Journal of Microbiology and Biotechnology
Vol. 5. No. 4, 241-243, 1995

NOTE

Increased Cell Surface Hydrophobicity of A Lipopolysaccharide-defective Mutant of *Bradyrhizobium japonicum*

PAE, KYEONG-HOON AND JAE-SEONG SO*

Department of Biotechnology, Inha University, Incheon 402-751, Korea

A lipopolysaccharide (LPS) defective mutant of *Bradyrhizobium japonicum* was characterized in terms of its cell surface hydrophobicity (CSH). By monitoring the kinetics of adhesion to hexadecane the LPS⁻ mutant was found to be far more hydrophobic than the wild type strain; the removal coefficients were 4.65 min^{-1} for the mutant, as compared with only 2.40 min^{-1} for the wild type. The possible role of cell surface hydrophobicity of *B. japonicum* in nodulation process is discussed.

Bradyrhizobium japonicum is a Gram-negative soil bacterium infecting soybean. Interaction between soybean and *B. japonicum* results in the formation of nodules, in which differentiated *B. japonicum* can fix atmospheric nitrogen into ammonia. Since the nodulation process requires intimate cell-to-cell interaction, it is evident that the physicochemical properties of the cell surface, such as hydrophobicity or hydrophilicity, play a major role (1, 3, 6, 8). In previous studies we have isolated and characterized a lipopolysaccharide (LPS) defective mutant of *B. japonicum* which shows a defective nodulation phenotype (5, 6). Subsequent chemical analysis of the mutant revealed that it was devoid of the high molecular weight portion of LPS (i.e. the O-antigenic part), supporting the notion that intact LPS is essential for nodulation. However, there have been reports indicating that the correct cell surface chemistry is less stringent, and that the overall physicochemical properties of the bacterial surface might be more important during the intimate interactions taking place during nodulation (3, 9). Microbial cell surface properties are determined by extracellular and cell-wall-associated compounds. In this study, we have investigated the effects of LPS alteration on cell surface characteristics including hydrophobicity, and have found that the mutant is far more hydrophobic than the wild type.

Bacterial strains, growth conditions and assay for cell surface hydrophobicity (CSH).

B. japonicum wild type strain 61A101C (6) and the LPS mutant strain JS314 (5, 6) were cultivated in AMA broth (per liter of deionized water; 10 g mannitol, 1 g yeast extract, 0.2 g $MgSO_4$-$7H_2O$, 0.2 g NaCl, 0.5 g K_2HPO_4, 0.005 g $FeCl_2$, pH 7.5) at 30 C. To measure the CSH, cells were harvested by centrifugation at 5,000×g, and resuspended in phosphate buffer (per liter of deionized water; 22.2 g K_2HPO_4-$3H_2O$, 7.26 g KH_2PO_4, pH 7.0) to give $O.D._{600}$=1.0. Cell surface hydrophobicity was determined by measuring the kinetics of adhesion to hexadecane as described by Rosenberg *et al.* (4). Briefly, bacterial suspensions were mixed with hexadecane as indicated and vortexed at room temperature for 5-s intervals. After each mixing period, the absorbance of the lower, aqueous phase was measured spectrophotometrically.

Increased CSH of LPS mutant.

We have previously reported the isolation and characterization of a LPS-defective mutant of *B. japonicum* (6). The mutant completely lacks the O-antigenic part of the LPS and, upon infection, induces pseudonodules on soybean which are completely devoid of bacteria, supporting the notion that the intact LPS is essential for normal nodulation. One interesting observation made on the mutant was that the mutant cells tend to aggregate (data not shown), which prompted the present study on the physicochemical properties of the mutant cells including the hydrophobicity test. The relationship between the lack of the O-antigenic part of the LPS and the overall cell surface hydrophobicity was investigated by measuring the cell adhesion to hydrocarbon as described above. Fig. 1 illustrates the partitioning pat-

