한국환경위생학회지, 제23권 제2호 (1997)
Kor. J. Env. Hlth. Soc., Vol. 23, No. 2, pp 1~11 (1997)

The Effects of Metal Compounds on the Phospholipid Metabolism in *Bacillus subtilis*

Hye Young Ma, Kyung Suk Jung, Jae Seon Jang and Chong Sam Lee
Dept. of Biology, College of Natural Sciences, Sungshin Women's Univ., Seoul, Korea

*Bacillus subtilis*의 燐脂質 代謝에 미치는 金屬化合物의 效果

馬惠榮 · 鄭璟錫 · 張在善 · 李鍾三
誠信女子大學校 自然科學大學 生物學科

국문요약

Copper chloride 10 ppm, manganese chloride 100 ppm, nickel chloride 50 ppm을 각각 처리한 *Bacillus subtilis*를 배양하는 동안에 이들 세포에서 일어나는 인지질 생합성 및 지방산 조성의 변화를 대조구와 비교하여 분석하였다. 세포의 생장과 total lipid, phosphatidylethanloamine, phosphatidylcholine, phosphatidylglycerol, cardilolpin은 대조구에 비해 금속 화합물 처리구에서 저해되었는데 copper chloride가 가장 큰 억제 효과를 나타내었다. 그러나 phosphatidylinositol은 금속화합물의 영향을 받지 않았다. 인지질 생합성에 이용된 주요 지방산은 대조구에서는 palmitic acid(평균 19.00%)와 stearic acid(평균 9.58%)로 나타났다. 그러나 copper chloride 처리구는 palmitic acid(평균 17.38%)와 oleic acid (평균 15.99%)가 인지질 생합성에 이용된 주요 지방산이었고, manganese chloride 처리구는 palmitic acid(평균 15.00%)와 myristic acid(평균 14.24%), nickel chloride 처리구는 oleic acid(평균 17.87%)와 stearic acid(평균 13.78%)가 인지질 형성에 이용되었다.

Keywords : *B. subtilis*, Phospholipid, Fatty acid, Metal compounds

I. Introduction

Heavy metals have insignificanly influenced in the growth, morphology, the spore formation, the activity of the biochemical reaction of microorganism, etc.,[1] and on the cubic structure of nucleic acid, and it obstructs the oxidative phosphorylation.[2]

Heavy metals exist with the ion forms in the aqueous solution and so, they cause the problems of the environment pollution of the water ecosystem owing to the influence on the algae. In the higher plant, as these ions of the heavy metals were absorbed with water, it has influenced on photosynthesis.[3] Copper enhances the activities of the various enzymes. But, during the deficiency, the phenomenon of the etiolation by the reduction of chlorophyll is induced.[4] Then, the activity of Glutathione S-transferase (GST) in *Chlamydomonas reinhardtii* is inhibited by the heavy metals.[5] Manganese is inhibited the activity of cellulase in mold and showed more the strong action than copper.[6,7] In addition, if one injects manganese with EDTA below 0.3 mM to the erythrocyte of people or wheat leaf, the activity of aminoaevulinic acid dehydratase is inhibited.[8] Manganese and nickel give rise to the abnormal division of the root by causing the abnormality of the mitosis in the root of *Vicica faba*.[9,10] If nickel is absorbed in stomach with a very small amount, it causes the oversensitive reaction and skin allergy, and gives rise to the lung cancer.

In most microorganisms, not only the composition and the content of phospholipid, which is the membrane component, are influenced by the environment change such as temperature, pH, the component of the culture media and the growing period etc.,[11] but also it changes the function of the catalyst of the enzyme complex

of phospholipid as the substrate of the membrane.[12] Then there is the report about the various factors to have influence on the content of phospholipid and the composition of their fatty acid in the various organisms.

This study was analyzed to compare with the control on the biosynthesis of phospholipid and their fatty acid composition in *B. subtilis* treated with copper, nickel, and manganese during the culture.

II. Materials and Methods

1. Culture of B. subtilis

B. subtilis (ATCC 25922) precultured in the nutrient agar media for 24 hour at 37℃. The precultured cells were inoculated the nutrient broth treated with copper chloride (10 ppm), manganese chloride (100 ppm), and nickel chloride (50 ppm), respectively, and shaking cultured for 24 hour at 37℃ (130 ppm).

2. Extraction of total lipid

Total lipid in cells harvested at the beginning and the middle phase of the culture was extracted according to the modified method described by Bligh and Dyer method.[13] The harvested cells were added chloroform : methanol(1:2, v/v), shaked for 30 min., and added, mixed the same quantity of distilled water and the separated chloroform layer filtered by using Whatmann No. 1 filter paper and extracted total lipid. After the methanol layer, upper layer, was added and mixed with chloroform again, total lipid re-extracted by the filtration using the same filter paper in the separated chloroform layer. After the extracted total lipid dried in 45-50℃, dry weight measured.

