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Accumulation of Plant Galactolipid Affects Cell Morphology of *Escherichia coli*

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Monogalactosyldiacylglycerol (MGDG) is a major constituent of thylakoid membrane in chloroplasts. Therefore, it is considered to have an important role in the maintenance of the complicated structure of the thylakoid membrane. We have succeeded in cloning the enzyme for MGDG synthesis and overexpressed it in *Escherichia coli.* In this study we analyzed the morphology of the *E. coli* harboring the gene. The fatty acid composition of its membrane lipids did not differ between the wild type and transformant, except for the appearance of MGDG. However, transformant cells appeared to be elongated. DAPI staining revealed the entire intracellular region of filamentous cells to be stained; therefore, the elongation of the cells is probably due to a defect in cell division. Atomic force microscopy revealed that the transformant had a smooth but scratched surface. It was concluded that the excessive accumulation of a non-bilayer lipid, MGDG, interfered with the translocation of proteins across the plasma membrane, including those for cell division.

Key Words: **monogalactosyldiacylglycerol; atomic force microscopy; DAPI staining; fatty acid composition; *Escherichia coli;* non-bilayer lipid.**

Galactolipids are major constituents of the thylakoid membrane of chloroplasts. Monogalactosyldiacylglycerol (MGDG), which constitutes 50% of all the lipids in this photosynthetic membrane, is considered the main factor determining the physicochemical properties and structure of thylakoid membrane (1). MGDG is synthesized from uridine diphosphate (UDP)-galactose and 1,2-*sn*-diacylglycerol by a MGDG synthase, UDP-galactose: diacylglycerol galactosyltransferase (EC 2.4.1.46) (2). We previously cloned a MGDG synthase from cucumber and confirmed that on overexpression of the enzyme in *Escherichia coli,* MGDG was accumulated to a level equivalent to 17.4 mol% of the total amount of lipid (3). This transformant also exhibited a slower rate of growth suggesting that accumulation of MGDG affects cell multiplication. It was reported that abnormal synthesis of phosphatidylethanolamine (PE) resulted in an elongation of *E. coli* cells (4, 5). Since MGDG and PE are known as non-bilayer lipids, that is, they do not self-assemble into a bilayer in aqueous solution, it is possible that accumulation of MGDG in *E. coli* membrane also induces morphological changes in the cell. Moreover, an excess of non-bilayer lipids may lead to the formation of hexagonal phase lipids and affect the structure of the cell surface.

To study the effect of accumulation of MGDG on *E. coli* cells in more detail, we analyzed the fatty acid composition of membrane lipids and observed the cell morphology with light microscopy and atomic force microscopy (AFM). AFM, invented in 1986 by Binnig *et al.* (6), is considered one of the best options for studying surfaces at high resolution. However, few reports have been published on the morphological changes to bacterial cells in response to drugs (7–9). In this report, we present evidence of morphological changes in a transformant of *E. coli* caused by an accumulation of plant galactolipid.

MATERIALS AND METHODS

Extraction and analysis of lipids. Both control and transformant cells of *Escherichia coli* XL 1-Blue (MRF′) were cultured in Luria–Bertani (LB) medium and grown in a shaking incubator at 37°C for 16 h before being diluted 10-fold with LB medium. After 3 h incubation, isopropyl 1-thio-β-D-galactoside (IPTG) was added to a final concentration of 1 mM to induce the expression of fusion proteins and cells were incubated for 2 more hours. They were then harvested and washed twice with 1.2% NaCl. Total lipids were extracted as described by Bligh and Dyer (10) and separated by TLC on silica gel plates (Silica Gel 60, Merck, Darmstadt, Germany) with two solvent systems: chloroform:methanol:acetic acid (65:25:10) for the separation of phospholipids and chloroform:methanol:water (65:45:2) for

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the separation of galactolipids. For the analysis of fatty acid composition, gas chromatography was applied using pentadecanoic acid as a control.

DAPI staining. Cells were cultured as described above with the exception that IPTG was added to the culture medium from the beginning of incubation. DAPI staining was performed as described by Hiraga *et al.* (11).