*Corresponding Author
Key words: *B. japonicum*, LPS, hydrophobicity, nodulation

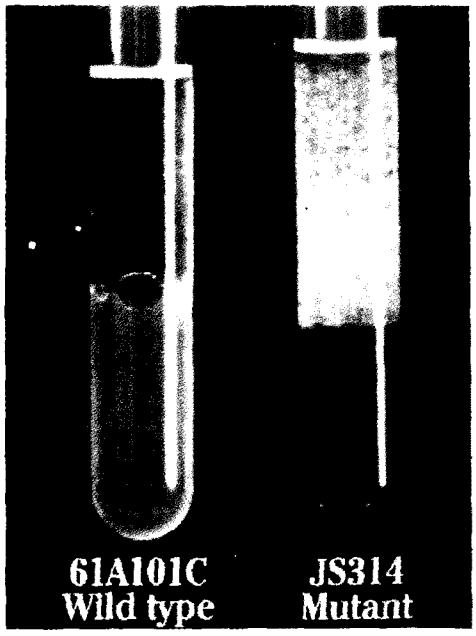

Fig. 1. Partitioning of cells of wild type and LPS mutant strains to hexadecans.
Equal volumes (2.5 ml) of cell suspensions and hexadecane were mixed and allowed to separate for 2 min.

terns of two strains. Upon mixing with hexadecane the mutant cells were immediately, and nearly completely, removed from the aqueous phase layer while the wild type cells remained. To compare the hydrophobic surface properties of the strains more precisely, we studied the kinetics of adhesion (i.e. removal rate) of cells to hexadecane. Wild type cells were far less hydrophobic than the mutant cells were (Fig. 2). For example, whereas 90% of the mutant cells were removed by 250 μl of hexadecane after 25 s of mixing, only 20% of the wild type cells were removed under the same conditions. Removal rates of wild type and LPS mutant strains were obtained from the slopes of the adhesion plots (Fig. 2A,B). When the removal rates were plotted as a function of the hexadecane-to-water volume ratio, the slopes gave the removal coefficient values for the two strains. The removal coefficient was 4.65 min^{-1} for the mutant, as compared with only 2.40 min^{-1} for the wild type. This result clearly indicates that the lack of the O-antigenic part of the LPS makes the surface of the cells more hydrophobic.

There have been a number of reports on the nodulation defective mutants of rhizobia which are concomittantly defective in their LPS structure (1, 6, 9). However, it still is not clear how the LPS defect affects the nodulation process. This study demonstrats that the change in surface hydrophobicity is cor-