3. Separation and identification of phospholipid

The major phospholipids, such as PE, PC, PI, PG, and CL were separated from total lipid by thin layer chromatography(TLC, Desaga). Glass plates(20×20 cm) used in TLC were coated with a 0.25 mm thicks of silica gel(Merck), dried in the room temperature and activated in 110-120℃ dry oven for 60 min. before the use. Chloroform - methanol-28% ammonia water (65:25:2, v/v/v) for the first expansive solvent and chloroform-acetonemethanol-acetic acid-distilled water (3:4:1:1:0.5, v/v/v/v/v) for the second expansive solvent were used according to the two-one dimensive method.[14] The phospholipids were identified to compare with the standard chemicals (Sigma). The developer were used the saturated butanol mixed with the 0.2% ninhydrin for PE, the dragendroff reagents for PC, the periodata-schiff's reagent for PI, and the 20% sulfuric acid mixed with ethanol for PG and CL, respectively.[15]

4. Methyl esterification of fatty acid

The separated phospholipids were methylated by the method of Allen and Good[16] in order to the analysis the compositions and the contents of fatty acid. Phospholipids separated from each plates were added 5 m*l* methanol contained 5% sulfuric acid and heptadecanoic acid as internal standard, dried for 120 min. in 60-70℃ dry oven, cooled for and then the same quantity of distilled water was added and shaked. The homogenates were added 2 m*l* hexane and separated the hexane layer after shaking powerfully. This separation procedures were repeated 3 times. The separated haxane layer was added 5 m*l* the saturated sodium bicarbonated and separated the hexane layer after shaking. The contents of fatty acid methyl ester contained in each phospholipids measured after the separated hexane layer dried.

5. Analysis of fatty acid

The compositions and the contents of fatty acids composed of each phospholipid were analyzed by gas chromatography(GC, Varian 3300). The identification of fatty acids such as lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), palmitolic acid (16:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3) were resolved by comparing the standard chemicals (sigma). The used column, such as stainless steel column(3mm×3m), used 15% DEGS(diethylglycol succinate) as packing material and H_2-flame ionization detector(FID) as GC detector. Analytical conditions were described as following;

Injection Port Temperature : 230℃

Column Temperature : 170℃
Detector Oven Temperature : 240℃
Carrier Gas : N_2(30 ml/min.)

III. Results

1. Growth

Growths were inhibited in the various metal compound treatments in comparison with the control as shown in Fig. 1.

Growth was inhibited average 63.36% in the copper chloride treatment, average 13.08% in the manganese chloride treatment, and average 20.19 % in the nickel chloride treatment to compare with the control, respectively.

2. Total lipid

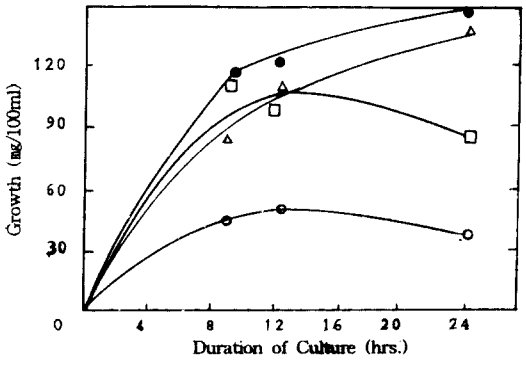

Fig. 1. Effects of metal compounds on the growth of *B. subtilis*. ● : control, ○ : copper chloride, △ : manganese chloride, □ : nickel chloride

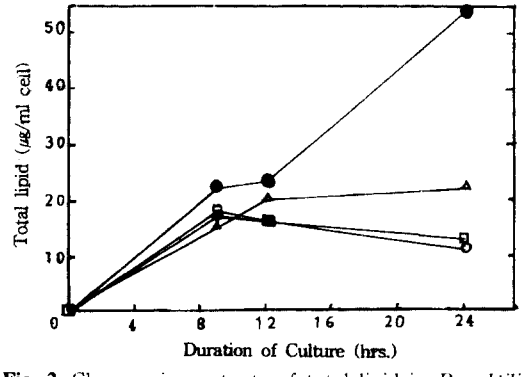

Fig. 2. Changes in contents of total lipid in *B. subtilis* treated with metal compounds during the cultivation. ● : control, ○ : copper chloride, △ : manganese chloride, □ : nickel chloride

The changes in the contents of total lipid in *B. subtilis* treated with the various metal compounds during the culture were showed in Fig. 2.

The contents of total lipid were decreased average 43.91% in the copper chloride treatment, average 39.41% in the manganese chloride treatment, and 34.27% in the nickel chloride treatment at the beginning of the culture to compare with the control. But the levels of total lipid in the copper chloride treatment and the nickel chloride treatment at the end phase of the culture were inhibited average 79.63% and average 74. 81%.

3. Phospholipid

As represented in Fig. 3 and Fig. 4, the contents of the various phospholipids in the control were contained 6.06% PE, 4.68% PC, 3.17% PI, 5. 44% PG, and 2.51% CL among total lipid.

It was showed that PE was decreased average 60.12% in the copper chloride treatment, average 21.81% in the nickel chloride treatment, and average 25.79% in the manganese chloride treatment to compare with the control during the culture.