Sample preparation for AFM imaging. Both control and transformant cells were cultured at 37°C for 16 h. Transformant cells grew relatively slowly, and had to be incubated for longer to obtain yields high enough for sample preparation. All cells were harvested by centrifugation and washed twice with Milli Q water. They were then suspended in 1 ml of the same solvent. An aliquot of 50 μl was deposited on a clean cover glass, rotated to distribute the droplet on the surface and allowed to air dry at room temperature. The cover glass was then glued to a metal disc of a Nanoscope IIIa (Digital Instruments, Santa Barbara, CA) atomic force microscope. Silicon nitride contact mode cantilevers of a nominal spring constant of 0.06 N/m were used for imaging.

RESULTS AND DISCUSSION

In a prior study, we reported that monogalactosyldiacylglycerol (MGDG) was accumulated on the introduction of a MGDG synthase gene into *Escherichia coli* cells, to a level equivalent to 17.4 mol% of the total amount of membrane lipid (3). Here, we compare the fatty acid composition of membrane lipids of the transformants and wild types (Table 1). Wild type *E. coli* contains three phospholipids in the membrane, phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and cardiolipin (CL). There was no significant difference in the fatty acid composition of the phospholipids between the control and transformant, though the MGDG of the transformant cells contained relatively more unsaturated fatty acids (UFA). The UFA of transformants constituted 46.4% of MGDG, 37.3% of PE, 43.3% of PG and 44.9% of CL. Although the amount of UFA in total lipid changed little, that in PE, the major phospholipid, decreased significantly in the transformant. Thus, cucumber MGDG synthase expressed preferentially in *E. coli* incorporated diacylglycerol moiety which contained unsaturated fatty acids at the *sn*-1 and *sn*-2 position. These results are consistent with a previous study (12) which analyzed partially purified spinach MGDG synthase.

During the present study, we found that *E. coli* cells overexpressing MGDG synthase accumulated galactolipid in their membrane and grew at a remarkably slow rate compared to the wild type cells. Since wild type *E. coli* does not have any glycolipid in its cytoplasmic membrane, we predicted a morphological change in the transformants. We observed cell shape by light microscopy and found that the overexpresser appeared longer than the wild type (Fig. 1). To determine whether cell elongation occurred as a result of either a defect in DNA replication or cell division, we performed DAPI staining for transformant cells. Figures 1A and 1B show a typical image of elongated cells of the MGDG overexpresser and DAPI staining. The entire intracellular region of filamentous cells was stained by DAPI, indicating that there was no defect in the segregation of the two copies of genomic material that resulted from karyogenesis. Therefore, the elongation is most

