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Improvement of Permeability to Organic Solvent in *Escherichia coli* for a Toxicity Biosensor

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Summary

The outer membrane (OM) of gram-negative bacteria acts as an effective permeability barrier against noxious agents including several antibiotics and organic solvents, and lipopolysaccharide (LPS) is the key molecule for this function.

Outer membrane modified mutants (M1-166, M2-42, M3-21) of *E. coli* DH5α/pBS1 were selected through a mutation using EMS (ethyl-methane-sulfonate). Among the selected mutants, M3-21 was twice as sensitive as LumisTox® to benzene and M2-41 was 8 times as sensitive as LumisTox® to toluene. To identify the structural change in the membrane by mutation, the relative cell surface hydrophobicities and the absorption of the crystal violet to the organisms were measured. All the mutants absorbed more crystal violet than their parent and the absorption of crystal violet increased in cell walls as carbohydrate of lipopolysaccharide decreased. When the cell surface hydrophobicities of DH5/pBS1 and its mutants were measured by the BATH, the hydrophobicities of mutants increased compared to their parent in several organic solvents. The difference of lipopolysaccharide between DH5/pBS1 and its mutants was identified by various ways such as the SDS-PAGE gel, the screening of LPS molecular weights, the mass spectrometry, and MALDI-TOF.

Introduction

Many kinds of organic solvents are generally regarded as extremly toxic to living organism and therefore impose the stress in the contaminated environment. Bacterial bioluminescence assays have been developed to provide rapid, cost-effective and accurate toxicity data for environmental science and engineering applications (Melissa and Bundy, 1999). The LumisTox® test, which is similar to Microtox test, is based on the reduction in light emitted by a nonpathogenic strain of luminescent marine bacteria *V. fischeri* upon exposure to a toxic sample (Ribo and Kaiser, 1987).

But these systems have several demerits; for example, the sensitivity of the assays is often reduced due to low temperature and high NaCl concentrations required by this marine organism. To overcome these problems, many papers were published regarding the utilization of the recombinant *E. coli* as a biosensor. The recombinant *E. coli* DH5α/pBS1 was made genetically by cloning the genes encoding bacterial luciferase and aldehyde substrate in this study. The genes originated from *Photohabdus luminescens*.

In this study, we studied on the improvement of permeability to oranic solvent in recominant *E. coli* harboring *lux* operon through lipopolysaccharide mutation. The effect of LPS mutation in a toxicity

biosensor and the structural change in the membrane by mutation were investigated.

Materials and Methods

Selection of lipopolysaccharide deleted mutants using chemical mutagen

EMS (ethly-methane-sulfonate) was used as a mutagen and added to the cell suspension at a concentration of 1.6% (vol/vol). Mutants with modified outer membrane were selected by the sensitivity test with 0.4% bile salt.

Toxicity tests for organic solvents

Phenol, benzene, toluene and p-xylene were diluted to appropriate concentration, and used in the toxicity experiments. Experimental data were expressed as the inhibitory concentrations EC50 inducing a 50% luminescence inhibition at 10 min.

Comparison of organic solvent permeability between the parent and its mutants

Hydrophobicity was measured by the BATH (Bacterial Adhesion to Hydrocarbon) method (Aono and Kobayshi, 1997). The absorption of the crystal violet to the organisms were measured by Gustaffson's method (1973).

Isolation of lipopolysaccharide and analysis the differences of lipopolysaccharide between the parent and its mutants

Lipopolysaccharide was isolated from cell-wall preparations by a modification of the method of Osborn (1962) and Kokeguchi (1990). Heptose was quantified by the colorimetric method of Wright & Rebers (1972). Total hexose was assayed by the colorimetric method of Wright & Rebers (1972) and Diche *et al.* (1949). SDS-PAGE gel of lipopolysaccharides was carried out according to the Laemmli procedure (1970). The gel was silver stained using Bio-Rad Kit by the method described by Hitchcock and Brown (1983). *O*-deacylated LPS samples were analyzed by Nancy (2000) on a Voyager or an Elite MALDI-TOF instrument (PE Biosystems, Framingham, MA) equipped with a nitrogen laser (337 nM).

Results

Mutants (M1-166, M2-42, M3-21) with modified outer membrane of *E. coli* DH5α/pBS1 were selected after a mutation using EMS (ethyl-methane-sulfonate). The observed responses of the selected mutants (M1-166, M2-41, M3-21) to organic solvents were measured after 10 min. exposure. In comparison with the commercial LumisTox®, M3-21 was more than 2 times sensitive to benzene and M2-41 was more than 8 times sensitive to toluene. But in the case of *p*-xylene, M2-41 was a half as sensitive as LumisTox®.

The structural changes in the membranes of the selected mutants were identified by comparison of the absorbed amount of crystal violet and the hydrophobicities of the strains. All the mutants absorbed more crystal violet than the parent. The cell surface hydrophobicities of mutants were higher than the parent.

The lipopolysaccharide of DH5α/pBS1 and its mutants was purified using a mixture of hot phenol-water. Purified LPS accounted for 1.96, 2.48, 1.96 and 2.0% of dry weights of the DH5α/pBS1, M1-

166, M2-41 and M3-21, respectively. To identify the difference in LPS of DH5α/pBS1 and its mutants, the assays of heptose and hexose in lipopolysaccharide were examined. All the mutants showed decreased mass of heptose and hexose compared to the parent strain.

Silver-stained SDS-PAGE gels of hot phenol-water-extracted LPS from DH5α/pBS1 and its mutants exhibited R-form LPS. There were differences between the parent and its mutants in the banding form of their lipopolysaccharide. The MALDI-TOF spectra of these *O*-deacylated LPS samples revealed the difference in molecular weights and heterogeneity of LPS between DH5α/pBS1 and its mutants.

References

1. Aono, R. and H. Kobayshi. 1997. Cell surface properties of organic solvent-tolerant mutants of *Escherichia coli* K-12. *Appl. Environ. Microbiol.* **63:** 3637-3642.
2. Kokeguchi, S., Tsutsui, O., Kato, K. and Matsumura, T. 1990. Isolation and characterization of lipopolysaccharide from *Centipeda periodontii* ATCC 35019. *Oral. Microbiol. Immunol.* **5:** 108-112.
3. Melissa, G. S. and K. J. Bundy. 1999. Toxicity measurement of orthopedic implant alloy degradation products using a bioluminescent bacterial assay. *J. Biomed. Master. Res.* **45:** 6-15.
4. Nancy, J. P., T. H. Miller, J. J. Engstrom, W. Melaugh, R. McLaughlin, M. A. Apicella, and B. W. Gibson. 2000. Characterization of chimaric lipopolysaccharides from *Escherichia coli* strain JM109 transformed with lipopolysaccharide synthesis genes (*lsg*) from *Haemophilus influenzae*. *J. Biolog. Chem.* **7:** 4747-4758.
5. Ribo, J. M. and K. L. E. Kaiser. 1987. *Photobacterim phosphoreum* toxicity bioassay. I. Test procedures and applications. *Toxicity Assess.* **2:** 305-323.