

## Identification of the PEG-Induced Proteins by 2D-Gel Electrophoresis and Mass Spectrometry in *Sphingopyxis macrogoltabida* strain 103

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### ABSTRACT

*Cell-free extracts from a PEG 4000-utilizing bacterium, Sphingopyxis macrogoltabida strain 103, grown on glucose and PEG 4000 medium were separated into cytoplasmic, membrane-bound and signal protein fractions. Each fraction was analyzed by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). A total of 19 differentially-expressed proteins by PEG were in-gel trypsin digested and the digestion mixtures were analyzed by MALDI-TOF mass spectrometry to determine the molecular masses of the resulting tryptic peptides. Ten proteins in the cytoplasmic fraction showed homology to fatty acyl CoA synthetase, IEA two-component response regulator, permease, LacI-transcription regulator, galactinol synthase, coenzyme PQQ synthesis protein, transcription regulator, translation-initiation factor like protein, CheY-like two-component response regulator and hypothetical protein. Three proteins in the membrane-bound fraction were identified as LysR-transcription regulator, GutR-transcription regulator and two-component response regulator. Six proteins in the signal protein fraction were polyphosphate kinase, ATP sulfurylase, amino acid permease, sigma-54 dependent transcription regulator, fatty-acid-CoA ligase and LysR-transcription regulator. These proteins are expected to be relevant to PEG metabolism by S. macrogoltabida strain 103.*

**Key words:** PEG degradation, *Sphingopyxis macrogoltabida*, 2D-PAGE, MALDI-TOF mass spectrometry

### INTRODUCTION

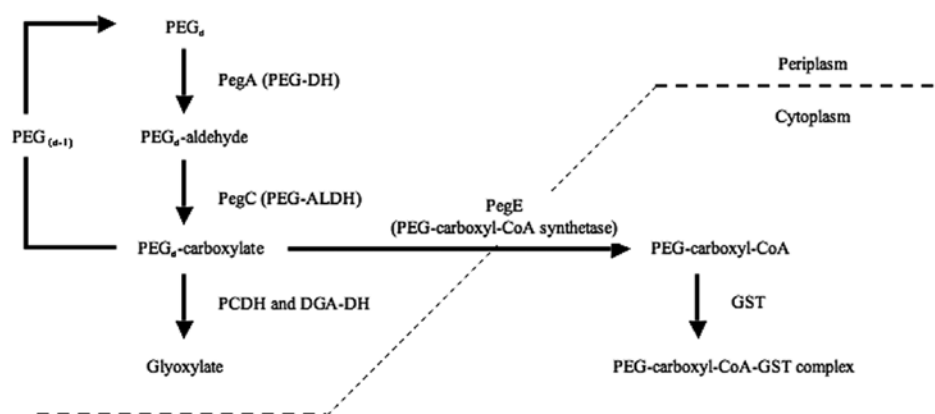
Polyethylene glycols [PEGs, HO(CH<sub>2</sub>-CH<sub>2</sub>O)<sub>n</sub>H] are man-made polymers that have been widely used as commodity chemicals in various industrial products such as pharmaceuticals, cosmetics and lubricants and as raw materials in the synthesis of nonionic surfactants and polyurethanes. Since they are water-soluble,

they finally show up in natural streams or wastewater systems as dilute solution, which can neither be recycled nor incinerated. Because of their random coil formation (Cox, 1978), the alcohol groups at the termini are randomly distributed in the space taken up by the macromolecule, resulting in the difficulty of attacking the termini by enzymes. Moreover, from a chemical point of view, aliphatic ether bonds are very stable, therefore, the compounds have long retention (Bailey and Koleske, 1976). Hence, microbiological degradation appears to be the only means to decompose this group of polymers.

