한국환경위생학회지, 제23권 제2호 (1997)
Kor. J. Env. Hlth. Soc., Vol. 23, No. 2, pp12~23 (1997)

The Effects of Metal Compounds on the Biosynthesis of the Galactolipid and Composition of Fatty Acids in *Escherichia coli* and *Bacillus subtilis*

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*Escherichia coli*와 *Bacillus subtilis*의 糖脂質 生合成과 脂肪酸 助成에 미치는 金屬化合物의 效果

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국문요약

Potassium dichromate(PD, 500 ppm, 500 ppm), potassium chromate(PC, 500 ppm, 500 ppm), cobalt chloride(CC, 100 ppm, 10 ppm), methylmercuric chloride(MC, 100 ppm, 10 ppm)을 처리한 배지에 *E. coli*와 *B. subtilis*를 배양하는 동안에 당지질과 galactose의 생합성 및 지방산 조성을 대조구와 비교 분석하였다. 금속화합물 처리구에서의 생장과 MGDG, DGDG의 함량 및 total lipid는 대조구에 비해 감소하였다. 또한 당지질 형성에 이용된 galatose의 함량은 억제되었다. *E. coli*와 *B. subtilis*에서 MGDG와 DGDG 합성에 이용된 주요 지방산은 다양한 변화를 나타내었다.

Keywords : Galactolipid, Fatty acid, Galactose, *E. coli*, *B. subtilis*

I. Introduction

Methylmercuric chloride, which is known as chief contaminator in biosphere, inhibited the growth of *Chlorella*, decreased to the synthesis of thymidine and uridine, and brought about the scission of the single strand in DNA.[1] Also, mercury as well as cadmium and lead inhibited the activities of many enzyme having the functional sulfhydryl groups and the oxidative phosphorylation[2], and photosystem I[3,4] and photosystem II[5] in photosynthesis. All the hexavalent chromium compounds cause the mutation as a results of the direct reaction of the bacterial DNA, and the growth of bacteria treated with 400-800 μg/plate during the culture was inhibited significantly.[6]

The synthesis of nucleic acid in Hamster fibroblasts, which is cultured in medium treated with potassium dichromate, were predominantly decreased because this metal compound interacted with the cell membrane and the specific biological ligands attached in DNA.[7] Also, cobalt ion (10 mM) hindered the active transport of magnesium ion into *E. coli*.[8,9] The action of acetylornithinase, which catalyzes the last reaction of the ornithine synthesis, was inhibited by calcium, magnesium, manganese, iron, copper, zinc, nickel etc., whereas was enhanced by cobalt ion.[10] The biosynthesis of the unsaturated fatty acid was inhibited owing to decreasing of the activity of bound-induced enzyme of the unsaturated fatty acid by 3-decanoyl-N-acetylcysteamine. Many fatty acids involved in galactolipid, are characterized by high in saturation because their structure contained the double bond. The unsaturation reactions of galactolipid occur in chloroplasts or endoplasmic reticulum depending upon the types of galactolipid, respectively.[11,12]

Galactolipids are seperated from the eukaryotic galactolipid (including C-18 fatty acid located No.2 of glycerol backbone) and the prokaryotic galactolipid (including C-16 fatty acid located No.2 of glycerol backbone) depending upon the synthetic sites of diacylglycerol or phosphatic acid contributed to the fundamental skeleton.

It was confirmed that the electron source needed for the unsaturation reaction of the prokaryotic galactolipid is ferredoxin, electron carrier of photosynthesis, by experiments *in vitro* using the chloroplasts isolated from the spinach leaf[13] and the thylakoid envelope isolated from cyanobacteria[14] and the electrons is supplied by the desaturase, too.[15]

It was many reports that the metal compounds bring about the destruction of the order of environments and the natural ecosystem due to rapid development of the recent industry. As we considered above, there were many articles on the effects on the lipid metabolism by the various environmental conditions and on the cellular metabolism by the metal compounds, but the report on the biosynthesis of galactolipid, galactose and the fatty acid composition in the bacterial cells by the metal compound is few.

This study analyzed the effects of the metal compounds on the biosynthesis of galactolipid and galactose and the fatty acid composition.

II. Materials and Methods

1. Cultivation of *E. coli* and *B. subtilis*

E. coli(ATCC 25922) and *B. subtilis*(ATCC 5533) inoculated in the nutrient broth treated with PD (500 ppm, 500 ppm), PC (500 ppm, 500 ppm), CC (100 ppm, 10 ppm), MC (100 ppm, 10 ppm), respectively, were incubated at 37℃ for 4 days in the shaking incubator(130 rpm). The growth was measured by the dry weight.

2. Extraction of total lipid

Total lipid was extracted from the collected cells by the method of Bligh & Dyer.[16] After cells were mashed, added chloroform:methanol(1:2, v/v) and shaked and added the same quantity of distilled water, mixed and leaved. Total lipid in the separated chloroform layer was extracted as filtering through the Whatmann No.1 filter paper. Total lipid in the residual methanol layer was re-extracted in the same manner. These extracted materials were dried at 40-50℃ in the dry oven and the dry weight was estimated.