The content of PC was inhibited average 53.12 % in the copper chloride treatment, average 23.00 % in the manganese chloride treatment, and average 16.55% in the nickel chloride treatment during the cultivation. It was observed that the level of PI was reduced average 80.56% in the copper chloride treatment, average 21.46% in the nickel chloride treatment, and average 27.18% in the manganese chloride treatment to compare with the control. PG was decreased average 71.62 % in the copper chloride treatment, average 27.99 % in the manganese chloride treatment, and average 49.03% in the nickel chloride treatment with comparison to the control. The level of CL was hindered average 31.67% in the copper chloride treatment, average 55.46% in the manganese chloride treatment, and average 11.43% in the nickel chloride treatment during the culture.

4. Total fatty acid methyl ester

As showed with Fig. 5, the contents of total fatty acid methyl esters in *B. subtilis* were reduced

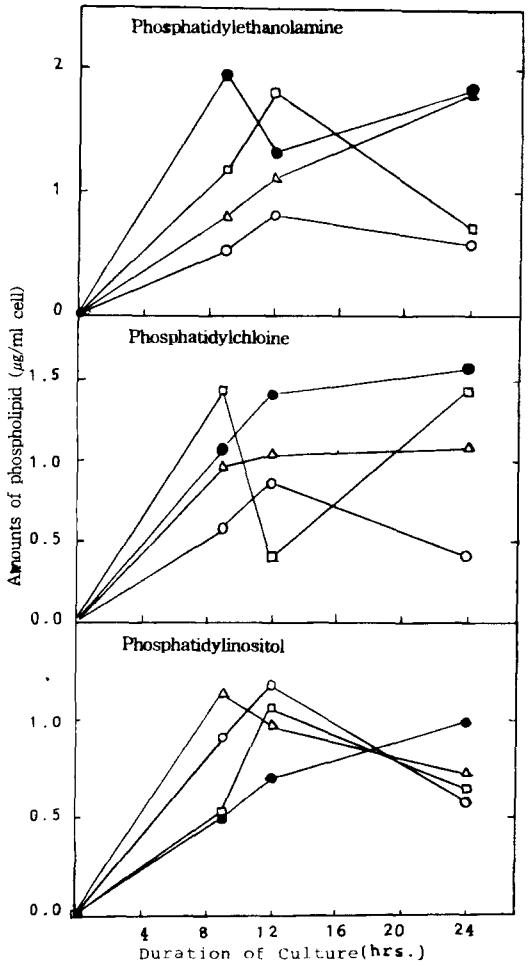

Fig. 3. Changes in contents of various phospholipid in *B. subtilis* treated with metal compounds during the cultivation. ● : control, ○ : copper chloride, △ : manganese chloride, □ : nickel chloride

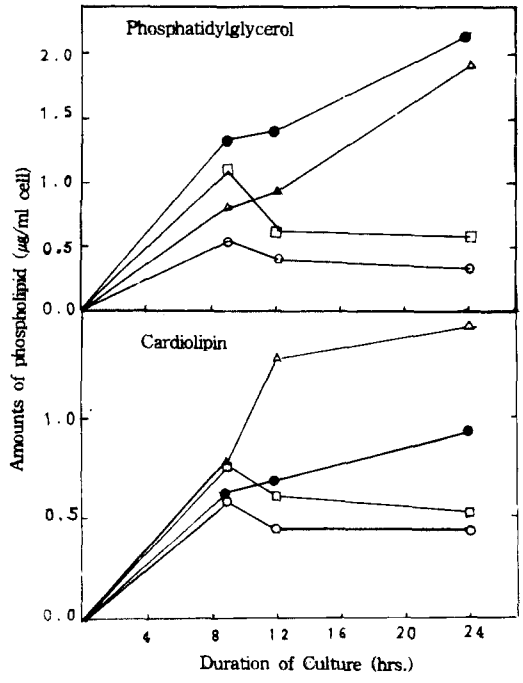

Fig. 4. Changes in contents of various phospholipid in *B. subtilis* treated with metal compounds during the cultivation. ● : control, ○ : copper chloride, △ : manganese chloride, □ : nickel chloride

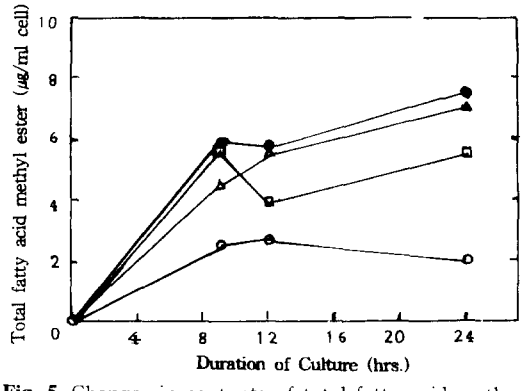

Fig. 5. Changes in contents of total fatty acid methylesters in *B. subtilis* treated with metal compounds during the cultivation. ● : control, ○ : copper chloride, △ : manganese chloride, □ : nickel chloride

average 69.90% in the copper chloride treatment, average 24.84% in the manganese chloride treatment, and average 10.95% in the nickel chloride treatment during the culture.