Biodegradation of PEG was first reported on a PEG 400-utilizing bacterium (Fincher and Payne, 1962). Since then, many reports on aerobic PEG-utilizing bacteria have been made on various molecular sizes of PEG (Haines and Alexander, 1975; Ogata et al., 1975; Hosoya et al., 1978; Obradors and Aguilar, 1991; Takeuchi et al., 1993; Kawai and Takeuchi, 1996). The well-documented examples are found in PEG-utilizing Sphingomonads, the metabolically-versatile bacteria that can utilize many types of xenobiotic recalcitrant compounds as well as a wide range of naturally-occurring organic compounds. Many Sphingomonads are able to degrade PEGs with average molecular weights of more than 4,000 as a sole carbon and energy source. PEG metabolization has been reported either in an axenic, e.g., *Sphingomonas macrogoltabidus*, a PEG 4000 utilizer (Kawai and Enokibara, 1996) or in a PEG 20,000-utilizing mixed culture, e.g., *Sphingomonas terrae* with a *Rhizobium* species, *Agrobacterium* species or *Methylobacterium* species (Kawai and Yamanaka, 1986; Takeuchi et al., 1993). Both Sphingomonads were reidentified as *Sphingopyxis macrogoltabida* and *Sphingopyxis terrae*, respectively (Takeuchi et al., 2001).

The metabolism of PEG by Sphingomonads has been previously proposed as responsive of the peg operon (Charoenpanich et al., 2006) and the genes located downstream of the operon (Somyoonsap et al., 2008). First, PEG was aerobically degraded by successive oxidation of a terminal alcohol group to an aldehyde and a carboxylic group (Fig. 1). An ether bond adjacent to a carboxyl methyl group is split to yield glyoxylic acid, resulting in depolymerization by one glycol unit (Enokibara and Kawai, 1997; Kawai, 2002). All the steps occurred in periplasm were catalyzed by three enzymes (i) PEG dehydrogenase, PEG-DH (PegA) (Sugimoto et al., 2001), (ii) PEG-aldehyde dehydrogenase, PEGAL-DH (PegC) (Ohta et al., 2005) and (iii) PEG-carboxylate dehydrogenase, PCDH (Somyoonsap et al., 2008) or digoxycid dehydrogenase, DGA-DH (Yamashita et al., 2004). Then, PEG-carboxylate was changed to PEG-carboxyl-CoA by the action of PEG-carboxyl-CoA synthetase, PegE (Tani et al., 2008) and moved into cytoplasm before being detoxified by glutathione-S-transferase, GST (Somyoonsap et al., 2008). All enzymes responsive for the upstream metabolism of PEG have been proved as inducible enzymes triggering from the presence of PEG (Charoenpanich et al., 2006; Somyoonsap et al., 2008). However, the mechanism of PEG uptaking and a downstream metabolism after PEG-carboxyl-CoA-GST complex is still doubtful. Thus, it is possible to find the other proteins relevant to PEG uptaking or downstream metabolism from the unique expressed protein after induction of PEG. Since two-dimensional polyacrylamide gel electrophoresis (2D-PAGE)

(Klose, 1975; O' Farrell, 1975) is a powerful tool to study the changes in cellular protein expression (Choe and Lee, 2000) and thousands of different proteins can be separated, information such as the protein pI, the apparent molecular weight and the amount of each protein can be obtained and used for identification. Hence, the objective of this study is to understand the entire complement of the proteins which expressions have been induced by PEG. A combination of 2D-PAGE and MALDI-TOF mass spectrometry was used for separation of differentially-expressed proteins and their identification.



**Figure 1.** Enzymes involved in PEG metabolism in *S. macrogoltabida* strain 103 (Somyoonsap et al., 2008).

## MATERIALS AND METHODS

### Cell cultivation and harvesting

*S. macrogoltabida* strain 103 (Takeuchi et al., 1993; Takeuchi et al., 2001) was cultivated at 28°C in PEG 4,000 medium and glucose medium (Kawai et al., 1985) until late exponential phase ( $OD_{600}$  in the range of 0.6 to 0.8). Cells were collected by centrifugation at  $5,000 \times g$  for 10 min at 4°C, washed with 0.9% NaCl, and stored at -80°C until use.