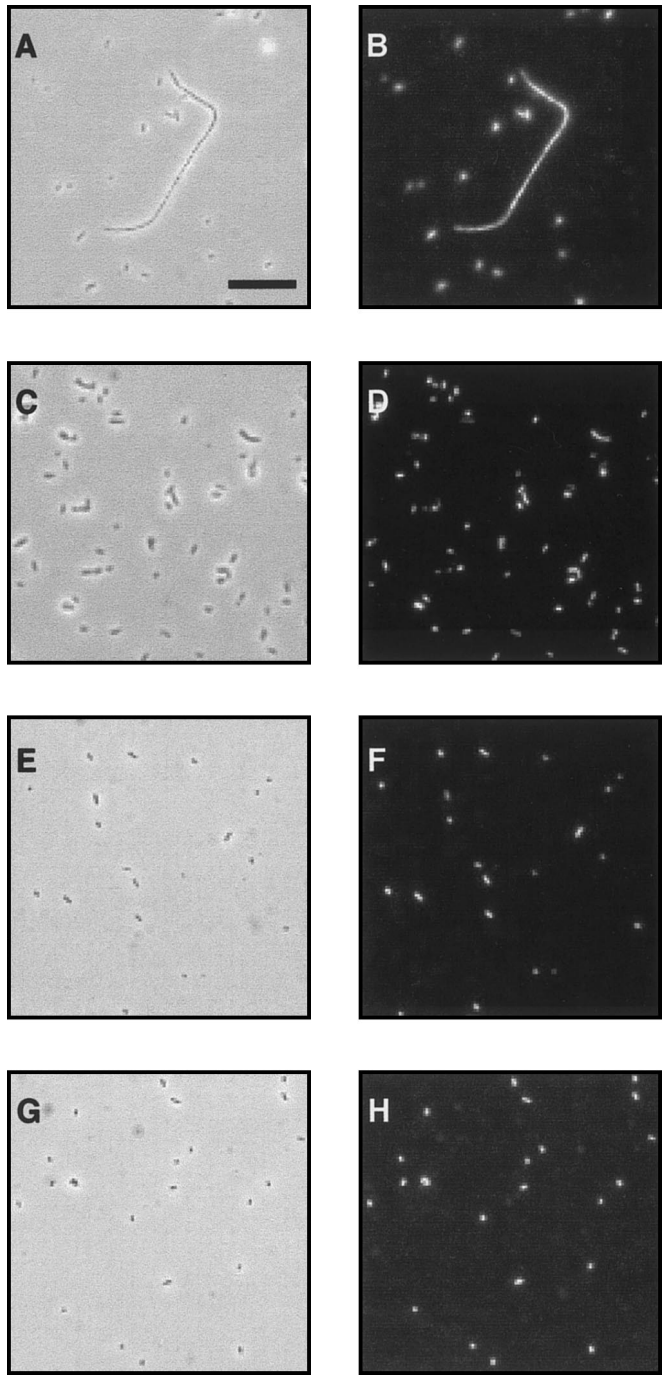

FIG. 1. DAPI staining of elongated cells. (A) Transformant cells grown in presence of IPTG, (B) DAPI staining of A, (C) morphology of transformant cells, (D) DAPI staining of C, (E) control cells grown in presence of IPTG, (F) DAPI staining of E, (G) morphology of control cells, (H) DAPI staining of G. Bar in column A indicates 10 μm. Magnification of all panels is the same.

TABLE 1

Fatty Acid Composition of Total and Individual Membrane Lipids from *E. coli* (mol%)

Lipid		Fatty acid							UFA[a]/SFA[b] ratio
		14:0	14:1	16:0	16:1	18:0	18:1	others	
TL	GEX-3X	4.0	0.5	51.6	21.5	0.9	15.5	6.1	0.75
	Transformant	4.7	0.4	51.3	21.2	0.9	14.8	6.6	0.72
PE	GEX-3X	5.4	0.7	53.4	21.3	0.8	11.4	7.0	0.66
	Transformant	5.3	0.8	55.5	17.7	0.8	11.9	8.0	0.59
PG	GEX-3X	2.4	1.2	48.8	20.0	1.5	22.1	4.0	0.87
	Transformant	2.3	1.4	48.5	18.1	1.8	23.6	4.3	0.85
CL	GEX-3X	4.1	3.0	48.4	19.9	1.8	18.0	4.8	0.81
	Transformant	4.0	4.3	47.8	18.6	2.0	19.4	3.8	0.81
MGDG	GEX-3X	—[c]	—	—	—	—	—	—	—
	Transformant	4.1	4.6	45.7	20.6	2.6	17.2	5.1	0.87

Note. TL, total lipids of *E. coli* cell membranes; GEX-3X, *E. coli* transformed with pGEX-3X; transformant, *E. coli* transformed with pGEX-3X/MGDG synthase.

[a] Unsaturated fatty acids.

[b] Saturated fatty acids.

[c] Not detected.

probably due to a defect in cell division which leads to the formation of coenocytic cells. We emphasize that the transformant celis (Figs. 1C and 1D) are basically longer than control cells (Figs. 1E–1H) even in culture with no IPTG. As indicated above, MGDG is a non-bilayer lipid which forms a hexagonal II structure (13). In the transformant used in this study, both MGDG and PE, other non-bilayer lipids, constituted 87.4% of total lipids (3). It is likely that this abnormal lipid composition affected the membrane structure of *E. coli* and resulted in inhibition of cell division. To observe the structure in more detail, we collected high resolution images of unstained, uncoated dried cells using atomic force microscopy (AFM).

