

# Enhanced Rheological and Structural Properties of Exopolysaccharide from *Rhizobium leguminosarum* VF39 through NTG Mutagenesis

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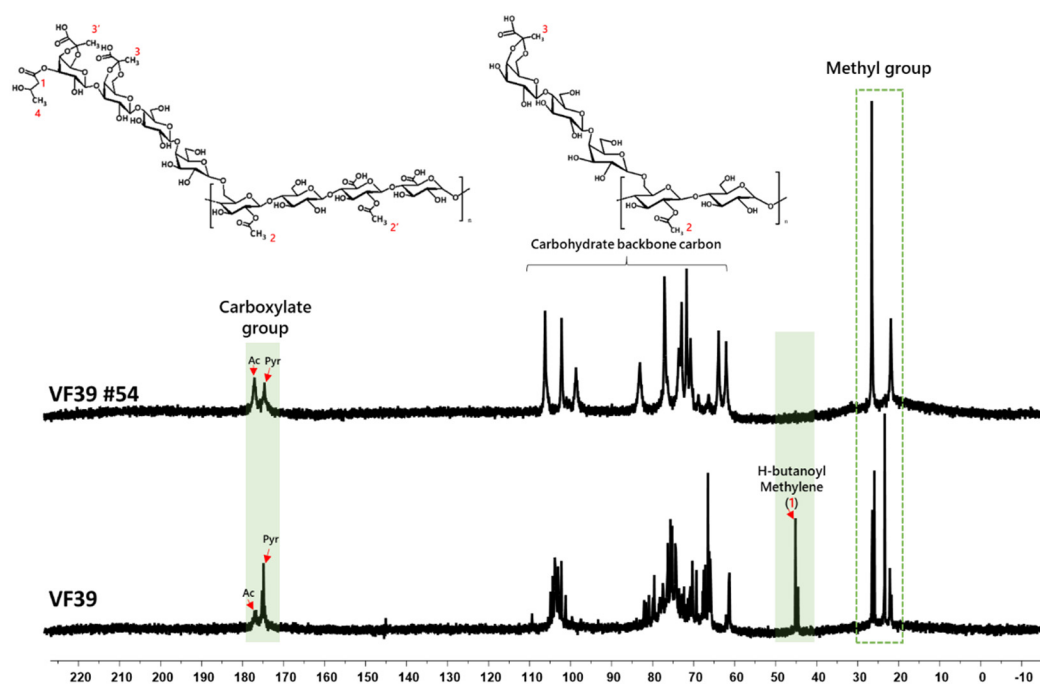


Figure S1. <sup>13</sup>C NMR of VF39, and #54 EPS. <sup>13</sup>C NMR spectra was measured at 80mg/ml concentration of sample solutions dissolved with D<sub>2</sub>O (99%).

The <sup>13</sup>C NMR spectrum of VF39 #54 showed the absence of the methylene peak at 45 ppm, which corresponds to the 3-hydroxybutanoyl functional group in the wild-type EPS [1].

