analyses.

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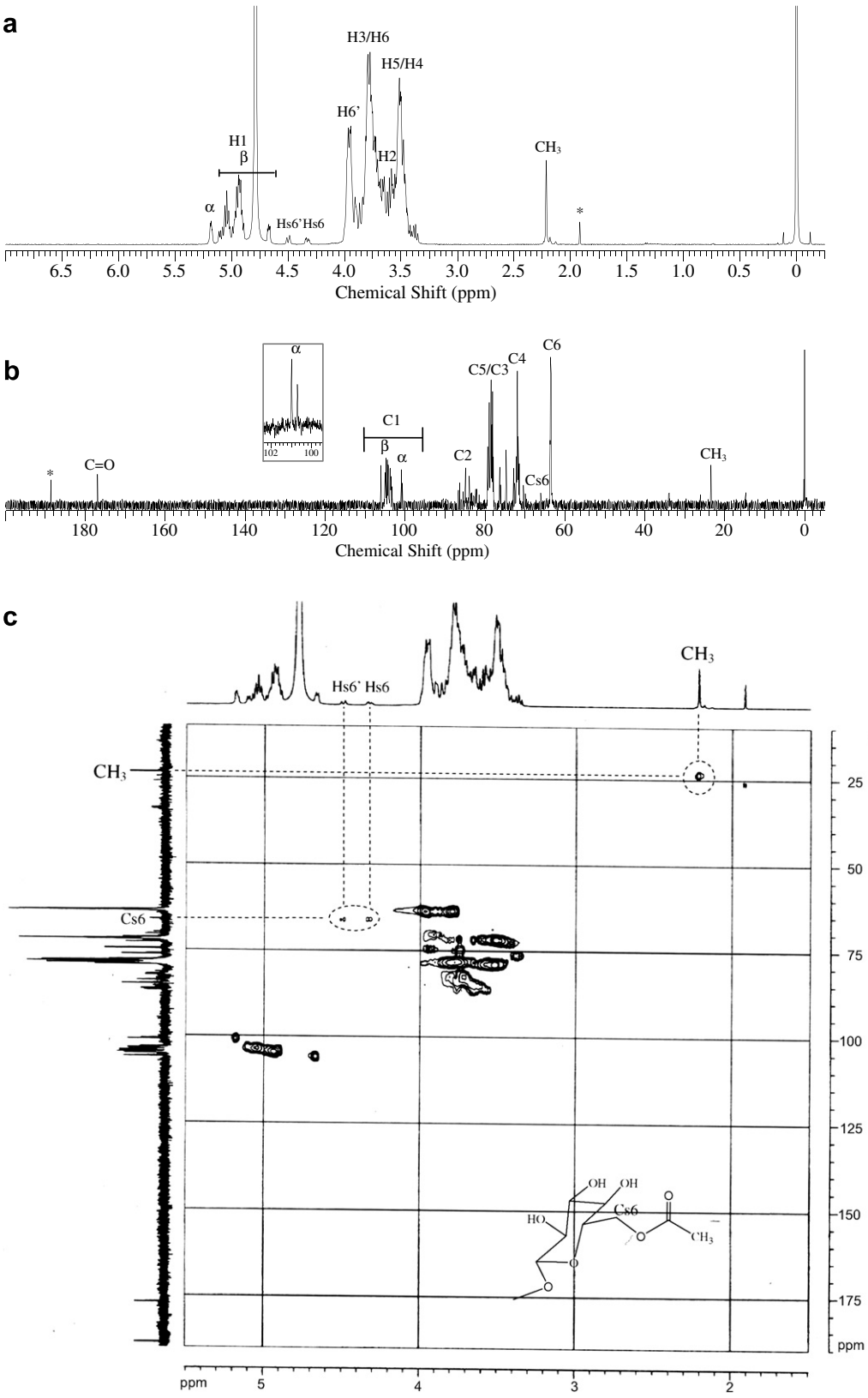

Figure 1. (a) ^{1}H NMR spectrum of the purified α-C13 of *R. solanacearum*. The asterisk (∗) peak at 1.92 ppm is an impurity in the sample. (b) ^{13}C NMR spectrum of the purified α-C13 of *R. solanacearum*. The inset spectrum from 100.00 to 102.00 ppm is enlarged to examine the resonances of C-1 carbons of α-glucose residue. The asterisk (∗) peak at 188.61 ppm is an impurity in the sample. (c) ^{1}H–^{13}C HSQC spectrum of the purified α-C13 from *R. solanacearum*. The H_s-6′, H_s-6 and C_s-6 indicate that the protons and the carbons of the glucose residues with acetyl ester are linked at position 6, respectively. The inset indicates the glucose acetylated at C_s-6.