3. Seperation and identification of galactolipid

Galactolipids from the extracted total lipid were seperated by thin layer chromatography (TLC, Desage).[17] After TLC glass plates (20×20 cm) were coated with 0.25 mm thicks of silica gel (Merck, 60G), dried in the room temperature and activated in 110-120℃ dry oven for 60 min. before the use. Galactolipids were separated by the two-one dimension methods.[18] The first expansive solvent made use of chloroform:methanol:water(65:25:4, v/v/v) and the second expansive solvent used for chloroform:aceton:methanol:acetic acid:water(100:40:20:20:10, v/v/v/v/v). The separated galactolipids were identified to compare with the standard chemicals(Sigma). Developing reagent used for the identification of MGDG and DGDG was 1-naphthol reagent.[11]

4. Methylesterification of fatty acid

To analyze the composition of fatty acid and the levels of galactolipid by gas chromatography (Varian 3400, GC), MGDG and DGDG were methylesterified according to Allen and Good method.[19] Galactolipid isolated from the TLC plate added to methanol:sulfuric acid:benzene(100:5:5, v/v/v), mixed solution, 4 m*l* the transesterification mixture and heptadecanoic acid (Sigma), internal standard and dried in dry oven at 70℃ for 60 minutes and cooled and shaked after 5 m*l* the distilled water added. After 2 m*l* the hexane solution added in this solution and shaked strongly, the hexane layer was seperated. This procedures were repeated 2 times. The contents of fatty acid methyl esters contained in each galactolipids were measured after the seperated hexane layer was dried.[18]

5. Analysis of galactose

The contents of galactose contained in each

galactolipid were analyzed by GC. Identification of galactose concluded to compare with the standard chmicals(Sigma). Analytical conditions using the GC were the same of fatty acid one.

6. Assay of fatty acids

The types and the contents of fatty acids composed of galactolipid were analyzed by gas chromatography. The various fatty acids were identified to compared with the standard, lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3) (Sigma). The used GC detector was $FIDH_2$-flame ionization detector, column MEGABORE DB-FFAP 15m was used to FFAP 530 μm IG, charged agent. Analytical conditions were described as following;

Injection Port Temperature : 200℃
Column Temperature : 180℃
Detector Oven Temperature : 250℃
Carrier Gas : N_2 (30 ml/min.)

III. Results

1. Growth

The growth of *E. coli* and *B. subtilis* treated with PC, PD, CC, MC during the culture were represented in Fig. 1, respectively. It was showed that the growth of *E. coli* was inhibited aver. 53.91% in the PC treatment, aver. 55.26% in the PD treatment, aver. 79.59% in the CC treatment and 48.25% in the MC treatment and in *B. subtilis* was decreased aver. 56.25% in the PC treatment, aver. 30.40% in the PD treatment, aver. 48.80% in the CC treatment and aver. 41.87% in the MC treatment during the cultivation.

2. Total lipid

The contents of total lipid in *E. coli* and *B. subtilis* in each metal compound treatments were showed in Fig. 2. As showed in Fig. 2, the contents of total lipids were decreased aver. 61.33% in the PD treatment, aver. 47.14% in the PC treatment, aver. 53.55% in the CC treatment and aver. 59.50% in the MC treatment in *E. coli* and aver. 60.43% in the PD treatment,

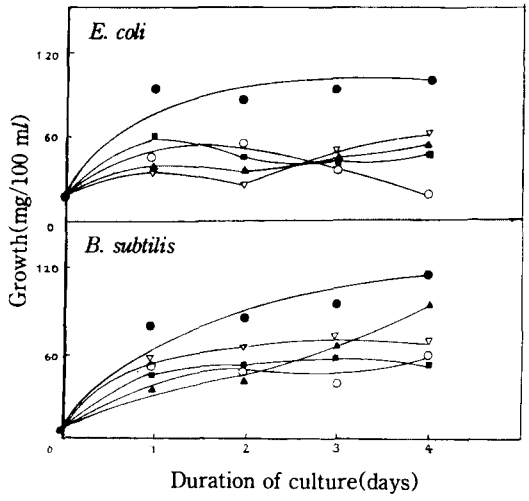

Fig. 1. Effects of various metal compounds on the growth of *E. coli* and *B. subtilis*. ● : control, ▲ : potassium chromate, ▽ : potassium dichromate, ○ : cobalt chloride, ■ : methylmercuric chloride

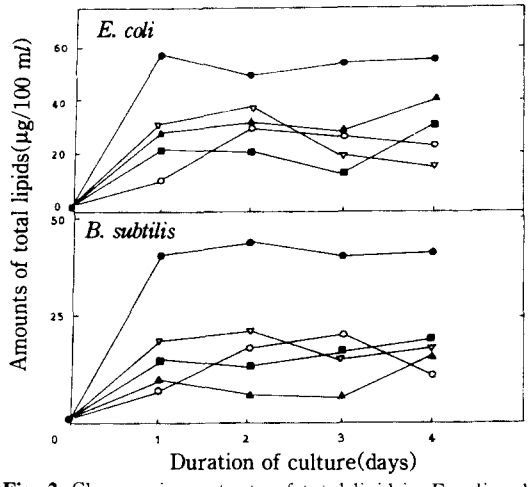

Fig. 2. Changes in contents of total lipid in *E. coli* and *B. subtilis* treated with various metal compounds during the cultivation. ● : control, ▲ : potassium chromate, ▽ : potassium dichromate, ○ : cobalt chloride, ■ : methylmercuric chloride

aver. 75.46% in the PC treatment, aver. 53.99% in the CC treatment and aver. 64.11% in the MC treatment to compare with the control in *B. subtilis*.