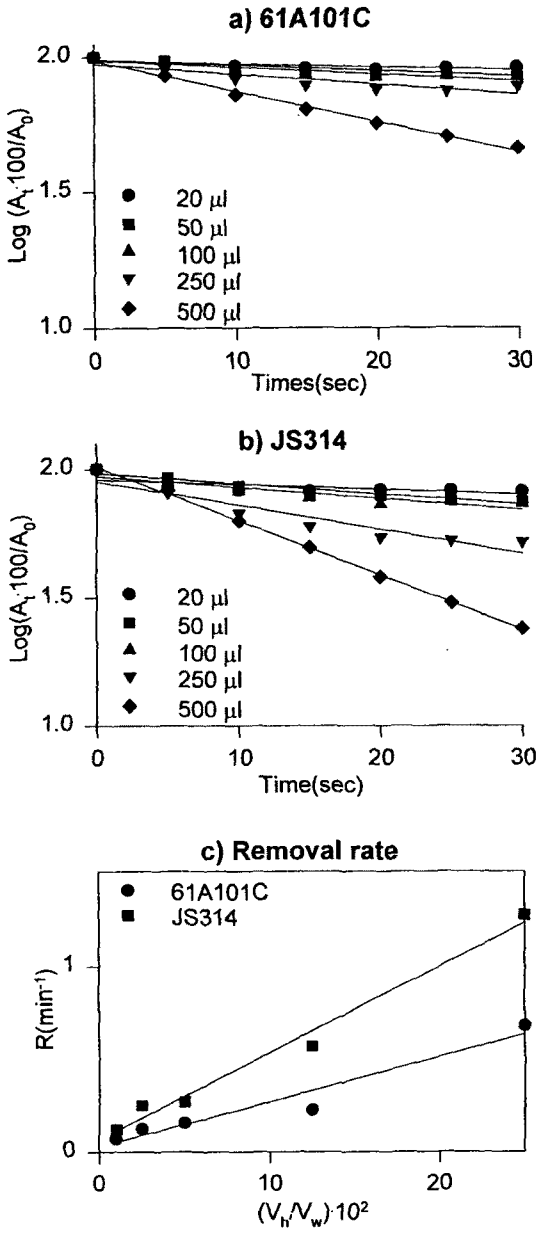

Fig. 2 Kinetics of adhesion of strain 61A101C (a) and JS 314 (b) to hexadecane, and removal rate (c) as a function of hexadecane-to-water volume ratio (v_H/V_W).
Washed bacterial suspensions (2 ml) were mixed with varying volumes of hexadecane, and at 5-s intervals the adhesion degrees were measured as described in the text (a, b). Results were presented as Log(A_t · 100/A_0) as a function of mixing time. Removal rates (c) of wild type and LPS mutant strains were obtained from the slopes of the adhesion plots (a, b).

related with the loss of the O-antigenic part of the LPS. Similar changes in cell surface properties of the LPS mutant of *Salmonella typhimurium* (7), *R. leguminosarum* (2), and *R. etli* (1) have also been observed. During interaction between plant and infecting rhizo-

bia it appears likely that the exact chemical structure of the cell surface would be less important than the overall physicochemical properties, such as cell surface charge and/or hydrophobicity. Therefore, it seems likely that the defects in the cell surface components (e.g. LPS) exert their effect rather indirectly by changing CSH and, thereby the subsequent interplay. This indirect or non-specific effect of cell surface components of rhizobia in the nodulation process was supported by the finding that defects in nodulation caused by exopolysaccharide (EPS) deficiency was complemented by gene regions responsible for LPS synthesis (3, 8).

Acknowledgements

This work was supported by a grant from the KOSEF (# 931-0500-003-2).

REFERENCES

1. Araujo, R. S., E. A. Robleto, and J. Handelsman. 1994. A hydrophobic mutant of *Rhizobium etli* altered in nodulation competitiveness and growth in the rhizosphere. *Appl. Environ. Microbiol.* **60**: 1430-1436.
2. De Maagd, R. A., C. van Rossum, and B. J. J. Lugtenberg. 1988. Recognition of individual strains of fast-growing rhizobia by using profiles of membrane proteins and lipopolysaccharides. *J. Bacteriol.* **170**: 3782-3785.
3. Putnoky, P., G. Petrovics, A. Kereszt, E. Grosskopf, D. Thi Cam Ha, Z. Banfalvi, and A. Kondorosi. 1990. *Rhizobium meliloti* lipopolysaccharide and exopolysaccharide can have the same function in the plant-bacterium interaction. *J. Bacteriol.* **172**: 5450-5458.
4. Rosenberg, M., D. Gutnick, and E. Rosenberg. 1980. Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. *FEMS Microbiol. Lett.* **9**: 29-33.
5. So, J.-S. 1991. Molecular cloning of a gene region from *Bradyrhizobium japonicum* essential for lipopolysaccharide synthesis. *FEMS Microbiol. Lett.* **83**: 299-303.
6. Stacey, G., J.-S. So, L. E. Roth, S. K. B. Lakshmi, and R. W. Carlson. 1991. A lipopolysaccharide mutant of *Bradyrhizobium japonicum* that uncouples plant from bacterial differentiation. *Mol. Plant-Microbe Interac.* **4**: 332-340.
7. Stendahl, O., L. Edobo, K.-E. Magnusson, C. Tagesson, and S. Hjerten. 1977. Surface-charge characteristics of smooth and rough *Salmonella typhimurium* bacteria determined by aqueous two-phase partitioning and free zone electrophoresis. *Acta Pathol. Microbiol. Scan. Sect. B.* **85**: 334-340.
8. Williams, M. N. V., R. I. Hollingsworth, S. Klein, and E. R. Signer. 1990. The symbiotic defect of *Rhizobium meliloti* exopolysaccharide mutants is suppressed by lpsZ$^+$, a gene involved in lipopolysaccharide biosynthesis. *J. Bacteriol.* **172**: 2622-2632.

(Received March 31, 1995)