外国語要旨

Bacteria surface modification for *in vivo* imaging

1. Introduction

Many kinds of bacteria reside within the body, particularly in the intestines, where they greatly affect the host's health and immune responses[1].

Bacteria are divided into two types (Fig. 1) according to their surface structures: Gram-positive and Gram-negative bacteria. Gram-positive bacteria, such as lactic acid bacteria, have thick peptidoglycan (cell wall) layers. The peptidoglycan consists of polysaccharides made up of GlcNAc-β(1-4)MurNAc repeating units and short peptide chains cross-linking these polysaccharides. Gram-negative bacteria, on the other hand, have thin peptidoglycan layers and an outer membrane.

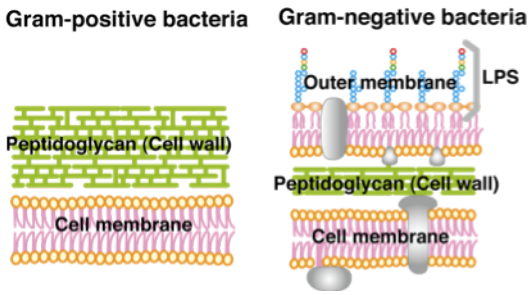


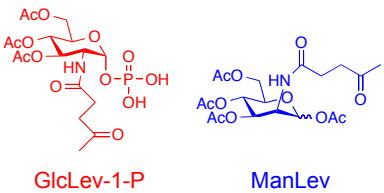
Fig. 1. Bacteria surface structure

2. The effect of bacteria cell-wall precursor (Ac_3GlcLev-1-P) for mammalian cells

We previously developed an approach to the display of a ketone moiety on the bacterial cell surface using a chemically synthesized cell-wall precursor, Ac_3GlcLev-1-P [2]. Lactic acid bacteria cultured in liquid growth medium containing Ac_3GlcLev-1-P, use the compound as a cell-wall precursor during cell-wall (peptidoglycan) biosynthesis. The displayed ketone moiety can then be used for further chemical modifications by specific coupling reaction of ketone and hydrazide. This method enables us to display various molecules, such as oligosaccharides and fluorophores, on the bacterial surface. Because the method uses peptidoglycan, which is a structure common to various bacteria, it is applicable to almost all kinds of bacteria, particularly to gram-positive bacteria, which have thick peptidoglycan layers on the surface.

Based on this approach, we now focus on the development of a new technique for *in vivo* bacterial imaging.

Human leukemic Jurkat cells and Caco-2 cells, often used as a model of the intestine, were used. ManLev, reported by the Bertozzi group[3] to be applicable to surface



display on mammalian cells, was used as a positive control for incorporation into mammalian cells. Cells were suspended in a medium, and Ac_3GlcLev-1-P was added to a final concentration 20 mM. The cells were then grown for 3 days at 37 °C in a 5% CO_2 atmosphere. Harvested cells were labeled with biotin-hydrazide (1 mM in PBS, 0.1% FBS, pH6.4), which binds specifically to the ketone group and then streptavidin Alexa Fluor 488 conjugate (5.6 mg /mL in PBS, 0.1% FBS, 0.1% sodium azide, pH7.4) via the surface-displayed ketone moiety. The fluorescent intensity of the cells was analyzed by flow cytometry (Fig. 2). Incorporation of the bacterial cell wall precursors into the mammalian cells is confirmed by fluorescence.

Fig. 2(A) shows phase-contrast micrographs of Jurkat cells after incubation for 3 days and Caco-2 cells after incubation for 4 days in the presence of Ac_3GlcLev-1-P, ManLev, or PBS alone. Few differences are observed in the incubated cells, suggesting that Ac_3GlcLev-1-P has no cytotoxic effect. Fig. 2(B) shows the fluorescence intensity of Jurkat cells and Caco-2 cells labeled with Alexa 488 after incubation with Ac_3GlcLev-1-P, ManLev, or PBS alone. The cells incubated with ManLev, but not the cells incubated with Ac_3GlcLev-1-P, showed strong fluorescence intensity, indicating that only ManLev was incorporated on the cell surface. The bacterial cell-wall precursor Ac_3GlcLev-1-P was not displayed on the mammalian cell surface, indicating that it has orthogonal properties in its incorporation into bacteria in the presence of mammalian cells.

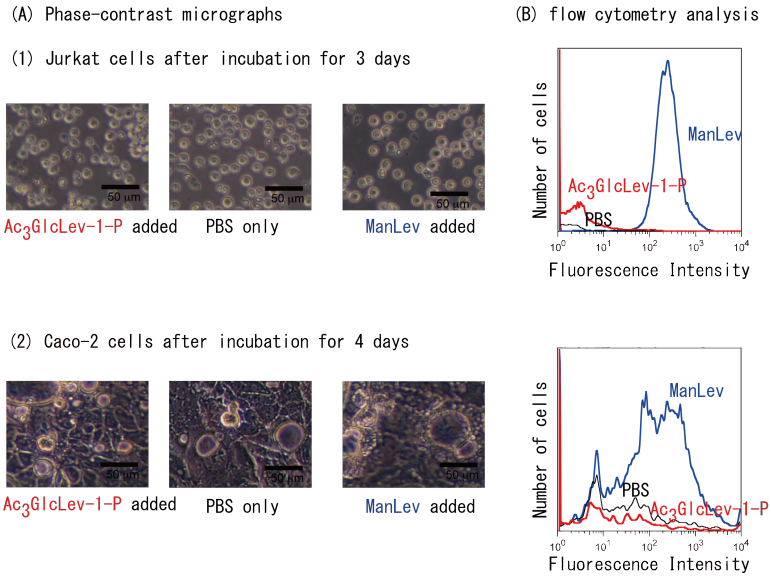
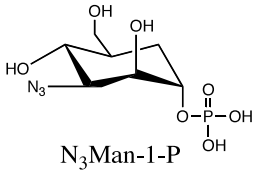