3. Total fatty acid methyl esters

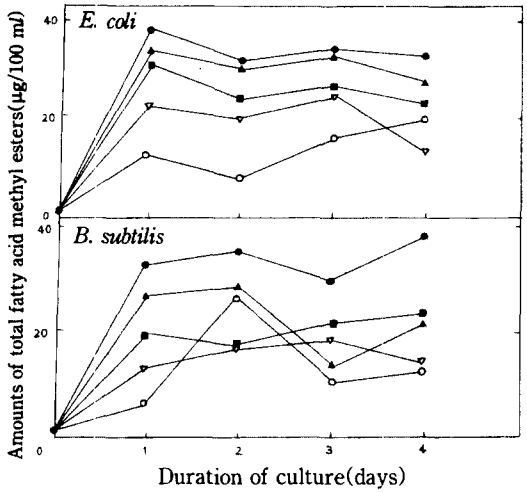

Fig. 3. Changes in contents of total fatty acid methylesters in *E. coli* and *B. subtilis* treated with various metal compounds during the cultivation. ● : control, ▲ : potassium chromate, ▽ : potassium dichromate, ○ : cobalt chloride, ■ : methylmercuric chloride

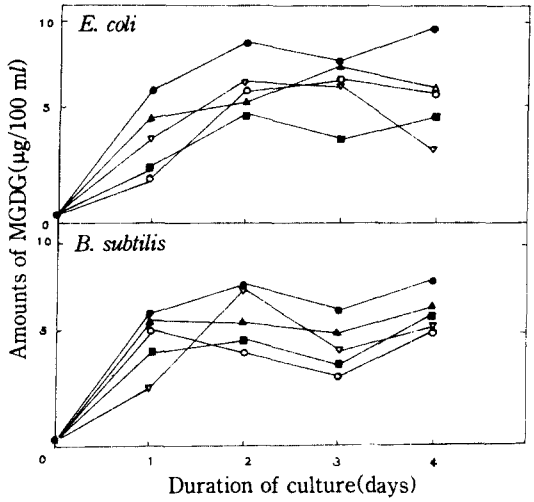

Fig. 4. Changes in contents of MGDG in *E. coli* and *B. subtilis* treated with various metal compounds during the cultivation. ● : control, ▲ : potassium chromate, ▽ : potassium dichromate, ○ : cobalt chloride, ■ : methylmercuric chloride

It was showed in Fig. 3 that the levels of total fatty acid methyl ester in *E. coli* were decreased aver. 28.75% in the PD treatment, aver. 7.81% in the PC treatment, aver. 40.01% in the CC treatment and aver. 57.50% in the MC treatment and in *B. subtilis*, aver. 40.51% in the PD treatment, aver. 42.22% in the PC treatment, aver. 55.55% in the CC treatment and aver. 49.79% in the MC treatment to compare with the control.

4. Galactolipid

The contents of galactolipid in each treatments during the culture were represented in Fig. 4 and 5. The levels of MGDG and DGDG contained total lipid in the control were 12.92% and 36.75% in *E. coli*, and 20.54% and 35.88% in *B. subtilis*, respectively. The biosynthesis of MGDG was inhibited 22.74% in the PC treatment, 46.77% in the PD treatment, 36.45% in the CC treatment and 32.90% in the MC treatment in *E. coli* and 43.72% in the PC treatment, 56.16% in the PD treatment, 36.45% in the CC treatment and 59.48% in the MC treatment in *B. subtilis* to compare with the control. The contents of DGDG in *E. coli* were decreased 44.10% in the PC treatment, 52.17% in the PD

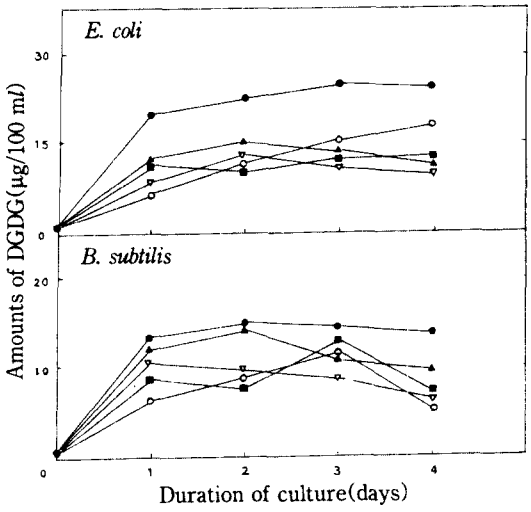

Fig. 5. Changes in contents of DGDG in *E. coli* and *B. subtilis* treated with various metal compounds during the cultivation. ● : control, ▲ : potassium chromate, ▽ : potassium dichromate, ○ : cobalt chloride, ■ : methylmercuric chloride

treatment, 58.62% in the CC treatment and 48.61% in the MC treatment. In *B. subtilis*, the biosynthesis of DGDG was hindered 12.41% in the PC treatment, 34.58% in the PD treatment, 35.65% in the CC treatment and 8.76% in the MC treatment in comparison with the control.