Figure 2 shows a typical image of control *E. coli* cells obtained by contact mode AFM. *E. coli* cells ranged in length from 2 to 5 μm and even reached 10 to 12 μm. Cells aggregated as a result of the dragging force of liquid meniscus during drying, however, individual cells were observed at lower concentrations. Bacterial cell wall is a rigid structure and it is possible to reveal the roughness of its surface by imaging with AFM (9, 14). The origin of these surface features is still obscure. Control cell surfaces appeared normal without deformations. On zooming in to see more detail, we observed a wavy surface as shown in Fig. 2B.

Figure 3A shows extraordinarily long transformant cells that can reach a length of 15 μm. This observation

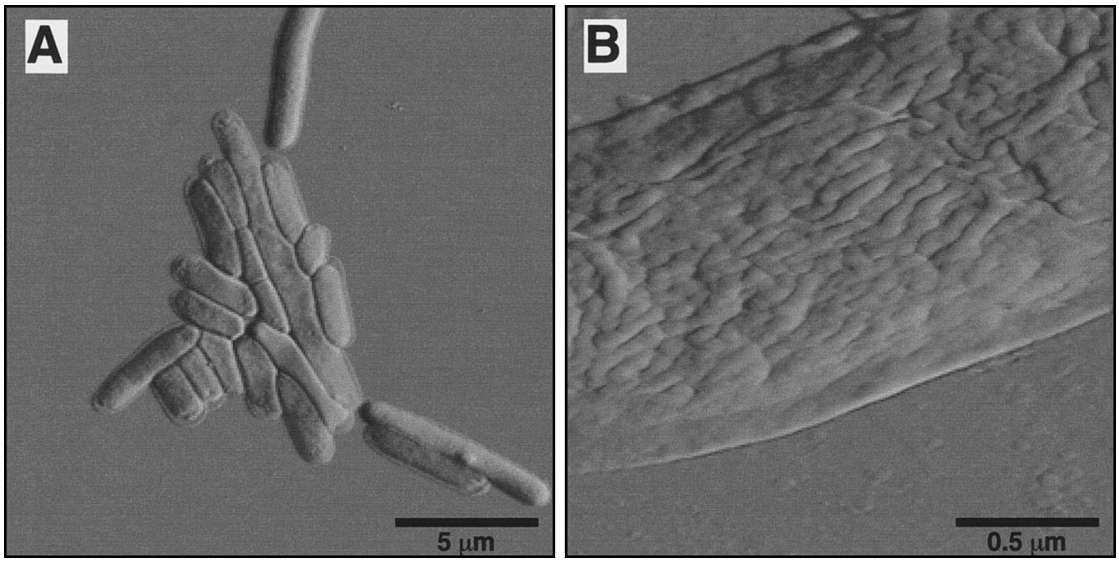

FIG. 2. AFM images of wild type control cells.