### Preparation of protein fractions

The cell pellets were resuspended in 5-ml of lysis buffer I (10 mM Tris-HCl, 1 mM EDTA at pH 8.0, 1 mM phenylmethylsulfonylfluoride, PMSF and 1 mM dithiothreitol, DTT) and then disrupted by ultrasonication as described previously (Charoenpanich et al., 2006). One-tenth volume of nuclease solution (1 mg DNaseI  $ml^{-1}$ , 0.25 mg RNaseA  $ml^{-1}$ , 50 mM  $MgCl_2$ , 24 mM Tris (base) and 476 mM Tris-HCl) was added and incubated on ice for 20 min to remove contaminated nucleic acid. Unbroken cells were removed by centrifugation at  $10,000 \times g$  for 30 min, below 4°C. The resultant supernatant was collected as a cytoplasmic fraction by centrifugation at  $40,000 \times g$  for 45 min, at 4°C. The pellet obtained was

dissolved in 5-ml lysis buffer II (40 mM Tris-HCl, 8 M urea, 50 mM DTT and 4% (w/v) 3-cholamidopropyl dimethyl-ammonio-1-propanesulfonate [CHAPS] at pH 8.0) and the solubilized membrane-bound protein (supernatant) was obtained by centrifugation as described above. The protein samples were precipitated by 9 volumes of acetone solution I (10% trichloroacetic acid (TCA) and 0.3% DTT in acetone) and kept overnight at  $-20^{\circ}\text{C}$ . The protein pellets were collected by centrifugation at  $5\,000 \times g$  for 5 min and washed with an appropriate volume of acetone solution II (0.3% DTT in acetone). The cytoplasmic and membrane-bound protein fractions were dissolved for 30 min in solubilization buffer I (8 M urea, 4% (w/v) CHAPS, 60 mM DTT, 2% (v/v) Bio-Lyte pH 3-10 [Bio-Rad], and 0.002% (w/v) bromophenol blue [BPB]) and solubilization buffer II (7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 60 mM DTT, 2% (v/v) Bio-Lyte pH 3-10, and 0.002% (w/v) BPB), respectively. An insoluble part and the contaminant from reagents were removed by centrifugation at  $15,000 \times g$  for 15 min. For preparation of the signal protein samples, a ready preparation signal protein extraction kit (Bio-Rad) was used with the cells grown on glucose and PEG 4000 medium. Protein concentration of the samples (supernatants) was measured with the Bio-Rad protein assay kit (Bio-Rad), using BSA as a standard.

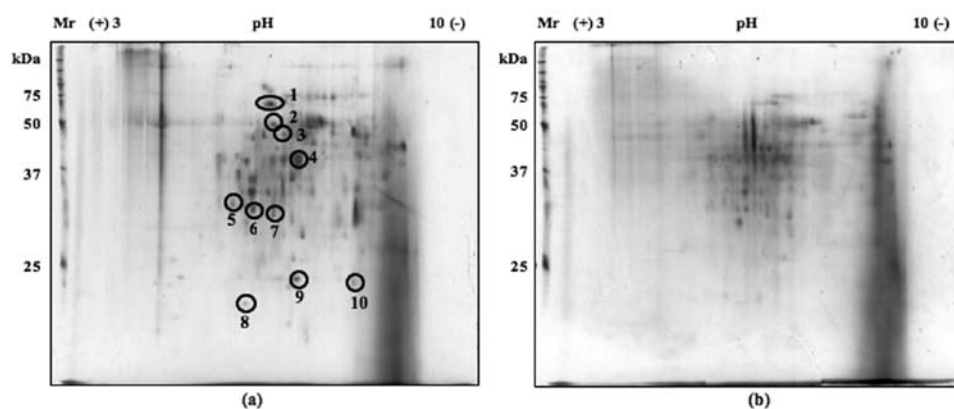
### **Two-dimensional electrophoresis, in-gel digestion, and MALDI-TOF MS analysis**