Table 1. Changes in contents of galactose in MGDG and DGDG in *E. coli* treated with various metal compounds during the cultivation

Duration of culture(days)	0	1					2				
Treatment Galactose	Cont	Cont	PD	MC	PC	CC	Cont	PD	MC	PC	CC
MGDG	0.74	19.05	12.74	15.12	18.75	18.77	24.01	13.50	16.52	21.05	24.00
DGDG	2.25	34.01	24.05	29.01	30.33	25.04	46.09	37.52	29.01	43.55	46.51
Duration of culture(days)	0	3					4				
Treatment Galactose	Cont	Cont	PD	MC	PC	CC	Cont	PD	MC	PC	CC
MGDG	0.74	27.72	24.06	14.22	17.24	25.90	26.25	24.78	13.50	20.52	18.60
DGDG	2.25	42.55	36.55	22.52	36.00	40.52	39.00	38.01	25.05	34.25	27.09

<NOTE> Unit : μg
<Abbre.> Cont. : Control, PD : Potassium Dichromat, MC : Methylmercuric Chloride, PC : Potassium Chromate, CC : Cobalt Chloride, MGDG : Monogalactosyldiacylglycerol, DGDG : Digalactosyldiacylglycerol

Table 2. Changes in contents of galactose in MGDG and DGDG in *B. subtilis* treated with various metal compounds during the cultivation

Duration of culture(days)	0	1					2				
Treatment Galactose	Cont	Cont	PD	MC	PC	CC	Cont	PD	MC	PC	CC
MGDG	0.74	19.50	12.74	15.12	18.75	18.77	24.01	13.50	16.52	21.05	24.00
DGDG	2.25	34.01	24.05	29.01	30.33	25.04	46.09	37.52	29.01	43.55	46.51
Duration of culture(days)	0	3					4				
Treatment Galactose	Cont	Cont	PD	MC	PC	CC	Cont	PD	MC	PC	CC
MGDG	0.74	27.72	24.06	14.22	17.24	25.90	26.25	24.78	13.50	20.52	18.60
DGDG	2.25	42.55	36.55	22.52	36.00	40.52	39.00	38.01	25.05	34.25	27.09

<NOTE> Unit : μg
<Abbre.> Cont. : Control, PD : Potassium Dichromat, MC : Methylmercuric Chloride, PC : Potassium Chromate, CC : Cobalt Chloride, MGDG : Monogalactosyldiacylglycerol, DGDG : Digalactosyldiacylglycerol

5. Galactose

The content of galactose in each treatment represented in Table 1, 2.

It was confirmed that the contents of galactose contained MGDG in *E. coli* and *B. subtilis* were decreased 48.44%, 23.08% in the PD treatment, 29.44%, 20.46% in the PC treatment, 46.48%, 10.46% in the CC treatment and 24.22%, 24.22% in the MC treatment to compare with the control, respectively. The galactose contained DGDG in *E. coli* and *B. subtilis* was inhibited 31.75%, 15.74% in the PD treatment, 4.81%, 10.99% in the PC treatment, 74.35%, 13.81% in the CC treatment and 12.10%, 17.50% in MC treatment in comparison with the control, respectively.

6. Fatty acid

The contents of lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3) used for MGDG and DGDG formation in *E. coli* and *B. subtilis* were analyzed.

The compositional changes of fatty acids in the various metal compound treatments were showed in Table 3, 4, 5 and 6. In case of the control of

Table 3. Changes in contents of fatty acid methyl esters of MGDG in *E. coli* treated with various metal compounds during the cultivation

Duratin of culture(days)	0	1					2				
Treatment / Fatty acid	Cont	Cont	PD	MC	PC	CC	Cont	PD	MC	PC	CC
Lauric acid(12:0)	-	-	-	-	-	-	-	-	45.27	13.86	8.4
Myristic acid(14:0)	1.44	7.89	10.12	5.61	7.91	8.91	1.75	14.03	2.21	0.08	5.6
Palmitic acid(16:0)	37.16	28.62	11.39	30.50	16.91	13.37	49.76	9.98	17.17	15.92	12.8
Palmitoleic acid(16:1)	-	14.05	21.07	10.14	-	-	0.35	4.08	2.34	-	-
Stearic acid(18:0)	3.02	10.03	-	-	12.05	1.03	7.16	10.02	11.57	5.67	-
Oleic acid(18:1)	9.56	-	8.36	9.87	10.11	22.79	-	-	-	22.59	7.28
Linoleic acid(18:2)	12.03	8.59	17.35	-	20.06	30.03	8.46	22.01	10.54	-	19.61
Linolenic acid(18:3)	15.34	13.52	-	15.78	9.58	10.27	3.99	28.93	-	10.14	29.04
Unknown	21.45	17.30	31.71	28.10	23.38	13.60	28.53	10.95	10.90	31.74	17.27
ToTal	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Duratin of culture(days)	0	3					4				
Treatment / Fatty acid	Cont	Cont	PD	MC	PC	CC	Cont	PD	MC	PC	CC
Lauric acid(12:0)	-	-	9.54	3.21	19.63	-	-	20.52	-	-	-
Myristic acid(14:0)	1.44	4.51	0.58	11.01	1.20	2.14	2.35	12.58	6.91	19.89	0.06
Palmitic acid(16:0)	37.16	34.09	14.01	43.95	12.21	10.83	40.13	15.13	17.21	31.42	29.16
Palmitoleic acid(16:1)	-	11.03	-	-	126.55	14.88	10.45	-	22.35	10.57	14.67
Stearic acid(18:0)	3.02	-	10.58	-	-	-	-	2.73	-	4.57	10.96
Oleic acid(18:1)	9.56	6.01	16.59	10.92	-	9.57	3.45	8.96	10.84	7.89	11.67
Linoleic acid(18:2)	12.03	14.51	-	11.08	17.09	20.16	15.89	21.37	18.76	-	5.67
Linolenic acid(18:3)	15.34	19.23	30.15	-	23.08	28.96	-	10.92	14.05	12.01	20.96
Unknown	21.45	10.62	18.55	19.83	10.24	13.46	27.73	7.79	9.88	13.65	6.869
ToTal	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