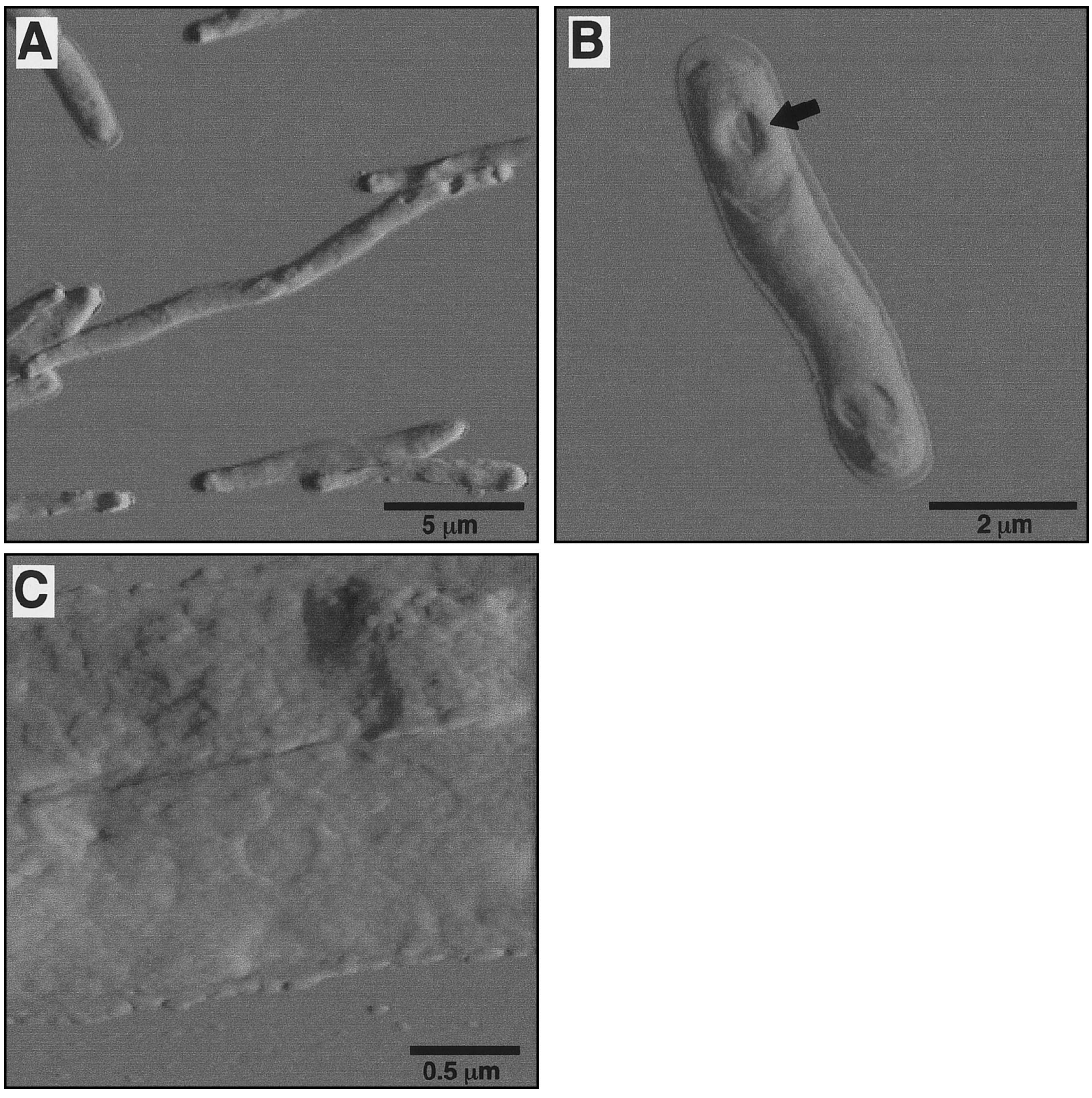

FIG. 3. AFM images of MGDG synthase overexpressing transformants. Arrow indicates the scratched surface.

is consistent with the results of light microscopy (Fig. 1). Furthermore, although the wild type *E. coli* showed smooth surfaces, all transformant cells were rough and some seemed to be scooped out (Fig. 3B). As mentioned above, MGDG is a non-bilayer lipid like PE, therefore, it is possible that an excess of non-bilayer lipids resulted in the hexagonal II structure in many places and in these scratched surfaces (15). Since MGDG is a major constituent of the thylakoid membrane of chloroplasts, we speculated that the internal structure of this transformant was changed. Ultrathin sections of the cells were prepared and analyzed by transmission electron microscopy (TEM). We could not observe any difference in the ultra structure of the transformants however (data not shown). This result indicates that major change occurred only on the cell surface including the cytoplasmic membrane. It is reported that YpfP, a protein similar to MGDG synthase from *Bacillus subtilis,* has activity to produce glucosyldiacylglycerols (16) and its null mutant made their cell length shorter than the wild type (17). The results showed that at least in this bacterium, the accumulation of glucolipid is essential to maintain cell length. In contrast, the present study showed that an excess of MGDG induced a longer cell shape in *E. coli.*

What causes the morphological change in the transformant cells that have accumulated MGDG? Figure 3C shows a magnified image of the transformant. In contrast to the control cell (Fig. 2B), a wavy surface could not be observed. This observation suggested that the proteins on the cell could not be translocated across the plasma membrane causing the drastic change in the cell surface. Rietveld *et al.* reported that non-bilayer lipids are required for efficient protein trans-

port across the plasma membrane of *E. coli* (18). It is, therefore, likely that the translocation of proteins, as well as cell division, was affected by an overaccumulation of non-bilayer lipids which resulted in the elongated cell.

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