5. Fatty acid

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) among fatty acids utilized for the biosynthesis of PE, PC, PI, PG, and CL in *B. subtilis* were analyzed.

The changes of the fatty acid composition during the PE formation represented in Table 1. The control was utilized for myristic acid(16.38 %), and palmitic acid(25.45%) at 9 hours of the

Table 1. Changes in contents of fatty acid methyl esters of phosphatidylethanolamine in *B. subtilis* treated with various metal compounds during the cultivation

Duratin of culture(hrs.)	0	9				12				24			
Treatment / Fatty acid	Cont	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$
Lauric acid (12:0)	7.77	11.18	3.54	4.30	0.11	6.41	2.14	2.53	3.49	1.75	3.54	7.63	3.36
Myristic acid (14:0)	1.13	16.38	7.41	0.52	0.24	5.57	9.18	0.33	1.12	6.53	9.36	0.32	8.21
Palmitic acid (16:0)	21.61	25.45	24.01	2.53	0.59	17.88	30.95	1.52	7.71	19.73	1.16	11.21	17.96
Palmitoleic acid (16:1)	0.91		4.65	10.27	1.85	6.43	1.20	1.78	61.95	-	30.07	3.80	21.51
Stearic acid (18:0)	8.82	0.69	4.14	-	-	15.90	2.40	58.12	4.83	20.73	3.03	60.39	-
Oleic acid (18:1)	-	10.48	7.53	38.15	68.72	-	27.62	21.63	-	-	-	5.21	-
Linoleic acid (18:2)	2.12	0.52	-	-	6.75	2.78	-	-	0.15	4.92	-	-	19.28
Linolenic acid (18:3)	1.82	5.37	-	-	-	-	13.74	-	-	-	7.77	-	-
Unknown	45.82	29.93	38.72	44.23	21.74	45.03	12.77	14.	20.75	46.34	45.07	11.44	29.68
ToTal	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

<NOTE> Unit : %, Cont : Control

Table 2. Changes in contents of fatty acid methyl esters of phosphatidylcholine in *B. subtilis* treated with various metal compounds during the cultivation.

Duratin of culture(hrs.)	0	9				12				24			
Treatment / Fatty acid	Cont	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$
Lauric acid (12:0)	3.56	11.07	2.53	6.87	11.70	7.15	3.19	0.39	5.52	9.44	7.55	1.21	5.68
Myristic acid (14:0)	7.68	16.58	9.63	18.30	8.76	6.15	15.22	13.09	3.17	6.69	10.48	0.31	14.76
Palmitic acid (16:0)	24.00	22.82	23.54	5.94	2.96	18.94	27.10	1.34	7.42	18.67	-	14.47	3.08
Palmitoleic acid (16:1)	1.33		-	1.10	-	4.83	-	1.59	-	3.12	17.06	5.87	36.79
Stearic acid (18:0)	24.05	0.93	5.86	-	5.70	18.19	-	-	19.04	15.27	9.67	-	-
Oleic acid (18:1)	-	10.95	23.42	29.67	10.55	-	22.67	68.36	-	-	16.97	69.87	-
Linoleic acid (18:2)	17.50	0.70	-		12.16		-	4.25	11.68	-	3.11		
Linolenic acid (18:3)	0.97	1.32	-	20.17	-	-	14.30	1.36	-	-	8.79	-	-
Unknown	20.94	35.63	35.02	17.95	48.17	44.74	17.52	9.62	53.17	46.81	32.37	8.27	39.69
ToTal	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

<NOTE> Unit : %, Cont : Control

culture, palmitic acid(17.88%) and stearic acid (15.90%) at 12 hours of the culture, and palmitic acid(19.73%) and stearic acid(20.73%) at 24 hours of the culture.

It was analyzed that palmitic acid and oleic acid were used for 24.01%, 17.53% and 39.95%, 27.62% at 9 hours and 12 hours of the culture, myristic acid(9.36%) and palmitoleic acid(30.07%) at 24 hours of the culture in the copper chloride treatment to compare with the control. In the manganese chloride treatment, palmitoleic acid(10.27 %) and oleic acid(38.15%) were utilized for at 9 hours of the culture, stearic acid(58.12%) and oleic acid(21.63%) at 12 hours of the culture, palmitic acid(11.21%) and stearic acid(60.39%) at 24 hours of the culture to compare with the control. Oleic acid(68.72%) and linoleic acid(6.75%) at 9 hours of the culture, palmitoleic acid(61.95%) and palmitic acid(7.71%) at 12 hours of the culture, palmitoleic acid(21.51%) and linoleic acid(19.28%) at 24 hours of the culture in the nickel chloride treatment to compare with the control.

Table 2 was recorded the composition of fatty acid during the PC biosynthesis. Fatty acids utilized for the PC formation in the control were myristic acid(16.58%) and palmitic acid(22.82%) at 9 hours of the culture, palmitic acid and stearic acid were 18.94% and 18.19% at 12 hours of the culture, 18.67% and 15.27% at 24 hours of the culture.