<NOTE> Unit : %

<Abbre.> Cont. : Control, PD : Potassium Dichromat, MC : Methylmercuric Chloride, PC : Potassium Chromate, CC : Cobalt Chloride, MGDG : Monogalactosyldiacylglycerol

E. coli, the use rate of palmitic acid and linoleic acid were 49.76%, 8.46% on the 2nd day of the culture and palmitic acid and linoleic acid were utilized 40.13%, 15.89% on the 4th day of the culture for the MGDG formation, respectively.

The fatty acids used for MGDG formation in the PD treatment in *E. coli* were 49.76% linolenic acid and 8.41% linoleic acid on the 2nd day of the culture and lauric acid and linoleic acid were utilized and 21.37%, 20.52% on the 4th day of the culture. It was analyzed in the PC treatment that oleic acid and palmitic acid were used 23.59% and 15.92% on the 2nd day of the culture and palmitic acid and myristic acid were 31.42% and 19.89% on the 4th day of the culture. In the CC treatment for the MGDG formation, linolenic acid and linoleic acid were utilized for 29.04% and 19.61% on the 2nd day of the culture and the use rate of palmitic acid and linolenic acid were 29.15% and 20.96% on the 4th day of the culture. Lauric acid and palmitic acid were used for 45.27 % and 17.17% on the 2nd day of the culture and palmitoleic acid and linoleic acid were utilized for 22.35% and 18.76% on the 4th day of the culture in the MC treatment .

In the biosynthesis of MGDG in the *B. subtilis* control, palmitic acid and lauric acid were used for 43.14% and 21.45% on the 2nd day of the culture and palmitic acid and linolenic acid were utilized for 28.39% and 16.04% on the 4th day of the culture. Palmitic acid and linolenic acid used for the MGDG formation in *B. subtilis* treated

Table 4. Changes in contents of fatty acid methyl esters of MGDG in *B. subtilis* treated with various metal compounds during the cultivation

Duratin of culture(days)	0	1					2				
Treatment / Fatty acid	Cont	Cont	PD	MC	PC	CC	Cont	PD	MC	PC	CC
Lauric acid(12:0)			22.12	8.28	3.88	9.56	21.45		7.69	3.22	
Myristic acid(14:0)	10.67	7.74	3.23	6.74	9.49	2.40	3.64	9.86	13.64	7.12	12.62
Palmitic acid(16:0)	38.38	41.60	10.64	20.19	25.05	33.21	43.14	27.46	25.43	23.69	24.99
Palmitoleic acid(16:1)	–	0.58	4.97	–	9.82	–	–	–		3.38	14.50
Stearic acid(18:0)	6.18	1.36	11.53	0.89	–	0.59	0.51	11.05	–	–	–
Oleic acid(18:1)	5.14	7.08	–	3.45	–	6.58	3.38	1.86	15.76	9.76	1.28
Linoleic acid(18:2)	9.58	4.56	8.20		10.64			17.34	8.04		7.08
Linolenic acid(18:3)	10.55		19.24	23.01	9.52	22.45	8.02	19.30	19.07	20.01	10.11
Unknown	19.50	37.08	20.07	37.44	31.60	25.21	19.86	13.13	10.37	32.82	29.42
ToTal	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Duratin of culture(days)	0	3					4				
Treatment / Fatty acid	Cont	Cont	PD	MC	PC	CC	Cont	PD	MC	PC	CC
Lauric acid(12:0)		21.04	9.02	9.65		4.28		21.82	10.48	9.71	
Myristic acid(14:0)	10.67	4.46	2.47	13.83	11.60	0.91	0.91	4.47	5.41	5.17	4.30
Palmitic acid(16:0)	38.38	26.38	46.68	41.95	41.69	40.22	28.39	35.50	13.46	40.16	38.99
Palmitoleic acid(16:1)	–	2.49	5.39	0.97	–	–	11.38	12.63	7.73	–	9.91
Stearic acid(18:0)	6.18	1.71	–	–	5.43	4.83		0.81	7.98	0.12	0.12
Oleic acid(18:1)	5.14	7.92	–	1.35	7.89	0.65	1.35	–	8.39	–	5.48
Linoleic acid(18:2)	9.58	–	0.39	7.06	0.88	5.29	6.78	–	3.14	1.23	
Linolenic acid(18:3)	10.55	15.12	4.58	8.34	10.31	17.81	16.04	0.83	17.70	9.96	17.53
Unknown	19.50	20.83	31.47	16.85	22.20	26.01	35.15	23.94	25.71	33.65	23.67
ToTal	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

