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Lack of O-Polysaccharide Renders *Bradyrhizobium japonicum* More Resistant to Organic Acid Stress

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Abstract In previous studies, we isolated an isogenic LPS$^-$ mutant of *Bradyrhizobium japonicum* 61A101C, which was completely devoid of O-polysaccharide and had altered cell surface characteristics. Subsequently, the mutated gene was identified, cloned, and used to complement the LPS$^-$ mutant strain JS314 to restore the phenotype. Since it has been reported that in *Escherichia coli* LPS O-polysaccharide is involved in resistance to an organic acid such as acetic acid under low pH (Barua *et al*., *Molecular Microbiology* **43:** 629–640, 2002), we compared the organic acid resistance of the three *B. japonicum* strains; wild-type 61A101C, the LPS$^-$ mutant JS314, and the complemented strain to determine whether the role of O-polysaccharide in the resistance to organic acid could be generalized. Growth of all three strains was inhibited by the presence of 3 mM acetic acid under acidic condition (pH 5.5). To our surprise, however, in the presence of 2 mM acetic acid, wild-type and the complemented strains did not grow while the LPS$^-$ mutant showed a significant growth. Therefore, unlike in *E. coli*, the lack of O-polysaccharide of LPS appears to render *B. japonicum* more resistant to organic acid.

Key words: *Bradyrhizobium japonicum*, lipopolysaccharide, O-polysaccharide, organic acid

In general, Gram-negative bacteria, such as *Escherichia coli* and *Salmonella* spp., have various devices of acid stress resistance mechanism combining to protect themselves [2, 5]. These terminologies for the acid survival mechanisms are divided into three systems depending on assay methods: acid tolerance response (ATR), the protection of cells by proton flux in exponential and stationary phase; acid habituation (AH), exponential phase ATR including many stress substances, such as glutamate, glucose, aspartate, FeCl, KCl, and L-proline; and acid resistance (AR), identified in Enterobacteriaceae including the effect of the σ^s and cAMP receptor protein-dependent oxidative system [4, 8, 13, 14]. It has been suggested that surface polysaccharides in *E. coli* are involved in organic acid resistance, which is a system different from the inorganic acid resistance system [1]. The inorganic acid stress is affected only by proton, but organic acid stress also acidifies the pH_i of the cell with accumulation of intracellular anion. It is the protonated form of organic acid that permeates the cytoplasmic membrane and deprotonates in the cytoplasm [2].

Lipopolysaccharide (LPS) is a cell surface component of Gram-negative bacteria, protecting themselves from their environment, and is composed of three domains: lipid A, the core which is an oligosaccharide consisting of an inner and outer region, and a distal repeating unit known as O-antigenic chain [3, 5, 10, 11]. In our previous study, we isolated an isogenic LPS$^-$ (*rfaF*$^-$) mutant of *B. japonicum* 61A101C, which was completely devoid of O-polysaccharide and had altered cell surface characteristics [10, 12]. Subsequently, we identified a gene region involved in LPS biosynthesis in *B. japonicum*, including *rfaF* (heptose transferase) and *rfaD* (heptose epimerase), and the *rfaF* gene was cloned and used to complement the *rfaF*$^-$ mutant JS314 to restore the phenotype [15]. It has recently been reported that in *E. coli*, LPS O-polysaccharide is involved in resistance to an organic acid such as acetic acid under low pH [1]. In order to find out whether the O-polysaccharide is also involved in the organic acid resistance in *B. japonicum*, we examined the organic acid resistance of the three *B. japonicum* strains (wild-type strain 61A101C, LPS mutant JS314, and the complemented strain JS314/pLps). First, we cultured

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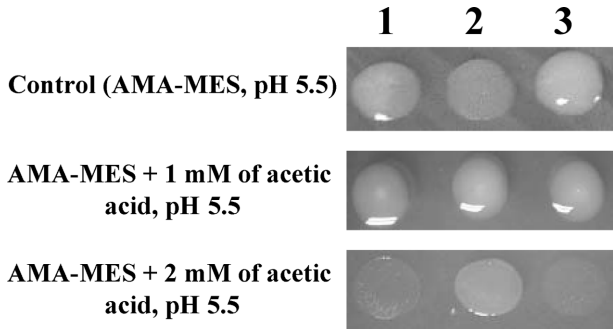

Fig. 1. Growth of *B. japonicum* strains on AMA-MES agar plate supplemented and non-supplemented with acetic acid under acidic condition (pH 5.5).
1, Wild-type (61A101C); 2, LPS mutant (JS314); 3, the complemented strain (JS314/pLps).