The 18-cm pH 3-10 and pH 5-8 immobilized pH gradients (IPGs) (Bio-Rad) were rehydrated at 50 V,  $20^{\circ}\text{C}$  for 12 h with 350  $\mu\text{l}$  of rehydration solution (8 M urea, 0.5% (w/v) CHAPS, 0.2% (w/v) DTT, 0.5% (v/v) Bio-Lytes pH 3-10, and 0.002% (w/v) BPB) that contained 1  $\mu\text{g}$  of each protein sample. IEF was conducted at  $20^{\circ}\text{C}$  as follow: S1: Rapid voltage at 100 V for 1 h, S2: Rapid voltage at 500 V for 1 h, S3: Rapid voltage at 10 000 V for 10 h, S4: Rapid voltage from 10,000 to 60,000 V, and S5: Rapid voltage at 500 V for 20 min. The limit voltage was 50  $\mu\text{A}$ /gel strip. After focusing, the gel strips were equilibrated with equilibration solution I (50 mM Tris-HCl at pH 8.8, 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS, 2% (w/v) DTT, and 0.002% (w/v) BPB) and equilibration solution II (50 mM Tris-HCl at pH 8.8, 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS, 2.5% (w/v) IAA, and 0.002% (w/v) BPB) by gentle shaking for 10 min, consequently. Then, the IPG was embedded onto a discontinuous SDS-PAGE gel (Laemmli, 1970) (10% separating gel, 5% stacking gel). Electrophoresis buffer consisted of 25 mM Tris-HCl at pH 8.3, 192 mM glycine, and 0.1% (w/v) SDS. Gels were run at constant current as 20 mA per gel until BPB moved into the separating gel, and then current was raised to 50 mA per gel until complete. The gels were pre-fixed in prefixative solution (20% (v/v) methanol and 7.5% (v/v) acetic acid) for 30 min, stained with staining solution (50% (v/v) methanol, 10% (v/v) acetic acid, and 0.05% Coomassie brilliant blue [CBB] R-250) for 45 min, and destained in destaining solution (5% (v/v) methanol and 7% (v/v) acetic acid) until the gel background become clear. The protein spots differentially appeared from cells grown on PEG 4000 were excised from the gel, in-gel digested with trypsin, and the amino acid sequences were identified by MALDI-TOF MS

analysis as described previously (Charoenpanich et al., 2006).

## RESULTS AND DISCUSSION

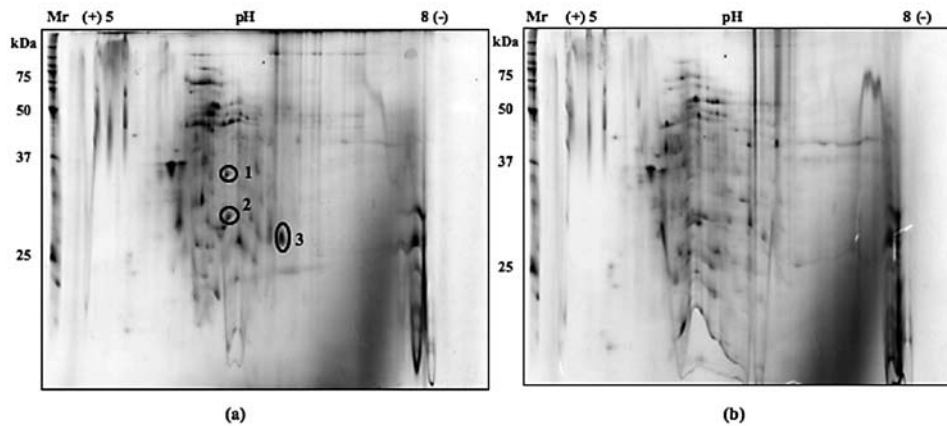
Cytoplasmic, membrane-bound and signal protein fractions were prepared from *S. macrogoltabida* strain 103 cells grown on PEG 4000 and glucose medium. The proteins from each fraction were separated on a series of 2D gels (Fig. 2-4) run in parallel with an initial pH gradient of pH 3 to pH 10 for cytoplasmic and signal proteins and pH 5 to pH 8 for membrane-bound proteins. Protein spots appeared in fractions obtained from PEG 4000-grown cells were cut from the gels, and digested with trypsin. The resulting digestion mixture was analyzed by MALDI-TOF MS to determine the molecular masses of the tryptic peptides. The sequences obtained were compared with those in databases. The possible identities are summarized in Table 1-3.