<NOTE> Unit : %

<Abbre.> Cont. : Control, PD : Potassium Dichromat, MC : Methylmercuric Chloride, PC : Potassium Chromate, CC : Cobalt Chloride, MGDG : Monogalactosyldiacylglycerol

with PD were 27.46%, 19.30% on the 2nd day of the culture and palmitic acid and lauric acid were utilized for 35.50%, 21.82% on the 4th day of the culture. In the PC treatment, the use rate of palmitic acid and linolenic acid were 23.69%, 20.01% on the 2nd day of the culture and linolenic acid 40.16%, 9.96% on the 4th day of the culture. Fatty acids used for the biosynthesis of MGDG in the CC treatment were 24.99% palmitic acid and 12.62% myristic acid on the 2nd day of the culture, palmitic acid and linolenic acid were 38.99%, 17.53% on the 4th day of the culture. Palmitic acid and linolenic acid were used for 25.43%, 19.03% on the 2nd day of the culture and 17.70%, 13.46% on the 4th day of the culture for the MGDG formation in *B. subtilis* treated with MC. In case of DGDG in the *E. coli* control, palmitic acid and linoleic acid were utilized for 15.94%, 17.07% on the 2nd day of the culture, palmitic acid and linolenic acid were used for 27.73%, 18.02% on the 4th day of the culture.

It was confirmed in the PD treatment that palmitic acid and oleic acid were utilized for 26.91%, 17.58% on the 2nd day of the culture for the DGDG formation. In the PC treatment, palmitic acid and linolenic acid were used for 26.23%, 13.79 % on the 2nd day of the culture, 21.12%, 28.64% on the 4th day of the culture. Palmitic acid and linolenic acid were used for 24.82%, 29.69% on the 2nd day of the culture for the DGDG biosynthesis in the CC treatment of *E. coli*, and linolenic acid and linoleic acid were utilized for 25.01%,

Table 5. Changes in contents of fatty acid methyl esters of DGDG in *E. coli* treated with various metal compounds during the cultivation

Duratin of culture(days)	0	1					2				
Treatment / Fatty acid	Cont	Cont	PD	MC	PC	CC	Cont	PD	MC	PC	CC
Lauric acid(12:0)	–	–	–	–	–	–	–	–	0.05	–	–
Myristic acid(14:0)	2.18	10.04	0.10	0.08	0.68	0.12	1.77	0.02	5.10	0.12	0.15
Palmitic acid(16:0)	35.86	19.31	11.12	28.94	18.30	31.05	15.94	26.91	112.89	26.23	24.52
Palmitoleic acid(16:1)	3.41	22.33	22.37	20.82	19.89	10.87	14.54	10.74	17.90	3.62	3.86
Stearic acid(18:0)	–	6.28	13.02	–	–	3.25	8.96	5.91	20.48	10.17	6.50
Oleic acid(18:1)	0.82	–	–	11.94	1.35	–	–	17.58	–	–	–
Linoleic acid(18:2)	3.43	0.85	9.02	6.18	14.31	12.81	17.07	–	11.54	12.13	12.30
Linolenic acid(18:3)	20.91	17.63	28.03	2.71	24.81	11.07	6.52	4.81	12.55	13.79	26.69
Unknown	33.39	23.56	16.34	29.33	20.66	30.83	35.20	34.03	21.49	33.94	22.98
ToTal	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Duratin of culture(days)	0	3					4				
Treatment / Fatty acid	Cont	Cont	PD	MC	PC	CC	Cont	PD	MC	PC	CC
Lauric acid(12:0)	–	–	1.67	–	14.60	–	–	23.90	–	–	–
Myristic acid(14:0)	2.18	24.11	0.06	0.27	0.01	2.19	1.20	–	0.08	0.08	0.10
Palmitic acid(16:0)	35.86	25.10	20.69	30.73	15.80	18.17	27.73	12.71	16.44	21.12	18.44
Palmitoleic acid(16:1)	3.41	0.10	22.20	12.03	–	3.73	9.49	1.93	22.29	–	0.89
Stearic acid(18:0)	–	7.13	28.05	18.96	5.61	10.01	10.48	1.22	–	12.28	6.53
Oleic acid(18:1)	0.82	10.14	–	3.59	20.65	–	–	–	2.89	3.41	10.18
Linoleic acid(18:2)	3.43	–	–	–	–	8.75	3.98	18.03	10.56	0.73	20.34
Linolenic acid(18:3)	20.91	29.83	16.73	6.91	8.96	27.02	18.02	29.21	18.24	28.64	25.01
Unknown	33.39	3.59	10.60	27.51	34.37	30.13	29.10	13.00	29.50	33.74	18.51
ToTal	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

<NOTE> Unit : %

<Abbre.> Cont. : Control, PD : Potassium Dichromat, MC : Methylmercuric Chloride, PC : Potassium Chromate, CC : Cobalt Chloride, DGDG : Digalactosyldiacylglycerol

20.34% on the 4th day of the culture. In case of the MC treatment, stearic acid and palmitoleic acid were used of 20.48%, 17.90% on the 2nd day of the culture, palmitoleic acid and linolenic acid were utilized for 22.29%, 18.24% on the 4th day of the culture. In the control during the biosynthesis of DGDG in *B. subtilis*, palmitic acid and linolenic acid were utilized for 26.41%, 15.54% on the 2nd day of the culture and palmitic acid and oleic acid were used for 21.88%, 19.17% on the 4th day of the culture. In the PD treatment, palmitic acid and myristic acid were used for 31.55%, 16.67% on the 2nd day of the culture, palmitic acid and lauric acid were utilized for 29.66%, 19.17% on the 4th day of the culture. In the PC treatment, palmitic acid and lauric acid were utilized for 35.43%, 15.20% on the 2nd day of the culture, palmitic acid and myristic acid were used for 42.55%, 7.80% on the 4th day of the culture. Palmitic acid and lauric acid were utilized for 35.43%, 15.20% on the 2nd day of the culture, palmitic acid and myristic acid were used for 42.55%, 7.90% on the 4th day of the culture. In the MC treatment, palmitic acid and lauric acid were used for 28.84%, 12.33% on the 2nd day of the culture, linolenic acid and palmitic acid were utilized for 21.08%, 18.63% on the 4th day of the culture.