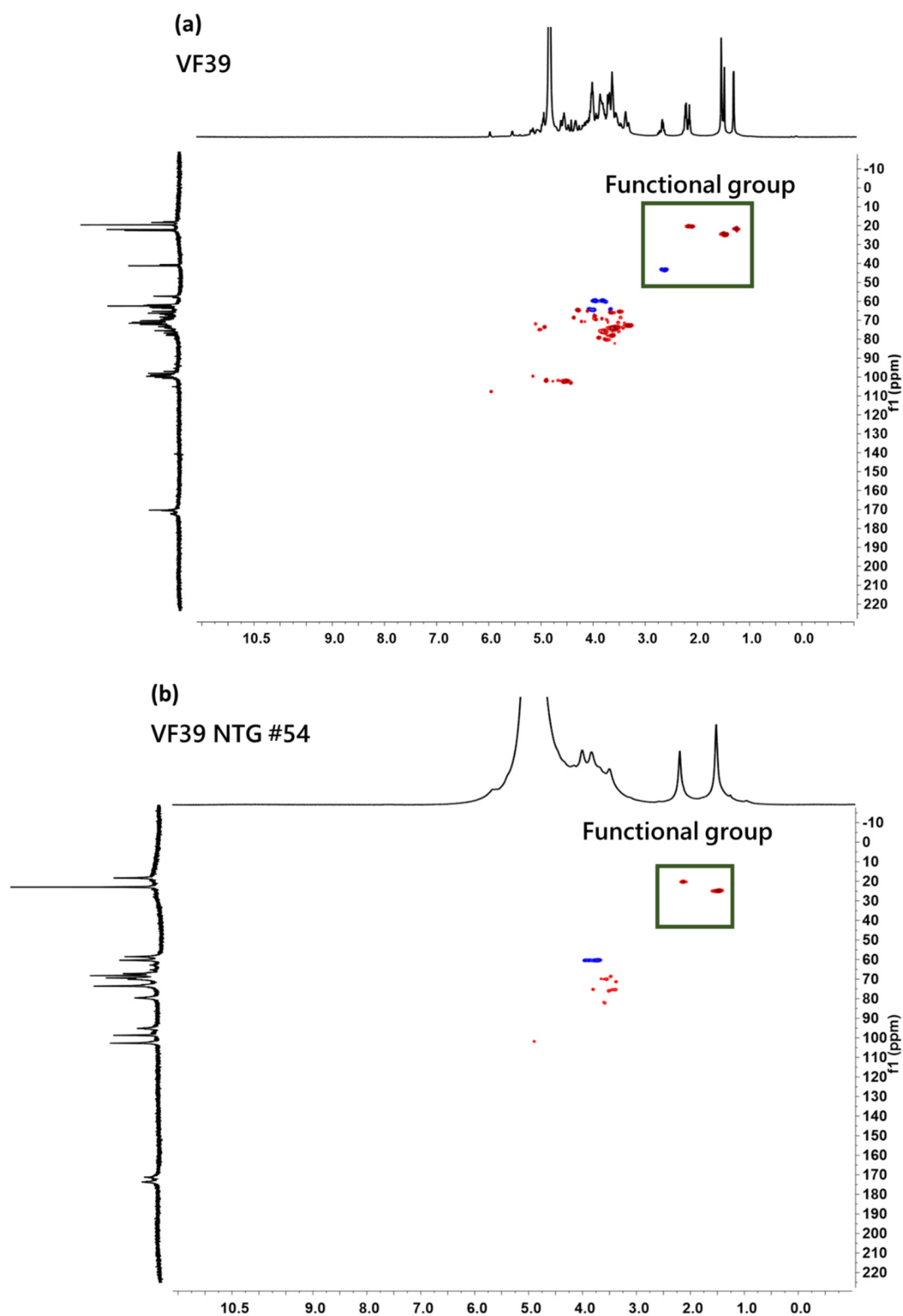


Figure S2. HSQC NMR spectra of VF39, and VF39 #54 EPS. HSQC NMR spectra was measured at 80mg/ml concentration of sample solutions dissolved with D<sub>2</sub>O (99%).

In Figure S2, the carbohydrate backbone structure of VF39 #54 EPS exhibited fewer signals compared to the VF39 wild type. This reduction was likely due to a decrease in the number of functional groups and mon-omers that constitute the backbone of VF39 #54 EPS. In this result, no signal for the 3-hydroxybutanoyl functional group was detected in the VF39 #54 EPS [2].