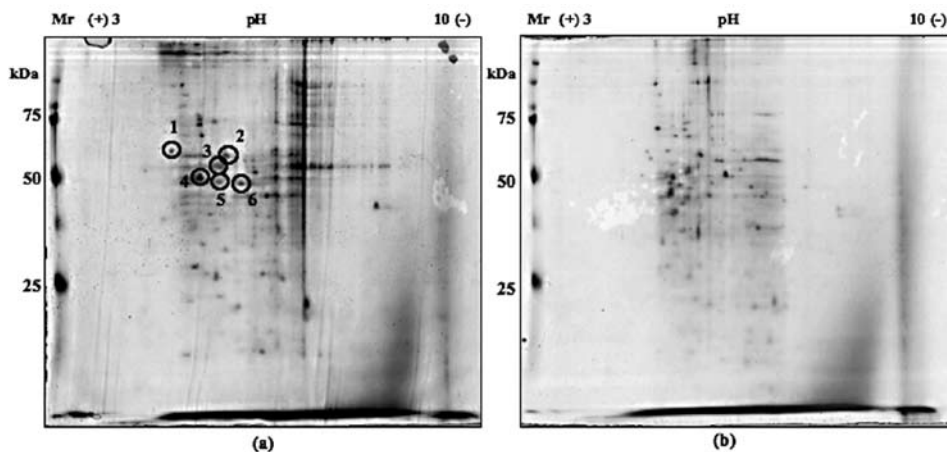
**Figure 2.** The cytoplasmic proteins in (a) PEG 4000- (b) glucose-grown *S. macrogoltabida* strain 103 separated by 2D electrophoresis using pH 3-10 IPG and 10% SDS-PAGE. Proteins were stained with CBB R-250. The different protein spots are circled and the identified proteins are labeled in Table 1.

Ten different protein spots from cytoplasmic fraction (Fig. 2) had molecular size of approximately 62.5 (pI 6.5), 51 (pI 6.6), 46 (pI 6.7), 39 (pI 7.0), 31 (pI 6.0), 30 (pI 6.2), 30 (pI 6.5), 12 (pI 6.5), 24 (pI 7.0) and 22 kDa (pI 8.0), respectively. Spots #1 and #2 corresponded to the fatty acyl CoA synthetase (61% identity) and the two-component response regulator (64%), respectively. Spot #3 was ascribed to permease (87%). Spots #4 to #10 corresponded to LacI transcription regulator (77%), galactinol synthase (77%), coenzyme PQQ synthesis protein (58%), transcription regulator (87%), translation-initiation factor like protein (85%), CheY-like two-component response regulator (87%), and hypothetical protein (88%), respectively. Three of the proteins identified in the membrane-bound fraction (Fig. 3), at 32 (pI 6.2), 30 (pI 6.3) and 27 kDa (pI 6.7), showed homology to LysR-transcription regulator (77%), GutR-transcription regulator (87%) and

two-component response regulator (71%), respectively. The signal protein fraction (Fig. 4) gave six different protein spots at approximately 62 (pI 4.5), 60 (pI 5.8), 55 (pI 5.5), 50 (pI 5.3), 49 (pI 5.7) and 48 kDa (pI 5.9), respectively. These protein spots corresponded to polyphosphate kinase, ATP sulfurylase, amino acid permease,  $\sigma^{54}$ -dependent transcription regulator, fatty acid CoA ligase and LysR-transcription regulator, respectively.