It was showed that palmitic acid and oleic acid were used 23.54%and 23.42% at 9 hours of the culture 27.10% and 22.67% at 12 hours of the culture, and palmitoleic acid(17.06%) and oleic acid (16.97%) at 24 hours of the culture in the copper chloride treatment to compare with the control. In the manganese chloride treatment, oleic acid and palmitoleic acid were utilized for 38.15% and 10.29% at 9 hours of the culture and stearic acid (58.12%) and oleic acid(21.63%) at 12 hours of the culture and stearic acid(60.39%) and palmitic aicd(11.21%) at 24 hours of the culture to compare with the control.

The fatty acids used for biosynthesis of phospholipid in the nickel chloride treatment were linoleic acid(12.16%) and lauric acid(11.70%) at 9

Table 3. Changes in contents of fatty acid methyl esters of phosphatidylinositol in *B. subtilis* treated with various metal compounds during the cultivation.

Duratin of culture(hrs.)	0	9				12				24			
Treatment / Fatty acid	Cont	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$
Lauric acid (12:0)	1.97	2.79	3.25	1.17	3.90	10.90	2.60	7.15	0.53	6.66	5.25	1.02	5.31
Myristic acid (14:0)	4.00	1.56	11.58	2.94	40.78	15.16	4.21	3.17	0.25	6.87	12.92	0.60	14.00
Palmitic acid (16:0)	9.54	20.50	32.96	15.55	8.93	16.20	20.61	1.86	1.79	20.92	-	5.60	3.50
Palmitoleic acid (16:1)	7.82	10.19	-	-	-	7.44	22.08	10.67	6.71	0.15	20.30	35.77	29.81
Stearic acid (18:0)	23.57	11.65	-	22.11	0.05	3.52	-	9.67	69.38	1.32	-	14.12	-
Oleic acid (18:1)	-	9.58	17.62	-	-	-	-	6.63	-	19.52	15.26	-	-
Linoleic acid (18:2)	16.69	9.10	-	15.57	0.19	12.96	-	11.92	-	2.71	-	15.90	-
Linolenic acid (18:3)	8.21	1.16	-	-	-	-	-	3.56	-	-	11.12	trace	-
Unknown	28.20	33.57	34.59	42.66	46.15	33.82	50.50	45.37	21.34	41.85	35.15	26.99	47.38
ToTal	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

<NOTE> Unit : %, Cont : Control

hours of the culture, stearic acid(19.04%) and linoleic acid(11.68%) at 12 hours of the culture, myristic acid(14.76%) and palmitoleic acid(36.79%) at 24 hours of the culture to compare with the control.

The composition of fatty acids utilized for the PI formation were represented in Table 3.

The control was used for palmitic acid(20.50%) and stearic acid(11.65%) at 9 hours of the culture, palmitic acid(16.20%) and myristic acid(15.16%) at 12 hours of the cultrue, palmitic acid(20.92%) and oleic acid(19.52%) at 24 hours of the culture. Palmitic acid(32.96%) and oleic acid(17.62%) at 9 hours of the culture, palmitic acid(20.61%) and palmitic acid(22.08%) at 12 hours of the culture, palmitoleic acid(20.30%) and oleic acid(15.26%) at 24 hours of the culture in the copper chloride treatment to compare with the control.

The fatty acids utilized for the PI formation in the manganese chloride treatment were stearic acid(22.11%) and linoleic acid(15.57%) at 9 hours of the culture, linoleic acid (11.92%) and palmitoleic acid(10.67%) at 12 hours of the culture, palmitoleic acid(35.75%) and linoleic acid(15.90%) at 24 hours of the culture.

The major fatty acids in the nickel chloride treatment were myristic acid(40.78%) and palmitic acid (8.93%) at 9 hours of the culture, stearic acid(69.38%) and palmitoleic acid(6.71%) at 12 hours of the culture, palmitoleic acid(29.81%) and myristic acid(14.00%) at 24 hours of the culture to compare with the control.

As showed with Table 4, fatty acids utilized for the PG formation were palmitic acid(20.24%, 14.02%) and oleic acid(16.59%, 10.52%) at 9 hours and 12 hours of the culture, respectively, and myristic acid(14.29%) and palmitic acid(17.29%) at 24 hours of the culture in the control. It was utilized for myristic acid(17.16%) and palmitic acid(18.72%) at 9 hours of the culture, palmitic acid(24.90%) and oleic acid(18.40%) at 12 hours of the culture, lauric acid(13.10%) and palmitoleic acid(16.08%) at 24 hours of the culture in the copper chloride treatment. PG in *B. subtilis* treated with manganese chloride was used for stearic acid(21.97%) and linoleic acid(19.35%) at 9