Fig. 2 Phase-contrast micrograph and flow cytometry analysis

3. The selective-labeling for Gram-negative bacteria

Next, we focused on strain-specific modification of the bacterial surface. O-antigens are often seen on the surface of Gram-negative bacteria, such as *Escherichia coli*, with the structures of O-antigen polysaccharides usually specific to each strain. The



N_3Man-1-P

O-antigen polysaccharide structure for *E. coli* O157 is known to be [->2-a-D-Rha4NAc-(1->3)-a-L-Fuc(1->)][4]. D-Rha is a relatively rare sugar among the various O-antigens, found in only three out of 180 strains of *E. coli*. Therefore, we chose a precursor of D-Rha, monophosphate-3-azido-3-deoxy-a-D-mannopyranoside-1-phosphate (N_3Man-1-P), as a base for surface modification. *E. coli* O157 (ATCC43888) was cultured in growth media with or without N_3Man-1-P, which was chemically synthesized according to a previously reported procedure. After incubation, bacteria were collected and the surface azides were labeled with a fluorophore (Fig. 3a). Flow cytometric analysis showed a stronger fluorescence for the bacteria incubated in the growth media containing N_3Man-1-P (Fig. 3b). These results suggest the possibility of strain-specific live imaging of bacteria (Fig. 3c).

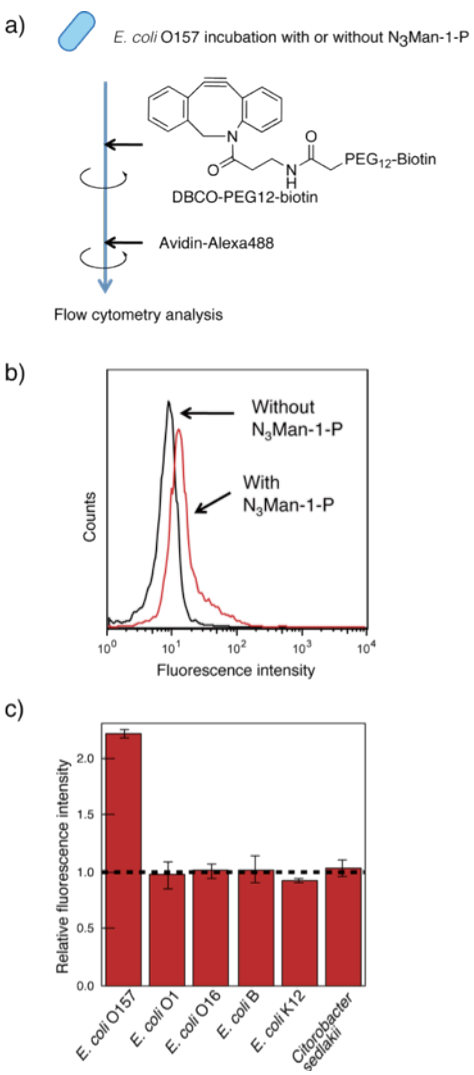


Fig. 3. (a) Experimental scheme of the flow cytometry analysis. (b) Histograms of E. coli O157 with or without N_3Man-1-P. The X axis represents fluorescence intensity (525 nm). The red line represents the bacteria incubated with 12.5 mM N_3Man-1-P, and the black line those without N_3Man-1-P. (c) Relative fluorescence intensity (525 nm) of each strain with N_3Man-1-P compared to without N_3Man-1-P. The relative fluo- rescence intensity was calculated by dividing the average fluorescence intensity of bacteria incubated with 12.5 mM N_3Man-1-P by that of bacteria incubated without N_3Man-1-P.

4. Conclusion

In summary, bacteria cell-wall precursor (Ac_3GlcLev-1-P) has orthogonal properties in its incorporation into bacteria in the presence of mammalian cells and mannosamine derivertive (N_3Man-1-P) labels only *E. coli* O157 amang the tested bacteria strain. These molecules may be applicable to the bacteria live imaging in microflora.

5. References

1) Kiyono Hirishi *et al*., Clinical Mucosal Immunology, Synergy International, Inc., pp. 2-16, **2010**. 2) Reiko Sadamoto *et al*., *Chem. Eur. J*., **2008**, *14*, 10192. 3) Eliana Saxon *et al*., *J. Am. Chem. Soc*., **2002**, *124*, 14893. 4) M. B. Perry *et al*., Biochem. Cell. Biol., **1986**, *64*, 21.