**Figure 3.** The membrane-bound proteins in (a) PEG 4000- (b) glucose-grown *S. macroglotabida* strain 103 separated by 2D electrophoresis using pH 5-8 IPG and 10% SDS-PAGE. Proteins were stained with CBB R-250. The different protein spots are circled and the identified proteins are labeled in Table 2.



**Figure 4.** The signal proteins in (a) PEG 4000- (b) glucose-grown *S. macroglotabida* strain 103 separated by 2D electrophoresis using pH 3-10 IPG and 10% SDS-PAGE. Proteins were stained with CBB R-250. The different protein spots are circled and the identified proteins are labeled in Table 3.



**Table 1.** Summary of proteins identified from the 2D-gel of PEG-induced cytoplasmic proteins in *S. macrogoltabida* strain 103, compared with the cells grown on glucose and PEG 4000<sup>a</sup>.

Spot #	Calculated masses	kDa	Expected protein	Source	% Identity	Accession no. in database <sup>b</sup>
1	GKCSVTPAGTLHLLGR	77	Fatty acyl CoA synthetase	<i>Leishmania</i>	61	Q9NKR2
2	TLLDSATVAAPGSSACTGDCR	52	Two-component response regulator	<i>Streptomyces coelicolor</i>	64	Q9X883
3	GAATCGAMGVMCAPSGMGKTR	42	Permease	<i>Pseudomonas syringae</i>	87	Q880Z1
4	FAAFFCYDNVSTKDWNAADR	37	LacI transcription regulator	<i>Corynebacterium</i>	77	AB193030
5	TGCSAFYESVTPVNSDK	37	Galactinol synthase	<i>Arabidopsis thaliana</i>	77	Q9FFA1
6	WGMFEHVNSCMHGWSDLLFT	42	Coenzyme PQQ synthesis protein	<i>Acinetobacter calcoaceticus</i>	58	P07782
7	WLGWCDVSCENHSVDCHKCR	37	Transcription regulator	<i>Candida albicans</i>	87	O94066
8	KVVAKFGKRKKEYTYEK-KRTCDYATK	12	Translation-initiation factor like protein	<i>Arabidopsis thaliana</i>	85	Q9FFT6
9	VAGSSNADASDYDVPYALEVK	26	CheY-like two-component response regulator	<i>Mesorhizobium loti</i>	87	Q985F8
10	FPFAVGCSLYSGDVEMAEPER	22	Hypothetical protein	<i>Caenorhabditis briggsae</i>	88	CAAC0100097

<sup>a</sup>The spot numbers listed in the first column correspond to the spot numbers in the 2D-gel in Figure 1.

<sup>b</sup>MS database (<http://dove.embl-heidelberg.de/Blast2/msblast.html>).

**Table 2.** Summary of proteins identified from the 2D-gel of PEG-induced membrane-bound proteins in *S. macrogoltabida* strain 103, compared with the cells grown on glucose and PEG 4000<sup>a</sup>.

Spot #	Calculated masses	kDa	Expected protein	Source	% Identity	Accession no. in database <sup>b</sup>
1	LKEHSVTRRARK	33	LysR-transcription regulator	<i>Silicibacter pomeroyi</i>	77	Q5LM95
2	GRVGERKPG-GPAAGGTAS-GAAEMK	29	GutR-transcription regulator	<i>Streptomyces coelicola</i>	87	Q8CJV4
3	AVGPSLVGC-PLMLLSRCSCR	25	Two-component response regulator	<i>Bacillus halodurans</i>	71	Q9KG43

<sup>a</sup>The spot numbers listed in the first column correspond to the spot numbers in the 2D-gel in Figure 2.

<sup>b</sup>MS database (<http://dove.embl-heidelberg.de/Blast2/msblast.html>).

**Table 3.** Summary of proteins identified from the 2D-gel of PEG-induced signal proteins in *S. macroglabrida* strain 103, compared with the cells grown on glucose and PEG 4000<sup>a</sup>.