B. japonicum strains in AMA liquid medium (10 g of mannitol, 1 g of bacto yeast extract, 0.2 g of magnesium sulfate, and 0.2 g of sodium chloride per liter of deionized water, pH 7.6) and spotted 5 µl of each cell culture broth on AMA-MES (3-[*N*-Morpholino]propanesulfonic acid) agar plate supplemented with 1 mM and 2 mM acetic acid under acidic condition (pH 5.5) along with non-supplemented acetic acid medium as a control. To our surprise, unexpected results were obtained, compared with those of *E. coli* O157:H7 [1]. As shown in Fig. 1, all three strains showed similar growth in control medium and 1 mM of acetic acid containing medium, whereas only JS314 showed a considerable growth in the presence of 2 mM acetic acid. To further confirm the results, growth kinetics of *B. japonicum* strains were determined in AMA-MES liquid medium supplemented with 2 mM acetic acid and non-supplemented medium as a control. In non-supplemented medium, all three strains showed similar growth rates (Fig. 2A), whereas JS314 showed significantly higher growth rate in medium supplemented with 2 mM acetic acid then the wild-type and complemented strains (Fig. 2B). Barua *et al*. [1] reported that wild-type *E. coli* strain showed growth in Luria-Bertani (LB)-MES liquid medium under acidic pH, whereas O-polysaccharide deficient strains did not. Also, they described that the RpoS-deficient mutant of *E. coli* grew at a rate similar to the parent strain in a medium supplemented with acetic acid, and their protection against acetic acid stress was different from mechanisms such as AR, ATR, and AH [1].

It is likely that the difference in LPS composition between *E. coli* and *B. japonicum* could lead to a different organic acid resistance mechanism, although no experimental evidence to this effect has been presented. Indeed, unlike in *E. coli*, mannose is known to be present in O-polysaccharide of *B. japonicum* strains [1, 7]. In summary, we found that the lack of O-polysaccharide of LPS rendered *B. japonicum* more resistant to organic acid, unlike in *E. coli*, and the proposed role of O-polysacchraide in protection of cells against organic acid stress should not be generalized.

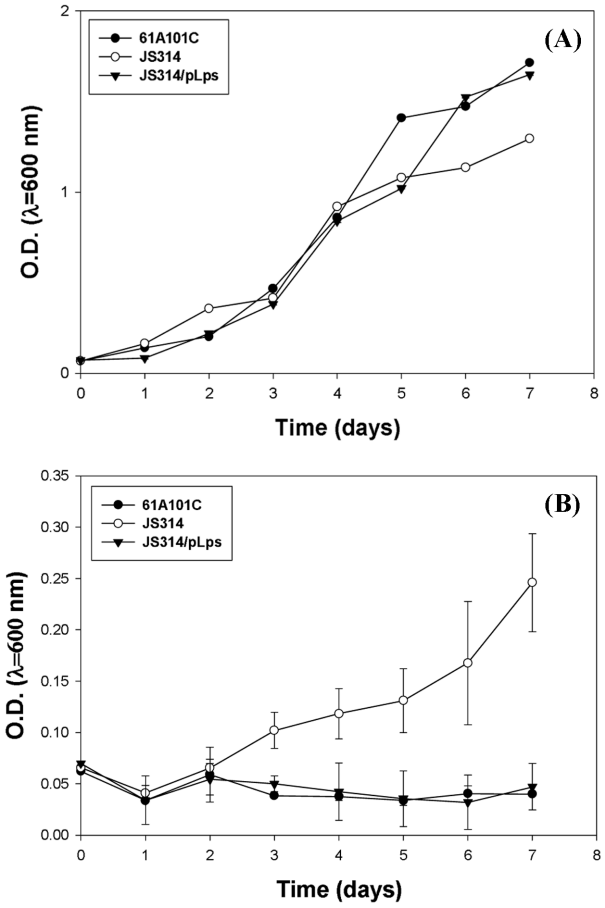

Fig. 2. Growth kinetics of *B. japonicum* strains in the liquid AMA-MES medium.
(A) The growth rate of the cells in AMA-MES liquid medium at low pH 5.5. (B) The growth rate of the cells in AMA-MES medium supplemented with 2 mM acetic acid (pH 5.5). (●), Wild-type; (○), LPS mutant; (▼), the complemented strain.