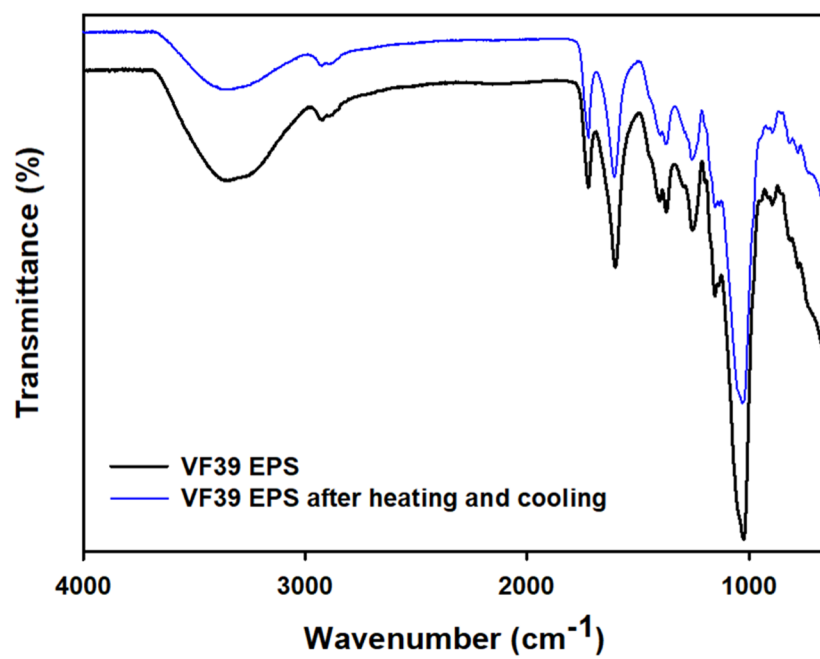


Figure S3. FT-IR spectra of VF39 EPS, and VF39 EPS after heating and cooling sample.

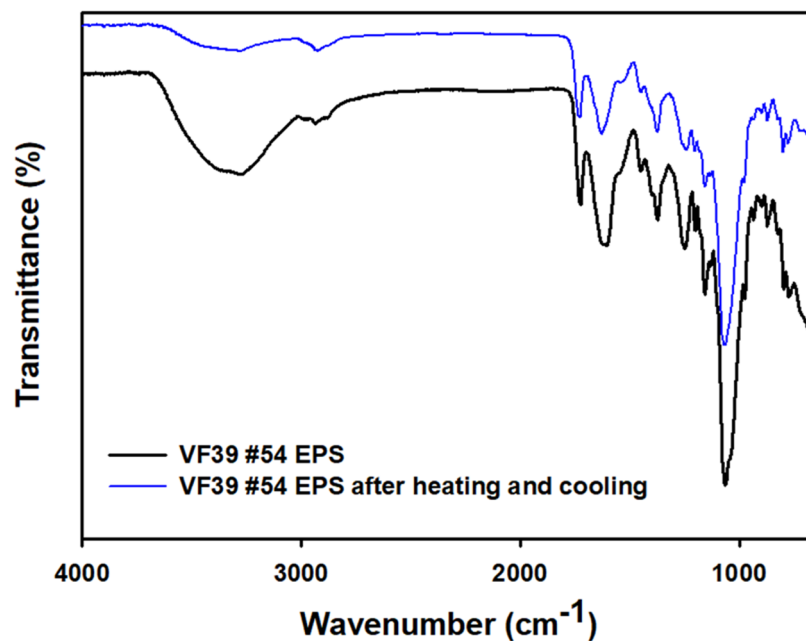


Figure S4. FT-IR spectra of VF39 #54 EPS, and VF39 #54 EPS after heating and cooling sample.

The preservation of the EPS structure after the heating and cooling process was confirmed using FT-IR. EPS sample 2 wt% solution was prepared by heating to 80°C and freeze-drying the cooled sample. In Figures S3 and S4, the FT-IR spectra appeared similar even after the heating process, showing that the structure of the sample was not destroyed.

#### Reference

- [1] Cremers, H.; Batley, M.; Redmond, J.; Wijfjes, A.; Lugtenberg, B.; Wijffelman, C. Distribution of O-acetyl groups in the exopolysaccharide synthesized by *Rhizobium leguminosarum* strains is not determined by the Sym plasmid. *Journal of Biological Chemistry* 1991, 266, 9556-9564
- [2] Gao, H.; Yang, L.; Tian, J.; Huang, L.; Huang, D.; Zhang, W.; Xie, F.; Niu, Y.; Jin, M.; Jia, C. Characterization and rheological properties analysis of the succinoglycan produced by a high-yield mutant of *Rhizobium radiobacter* ATCC 19358. *International Journal of Biological Macromolecules* 2021, 166, 61-70.