IV. Discussion

Mercuric compounds hinder the pyruvate metabolism and also the normal metabolisms owing to

Table 6. Changes in contents of fatty acid methyl esters of DGDG in *B. subtilits* treated with various metal compounds during the cultivation

Duratin of culture(days)	0	1					2				
Treatment / Fatty acid	Cont	Cont	PD	MC	PC	CC	Cont	PD	MC	PC	CC
Lauric acid(12:0)	-	12.07	-	-	-	10.06	-	-	12.33	15.20	-
Myristic acid(14:0)	13.39	3.04	3.74	8.85	15.51	9.70	5.85	16.67	-	2.93	-
Palmitic acid(16:0)	35.10	23.39	41.40	25.86	23.95	18.26	26.45	31.55	28.84	35.43	31.93
Palmitoleic acid(16:1)	7.44	-	5.61	4.15	8.01	-	4.94	1.34	8.02	-	11.20
Stearic acid(18:0)	-	-	15.77	7.25	4.85	0.27	1.29	6.79	7.97	-	4.59
Oleic acid(18:1)	10.02	11.34	-	-	-	8.28	-	-	9.18	3.28	7.34
Linoleic acid(18:2)	8.59	6.85	0.49	12.06	9.41	-	13.02	4.33	11.03	9.08	1.78
Linolenic acid(18:3)	0.68	8.59	9.57	18.22	17.06	17.03	15.54	10.76	-	13.87	10.57
Unknown	24.78	34.72	23.42	23.61	21.21	36.40	32.91	28.56	22.63	20.21	32.59
ToTal	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Duratin of culture(days)	0	3					4				
Treatment / Fatty acid	Cont	Cont	PD	MC	PC	CC	Cont	PD	MC	PC	CC
Lauric acid(12:0)			7.62	1.78	-	-	12.22	19.17	11.72	-	-
Myristic acid(14:0)	13.39	3.35	11.16	3.51	5.63	4.07	1.06	8.01	14.59	7.90	6.26
Palmitic acid(16:0)	35.10	23.89	30.19	31.99	35.58	29.47	21.88	29.66	18.63	42.55	44.24
Palmitoleic acid(16:1)	7.44	0.76	5.71	3.70	16.08	4.84	-	4.81	-	2.24	13.41
Stearic acid(18:0)	-	13.41	1.46	1.27	4.82	5.56	-	-	6.02	5.77	5.99
Oleic acid(18:1)	10.02	5.76	11.16	5.47	-	-	19.17	-	4.31	-	-
Linoleic acid(18:2)	8.59	19.07	3.05	3.03	-	1.81	9.98	0.93	-	0.63	1.57
Linolenic acid(18:3)	0.68	-	6.21	12.30	9.04	15.91	9.02	10.31	21.08	4.95	1.07
Unknown	24.78	33.76	23.44	36.95	28.85	38.34	26.67	27.11	23.65	35.96	27.46
ToTal	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

<NOTE> Unit : %

<Abbre.> Cont. : Control, PD : Potassium Dichromat, MC : Methylmercuric Chloride, PC : Potassium Chromate, CC : Cobalt Chloride, DGDG : Digalactosyldiacylglycerol

the interaction with lipoic acid, amino acid, protein and the thiol group of enzyme etc. And these compounds induce the chromosomal variety as influencing to the base or phosphate of nucleic acid and phenyl and the methyl mercuric derivatives retard for the synthesis of the spindle fiber during mitosis.[2] Hexavalent chromatin compounds give rise to the cytotoxic effects because of the interaction with the biomembrane and the specific biological ligands owing to the oxidation of the cellular constituents and the biosynthesis of amino acids and the accumulation of nucleotide is retard.[7] Cobalt ion hinders the active transport of magnesium ion into *E. coli*[8] and inhibits the growth, but some cells give rise to the retardation to the cobalt ion during the continuous culture because of the reduction of the energy-linked uptake of the toxicant cation.[20] It was showed in this experiment that the growth of *E. coli* and *B. subtilis* treated with the various metal compounds were decreased in comparison with the control. It was thought that the inhibitions of the metabolism by the hexavalent chromium compounds, of the growth by cobalt compounds and of mitosis by the methylmercuric chloride seem to action of the retarded elements of the growth of cells.[9] It is thought because of the metabolisms of the survival cells were persisted after the stationary phase that the growth of the control in *B. subtilis* was increased at 4th day of the cultures as showed in Fig. 2.[21]

Major Galactolipids contained in microorgan-

isms are MGDG, DGDG and TGDG, etc.