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

Duratin of culture(hrs.)	0	9				12				24			
Treatment / Fatty acid	Cont	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$
Lauric acid (12:0)	8.41	1.84	8.44	0.76	6.00	10.22	1.63	53.56	5.33	2.28	13.10	1.17	1.86
Myristic acid (14:0)	16.95	10.62	17.16	1.93	38.68	9.78	5.79	7.61	30.40	14.29	9.38	5.08	10.11
Palmitic acid (16:0)	19.82	20.24	18.72	12.96	9.65	14.02	24.90	0.88	8.23	17.29	2.17	29.20	12.92
Palmitoleic acid (16:1)	0.95	6.42				6.05	5.97	3.94		8.59	16.08	35.00	28.90
Stearic acid (18:0)	0.49	3.80	5.06	21.97	17.06	4.88	7.44	4.11	33.08	1.43	8.89	-	-
Oleic acid (18:1)	15.14	16.59	14.34			10.54	18.40			12.61	11.12		
Linoleic acid (18:2)	0.44	3.13		19.35	16.50	5.06		-	15.10	2.34	-	-	
Linolenic acid (18:3)	8.09	6.03		-	0.11		16.25	2.73	-	-	-	-	-
Unknown	29.71	31.33	36.28	43.03	12.00	39.45	19.62	27.17	7.86	41.17	39.26	29.23	46.21
ToTal	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

<NOTE> Unit : %, Cont : Control

Table 5. Changes in contents of fatty acid methyl esters of cardiolipin in *B. subtilis* treated with various metal compounds during the cultivation.

Duratin of culture(hrs.)	0	9				12				24			
Treatment / Fatty acid	Cont	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$	Cont	$CuCl_2$	$MnCl_2$	$NiCl_2$
Lauric acid (12:0)	1.63	1.26	2.47	8.65	0.53	1.04	0.74	0.51	1.38	7.04	9.15	6.47	5.96
Myristic acid (14:0)	4.84	4.96	7.07	0.48	26.86	3.21	7.98	4.02	1.58	10.08	10.34	31.43	14.78
Palmitic acid (16:0)	11.42	19.26	36.49	2.60	3.21	13.53	18.12	21.81	27.37	19.52	-	4.53	4.09
Palmitoleic acid (16:1)	6.02	12.89	-	14.50	-	10.41	9.04	8.85	3.44	19.14	18.54	17.45	32.53
Stearic acid (18:0)	20.95	8.74	-	-	12.71	21.37	4.05	8.41	-	15.29	2.39	7.82	-
Oleic acid (18:1)	-	9.79	22.20	19.68	4.75	-	13.07	8.80	43.07	-	19.58	-	2.22
Linoleic acid (18:2)	14.14	7.76	-	-	-	13.42	6.72	7.75	-	-	-	6.98	23.11
Linolenic acid (18:3)	1.45	5.32	-	-	5.19	-	-	2.75	-	-	12.14	-	trace
Unknown	39.55	30.02	31.77	54.09	46.75	37.02	40.28	37.10	23.16	28.93	27.86	25.32	17.31
ToTal	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

<NOTE> Unit : %, Cont : Control

hours of the culture, lauric acid(53.56%) and stearic acid(4.11%) at 12 hours of the culture, palmitoleic acid(35.00%) and palmitic acid(29.20 %) 24 hours of the culture.

In the nickel chloride treatment, myristic acid and stearic acid were utilized for 38.68% and 17.06% at 9 hours of the culture, 30.40% and 33.08% at 12 hours of the culture, respectively, and palmitic acid(12.92%) and palmitoleic acid(28. 90%) at 24 hours of the culture.

The changes in the compostion of fatty acid in *B. subtilis* treated with CL during the culture were represented in Table 5. Palmitic acid(19.26 %) and palmitoleic acid(12.89%) at 9 hours of the culture, stearic acid(21.37%) and palmitic acid(13.53%) at 12 hours of the culture, palmitic acid(19.52%) and palmitoleic acid(19.14%) at 24 hours of the culture in the control were used for the formation of CL. Palmitic acid and oleic acid were utilized for 36.49% and 22.20% and 18.12%, 13.07% at 9 hours and 12 hours of the culture, and palmitoleic acid(18.54%) and oleic acid(19.58 %) at 24 hours of the culture in the copper chloride treatment. In the manganese chloride treatment, palmitoleic acid and oleic acid were used for 14.50% and 19.68% at 9 hours of the culture, palmitic acid and palmitoleic acid 21.81% and 8.85 % at 12 hours of the culure, myristic acid and palmitoleic acid 31.43% and 17.45% at 24 hours of the culture. Myristic acid(26.86%) and stearic acid(12.71%) were used for at 9 hours of the culture, palmitic acid(27.37%) and oleic acid(43.07%) at 12 hours of the culture, palmitoleic acid(32.53 %) and linoleic acid(23.11%) at 24 hours of the culture in the nickel chloride treatment.