Recently, it has been reported that LPS-associated gene expression of *Helicobacter pylori* is induced by acid [9]. We observed in this study that, on AMA-MES agar plate supplemented with acetic acid, JS314 became more mucoid than the wild-type and the complemented strains. Also, in our previous study, we confirmed that the cell surface polysaccharide of *B. japonicum* was involved in precipitation of heavy metals such as Cd^{2+}, Cu^{2+}, Pb^{2+}, and Zn^{2+} [11]. Hence, further studies on the expression of genes involved in polysaccharide synthesis (such as transcription analysis, enzyme assay, and polysaccharide analysis) are required. We are particularly interested to find out whether organic acid induces genes involved in the biosyntheses of polysaccharide or oligosaccharde in *B. japonicum*, and the knowledge obtained could be applied to effective removal of heavy metals in environment.

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REFERENCES

1. Barua, S., Y. Takfumi, H. Tadao, Y. Keiko, T. Keizo, and O. Michio. 2002. Involvement of surface polysaccharides in the organic acid resistance of shiga toxin-producing *Esherichia coli* O157:H7. *Mol. Microbiol*. **43:** 629–640.
2. Cherrington, C. A., M. Hinton, G. C. Mead, and I. Chopra. 1991. Organic acids: Chemistry, antibacterial activity and practical applications. *Adv. Microb. Physiol*. **32:** 87–108.
3. Chung, T.-W., U.-H. Jin, and C.-H. Kim. 2003. *Salmonella typhimurium* LPS confers its resistance to antibacterial agents of baicalin of *Scutellaria baicalensis* george and novobiocin: Complementation of the *rfaE g*ene required for ADP-L-*glycero*-D-*manno*-heptose biosynthesis of lipopolysaccharide. *J. Microbiol. Biotechnol*. **13:** 564–570.
4. Foster, J. W. 1999. When protons attack: Microbial strategies of acid adaptation. *Curr. Opin. Microbiol*. **2:** 170–174.
5. Kim, S. K., C.-G. Lee, and H. S. Yun. 2003. Heavy metal adsorption characteristics of extracellular polysaccharide produced by *Zoogloea ramigera* grown on various carbon sources. *J. Microbiol. Biotechnol.* **13:** 745–750.
6. Heinrichs, D. E., J. A. Yethon, and J. P. C. Whitfield. 1998. Molecular basis for structural diversity in the core regions of the lipopolysaccharides of *Escherichia coli* and *Salmonella enterica*. *Mol. Microbiol*. **30:** 221–232.
7. Louch, H. A. and K. J. Miller. 2001. Synthesis of a low-molecular weight form of exopolysaccharide by *Bradyrhizobium japonicum* USDA 110. *Appl. Environ. Microbiol*. **67:** 1011–1014.
8. Lin, J., M. P. Smith, K. C. Chapin, H. S. Baik, G. N. Bennett, and J. W. Foster. 1996. Mechanisms of acid resistance in enterohemorrhagic *Escherichia coli*. *Appl. Environ Microbiol.* **62:** 3094–3100.
9. McGowan, C. C., A. Necheva, S. A. Thompson, T. L. Cover, and M. J. Blaser. 1998. Acid-induced expression of LPS-associated gene in *Helicobacter pylori*. *Mol. Microbiol.* **30:** 19–31.
10. Noh, J., D. H. Kim, E. T. Oh, and J.-S. So. 2002. Characterization of the *rfaD* gene region of *Bradyrhizobium japonicum* 61A101C. *J. Microbiol. Biotechnol.* **12:** 826–828.
11. Oh, E. T., H. S. Yun, T.-R. Heo, S.-C. Koh, K.-H. Oh, and J.-S. So. 2002. Involvement of lipopolysaccharide of *Bradyrhizobium japonicum* in metal binding. *J. Microbiol. Biotechnol*. **12:** 296–300.
12. Park, K.-M. and J.-S. So. 2000. Altered cell surface hydrophobicity of lipopolysaccharide-deficient mutant of *Bradyrhizobium japonicum*. *J. Microbiol. Methods* **41:** 219–226.
13. Price, S. B., C. M. Cheng, C. W. Kaspar, J. C. Wright, F. J. DeGraves, T. A. Penfound, M.-P. Castanie-Cornet, and J. W. Foster. 2000. Role of *rpoS* in acid resistance and fecal shedding of *Escherichia coli* O157:H7. *Appl. Environ. Microbiol*. **66:** 632–637.
14. Rowbury, R. J. and M. Goodson. 1998. Glucose-induced acid tolerance appearing at neutral pH in log-phase *Escherichia coli* and its reversal by cyclic AMP. *J. Appl. Microbiol*. **85:** 615–620.
15. So, J.-S., W.-S. Kim, and G. Stacey. 2000. Molecular characterization of a gene region involved in lipopolysaccharide biosynthesis in *Bradyrhizobium japonicum*: Cloning, sequencing and expression of *rfaf* gene. *FEMS Microbiol. Lett.* **190:** 109–114.