The changes of the structural material and the inhibition of the biosynthesis of galactolipid owing to the change of the permeability of the membrane and the metabolic capacity by the various metal compounds occured. And so, the biosynthesis of MGDG and DGDG were reduced to compare with the control.

The fatty acids used for the galactolipid synthesis in *Dunaliella salina* were characterized to have the high insaturation owing to including of the double bond in their chemical structure.[11,12] The contents of MGDG among lipid contained in the photosynthetic tissue of the plants as *Triform repens, Medicago sativa, Cucurbita pepo, Solenam lycopersium, Dactylis glomerta, Zea may* have the highest levels and the light harvesting complex, the oligomer form in the thylakoid envelope of chloroplast, contained MGDG as the lipid component. The reaction center of photosystem II contained only MGDG having the low unsaturation and these MGDG is not included in hexadecatrionic acid (16:3).

Because this phenomenon resulted from the other result to have very high contents of DGDG in *E. coli* and *B. subtilis*, it was showed the other compositional ratio of galactolipid depending upon a type of the plant and the microbe. And then the ratio of MGDG and DGDG in the thylakoid membrane is high in comparison with the stroma lamella. Also the microbe not include more galactolipid than the plant to compare with the result that the levels of MGDG and DGDG among total lipid in the *E. coli* and *B. subtilis* control were 45%-50% in this experiment. The major fatty acids constituted the chloroplast envelope of the mesophyll cells and the bundle sheath cells were 80% linoleic acid (18:2) and linolenic acid (18:3) and the major fatty acids composed of MGDG in the PD treatment in *E. coli* and DGDG of the MC treatment in *B. subtilis* were showed the same fatty acid pattern.

The inner envelopes of chloroplast were constituted to DGDG and the thylakoid envelopes were composed of very high contents of MGDG[22], but the major fatty acid used for galactolipid was linolenic acid. So, it was confirmed that the major fatty acids used in the control of *E. coli* and *B. subtilis*, the PC treatment of the *E. coli* MGDG and the MC treatment of the *B. subtilis* DGDG were the same pattern.

The changes in composition of the major fatty acid involved in galactolipid as well as the inhibition of the galactolipid biosynthesis were brought forth. These fatty acids have an effect on the growth of the various microbe depending upon their concentration and the chracterization. The growth of bacteria were decreased owing to the absorption of fatty acids by the change of the permeability of the cell when the unsaturated fatty acids were high concentration. The use of linoleic acid in the PD treatment and of myristic acid during the MGDG formation were increased in *E. coli*. *B. subtilis* used lauric acid in the PD treatment and myristic acid and stearic acid during the DGDG biosynthesis. In the PC treatment, *E. coli* almost used palmitic acid and linolenic acid, beside used oleic acid during the MGDG formation. In the CC treatment, *B. subtilis* used palmitic acid and palmitoleic acid during the formation of galactolipid and palmitoleic acid during the MGDG and DGDG biosynthesis. Otherwise, *E. coli* utilized for linoleic acid during the MGDG synthesis and oleic acid during the DGDG synthesis. In the event of the MC treatment, the use of palmitic acid and palmitoleic acid during the galactolipid biosynthesis and lauric acid during the MGDG formation were increased in *E. coli*. On the other hand, the use of palmitic acid, lauric acid and myristic acid during the MGDG formation and myristic acid during the DGDG synthesis were increased in *B. subtilis*. As a result, it was analyzed that the bacterial cells treated with the various metal compounds used the unsaturated fatty acids for biosynthesis of galactolipid to comparison with the control.

So then, the reports that the gram negative bacteria contained the straight chain fatty acids were constituited to the unsaturated fatty acids (18:1, 18:2) and some 16:0[23,24] and the gram negative bacteria contained the branched chain fatty acids were constituted to the saturated fatty acid all together[23,25] were agreed with the results of this experiment that *E. coli* used palmitic acid

and the unsaturated fatty acid in the control and *B. subtilis* used almost the saturated fatty acid and some unsaturated fatty acid in control. That the control of *E. coli* and *B. subtilis* in this experiment used palmitic acid and linolenic acid during the galactolipid formation were accorded with the facts that corn and pea used linolenic acid (80%) in MGDG and palmitic acid and linolenic acid (90%) in DGDG.[22]

E. coli and *B. subtilis* treated with the various metal compounds during the cultivation used the various unsaturated fatty acid during the biosynthesis of galactolipid. So it was confirmed that the metal compounds have the predominant effects on the growth and the synthesis of total lipid and galactolipid in microorganisms.

Summary

The biosynthesis of galactolipid, galactose and the fatty acid composition in *E. coli* and *B. subtilis* treated with potassium dichromate(PD, 500 ppm, 500 ppm), potassium chromate(PC, 500 ppm, 500 ppm), cobalt chloride(CC, 100 ppm, 10 ppm) and methylmercuric chloride(MC, 100 ppm, 10 ppm) during the culture were analyzed to compare with the control. The growth rate of cells, the contents of monogalactosyldiglyceride(MGDG), digalactosyldiglyceride(DGDG) and total lipid in the metal compound treatments were lower as compared with the control. And too, the contents of galactose utilized for the biosynthesis of galactolipids in these strains in the various metal compounds treatments were inhibited. The fatty acids used for the MGDG and DGDG formation in *E. coli* and *B. subtilis* treated with each metal compounds during the culture were showed to the variant compositional change.

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