IV. Discussion

Because of adaptation to manganese chloride in *B. subtilis*, it is thought that a rather long logarithmic phase in the manganese chloride treatment were induced. Growth in the metal compound treatments in this experiment was increased at the begining of the culture and then decreased at the end period of the culture. This phenomena is concord with the results that the

assimilation of cadmium didn't take place at the beginning phase of the bacterial growth, but was generated at the later phase of the culture.[17] Not only the growths in *B. subtilis* treated with the metal compounds but also the biosynthesis of total lipid were inhibited. This result is thought because the metabolism was hindered owing to the decrease by the metal compound the activity of enzyme that participated to the lipid biosynthesis. The activity of cytosine deaminase hydrolyted the amino group positioned at the sixth carbon of cytosine was completely lost by copper and manganese in *Bacillus strearothermophilus*.[18] The contents of total protein in experiment on the effect of the cellular toxin in the alveolar macrophages treated with in nickel were predominantly decreased.[19] And the copper of the low concentration was showed the fatal effect at the egg developmental stage of salmon.[20] The content of PE in the copper chloride treatment was observed the highest inhibition rate (60.12%) to compare with the control. Also, the biosynthesis of PC was predominantly inhibited by copper chloride. This result is because the biosynthesis of PC via PE didn't ocurred owing to decreasing the content of PE within the cell decreased by the inhibition of the PE formation. That the biosynthesis of PI was increased during the culture have differed from other phospholipid. This phenomena was accordant with the Daum's[21] experimintal report that PI was supplied to the normal content of total lipid. And too, the contents of PG and CL were decreased in the copper chloride treatment. In the manganese chloride treatment, the biosynthesis of PG was more synthisized than the other metal compound treatments was similar to the control at the end phase of the culture. This consequence was concord with the report that the biosynthesis of PG was enhanced in spinach chloroplast treated with 2 mM $MnCl_2$ owing to the change as the additional materials when phospholipid is formed.[22] Lipid metabolism is influenced by the various environmental factor. The biosynthesis of phospholipid and their fatty acid composition get to be different by temperature, the growth phase, the energy metabolism, light, and pH etc.,[23] phospholipid have influence on the membraneous fluidity and structure.[24,25] In this experiment the biosynthesis of phospholipid and the fatty acid composition was seen a variety of the change by the various metal compounds. Not only the heavy metals exist the free state in the cellular organelle, but also is bounded the polyphosphate or protein like methallothionin.[26]

The biosynthesis of fatty acid was inhibited owing to the decrease of the activity of β-ketoacyl carrier protein synthetase in *E. coli* treated with cerulenin because of the turbidity of enzyme activity by the heavy metals.[27] The branched chain fatty acid was discovered in *B. subtilis* and *Pseudomonas maltojphilia*. Even in case of the same species of microorganism, the almost microorganisms having branched chain fatty acid contain the saturated fatty acid (16:0, 15:0).[28] These results are accordance with the report that the gram positive bacteria is formed the branched chain fatty acid and didn't make the unsaturated fatty acid during the culture.[29]

The above report was concord with this experimental data that it was contained some unsaturated fatty acid, but was analyzed the almost saturated fatty acid in the control. The utility of the unsaturated fatty acid in *B. subtilis* treated with the various metal compounds was increased and when the concentration of the unsaturated fatty acids was high, the permeability of bacterial cell was changeable and didn't absorb the nutrition, and was increased the effect of antibacterials. And so, it was confirmed that the unsaturated and the saturated fatty acid is necessary for the growth and the existence of the normal cells.[30]

Summary

The synthesis of phospholipid and the composition of fatty acid in *B. subtilis* treated with copper chloride (10 ppm), manganese chloride (100 ppm), and nickel chloride (50 ppm) during the culture were analyzed to compare with the control. The levels of growth, total lipid, phosphatidylethanolamine(PE), phosphatidylcholine(PC), phosphatidylglycerol(PG), and

cardiolipin(CL) in *B. subtilis* treated with copper chloride were decreased predominantly. But, the biosynthesis of phosphatidylinositol(PI) was not affected by the metal compounds. The major fatty acids utilized for the formation of phospholipid were palmitic acid(average 19.00%) and stearic acid(average 9.88%) in the control. In the copper chloride treatment, however, palmitic acid (average 17.35%) and oleic acid(average 15.99%) made use of the major fatty acid during the biosynthesis of phospholipids. It was showed that oleic acid(average 17.87%) and stearic acid (average 13.78%) in thee manganese chloride treatment, and palmitic acid(average 15.00%) and myristic acid(average 14.24%) in the nickel chloride treatment were utilized.

References

1) Song, H. I. and Yu, T. S. : Morphological changes of *Hansenula anomala* B-7 by cadmium ion, Kor. J. Microbiol., 29(6), 397-410, 1991.
2) Vallee, B. and Ulmer, D. D. : Biochemical effects of mercury, cadmium and lead, Ann. Rev. Biochem., 41, 91-128, 1972.
3) Chun, H. S., Kwon, Y. M. and Lee, C. B. : Comparison of toxic effects of mercury, copper and zinc on photosystem II of Borley chloroplasts, Kor. J. Bot., 36(3), 195-201, 1993.
4) Park, I. H. and Kwon, Y. M. : Effects of cupic sulfate on the chlorophyll fluorescene in isolated chloroplasts from chinese cabbage, Kor. Biochem. J., 19, 207-212, 1987.
5) Shon, J. G., Kang, S. Y., Lee, J. W., Chai, Y. G. and Choi, Y. K. : The effect of heavy metals on the activity of Glutathione S-transferase from Chlamydomonas reinhardtii, Kor. J. Microbiol., 31(6), 561-565, 1993.
6) Choi, M. J., Kim, Y. M. and Kim, W. S. : Influence of some metal ions on the cellulase activity, Kor. J. Microbiol., 14, 75-83, 1976.
7) Kim, W. S. and Lee, S. J. : Investigation of cellulase of microbiol origin[1], Studies on some properties of cellulase isolated from Alternaria sp. ibid., Kor. J. Microbiol., 14, 65-74, 1976.
8) Border, E. A., Cantrell, A. C. and Kilroe-Smith, T. A. : The in vitro effect of zinc and other metal ions on the activity of human erythrocyte aminolaevulinic and dehydratase, Environ. Res., 11, 319-325, 1976.
9) Umeda, M. and Nishimura, M. : Inducibility of chromosomal aberrations by metal compounds in cultured mammalian cells, Mutation Res., 67, 221-229, 1979.
10) Leonard, A., Gerber, G. B. and Jacquet, P. : Carcinogenicity, mutagenicity and teratogenicity of nickel, Mutation Res., 87, 1-15, 1981.
11) Knivett, V. A. and Cullen, J. : Some factors affecting cyclopropane and formation in Escherchia coli, Biochem. J., 96, 771-776, 1965.
12) Stadtlander, K. S. and Ahlers, R. J. : Influence of frowth condition on the composition of the plasma membrane fractions, J. Cellular Biochem., 20, 369-380, 1982.
13) Bligh, E. G. and Dyer, W. J. : A rapid method of total lipid extraction and purification, Can. J. Biochem. Physio., 37, 911-917, 1959.
14) Turner, J. D. and Rouser, G. : Precise quantitative determination of human blood lipids by thin-layer and triethylaminoethylcellulose column chromatography II Plasma lipids, Anal. Biochem., 38, 437-445, 1970.
15) Skipski, V. P. and Barllay, M. : Thin-layer chromatography of lipids, Methods Enzymol., 14, 530-598, 1969.
16) Allen, C. F. and Good, P. : Acyl lipids in photosynthetic systems, Methods Enzymol., 23, 523-547, 1971.
17) Yu, T. S., Park, J. M. and Song, H. I. : Effects of Tritonx-100 on intracellular accumulation of cadmium in Hansenula anomala, Kor. J. Microbiol., 25(2), 110-116, 1987.
18) Yu, T. S., Kim, T. H. : Some properties of partially purified cytosine deaminase from Bacillus stearothermophilus, Kor. J. Microbiol., 30, 305-309, 1992.
19) Aranyl, C. F., Miller, F. J., Andres. S., Ehrilich, R., Fenters, J., Garder, D. E. : Cytotoxicity to alveolar macrophages of trace metals absorbed on Fly-ash, Environ. Res., 20, 14-23, 1979.
20) Craik, J. C. A. and Harvey, S. M. : A preliminary account of metal levels in eggs of formed and wild Atlantic salmon and their relation to egg mortality, Aquaculture., 73(1-4), 309-322, 1988.
21) Daum, G., Kohlwein, S. D., Zinser, E. and Paltauf, F. : Effect of inositol starvation on glycerolipid metabolism in *Saccharomyces uvarum*, Biochem. Biophy. Acta., 753, 430-438, 1983.
22) Sparace, S. A. and Mudd, J. B. : Phosphatidylglycerol synthesis in spinach chloroplasts, characterization of the newly synthesized molecule, Plant Physiol., 70, 1260-1264, 1982.
23) Cronan, J. E. J. and Vagelus, P. P. : Metabolism and function of membrane phospholipids of *Escherichia coli*, Biochem. Biophy. Acta., 265, 25-

60, 1972.
24) Sato, N., Murata, N., Miura, Y. and Ueta, N. : Effects of growth temperature on lipid and fatty acid composition in the blue-green algae, Biochem. Biophy. Acta., 572, 19-28, 1979.
25) Sauer, A. and Hense, K. P. : On the light-dependence of fatty acid synthesis in spinach chloroplasts, Plant Physiol., 73, 11-15, 1983.
26) Hamer, D. H. : Metallothionein, Ann. Res. Biochem., 55, 913-951, 1986.
27) Cronan, J. E. J. and Gelman, E. P. : Physical propereies of membrane lipids, Biologicalrelevance and regulation, Bacteriol. Rev., 39, 232-256, 1975.
28) Kaneda, T. and Smith, E. J. : Relationship of primer specificity of fatty acid *de novo* synthesis to fatty acid composition 10 speices of bacteria and yeasts, J. Microbiol., 26, 893-898, 1980.
29) Kaneda, T. and Smith, E. J. : Fatty acids of the genus *Bacillus*, an example of branched-chain preference, Bacteriol. Rev., 41, 391-418, 1977.
30) Hsuchen, C. C. and Fringold, D. S. : Polyene antibiotics action on lecithin liposomes, effects of cholesterol and fatty acyl chains, Biochem. Biophys. Res. Commun., 51(4), 972-978, 1973.