Spot #	Calculated masses	kDa	Expected protein	Source	% Identity	Accession no. in database <sup>b</sup>
1	WRSENLAVEEDGAC-CHAAA	77	Polyphosphate kinase	<i>Streptomyces griseus</i>	66	Q9EUS8
2	NEEAHVRFDRLGWARHVA	64	ATP sulfurylase	<i>Aspergillus niger</i>	69	Q8NJNI
3	DWWANVWVMCELRCRDR	49	Amino acid permease	<i>Arabidopsis thaliana</i>	100	Q9FKS8
4	KCTGHDDVPKEVEAHT-NAGR	50	Sigma54-dependent transcription regulator	<i>Pseudomonas syringae</i>	75	Q87X96
5	NRWCTVVVPRFAPCSFMR	56	Fatty-acid-CoA ligase	<i>Rhodospseudomonas palustris</i>	87	Q6N9X4
6	TCATEGMGAGWFL-CEACAVKFR	33	LysR-transcription regulator	<i>Sinorhizobium meliloti</i>	100	Q92U75

<sup>a</sup>The spot numbers listed in the first column correspond to the spot numbers in the 2D-gel in Figure 3.

<sup>b</sup>MS database (<http://dove.embl-heidelberg.de/Blast2/msblast.html>).

Fatty acyl CoA synthetase (fatty acid CoA ligase), found in both cytoplasmic (spot #1) and signal protein (spot #5) fractions, plays a pivotal role in the transport and activation of exogenous fatty acids prior to their subsequent degradation or incorporation into phospholipids. The enzyme catalyzes the esterification of fatty acids into metabolically-active CoA thioesters concomitant with transport (Black et al., 1992). The *pegE* in the *peg* operon showed 30% homology to the putative acyl CoA synthetase in *Amycolatopsis sp* (GenBank accession No. CAC18323). The localization analysis of the protein from PSORT-B program (<http://psort.nibb.ac.jp/>) and the necessity of ATP for the reaction suggested that the protein was a cytoplasmic enzyme. The molecular mass subunit predicted from the *pegE* ORF and expressed protein (Tani et al., 2008) was 61 kDa at pI 5.4, which is similar to those found in 2D-gels. The permease, encoded by *pegD* (Charoenpanich et al., 2006) was homologous to glycoside / pentoside / hexuronide: cation symporters from various sources. The predicted molecular mass from the *pegD* ORF was 53 kDa at pI 6.5 that agreed well with those found in membrane-bound fraction by 2D-gels. Thus, the acyl CoA synthetase and permease found in cytoplasmic fraction as spot #1 or #3 as well as spot#5 or #3 in membrane-bound fraction might be the proteins encoded by *pegE* and *pegD* in the *peg* operon (Charoenpanich et al., 2006; Tani et al., 2008).

Two-component response regulator found in spot #2 and spot #9 of cytoplasmic fraction and spot #3 of membrane-bound fraction as well as the polyphosphate kinase and sigma54-dependent transcription regulator found in signal protein fraction suggested that the possibility of signal transduction system is contained in PEG degradation by *S. macroglabrida* strain 103. Signal transduction



generally involves the transferring of phosphoryl groups between proteins (Hoch and Silhavy, 1995). Many of these signals are sensed and transmitted to the transcription apparatus by pairs of proteins which belong to the family of two-component regulatory proteins. The stimulus is normally transferred between a group of proteins termed histidine kinase and another group of proteins called response regulators (Charoenpanich, 2008). As mentioned previously (Charoenpanich et al., 2006; Tani et al., 2007), transcription of the *peg* operon occurred when strain 103 was grown in PEG and only PEG could induce expression of the *pegA*. Long-chain PEG directly could not enter through the cell wall into cytoplasm and therefore could not induce the *peg* operon directly. Hence, a sensing protein posited on the cell surface might have significance. When a sensing protein recognizes the PEG molecule outside of the cell, the signal would be transmitted into the cytoplasm via phosphorylation. Subsequently, the phosphorylated regulator protein will bind to a promoter region of the genes and help RNA polymerase to start gene transcription. Moreover, it is known that  $\sigma^{54}$  is required for transcription of genes encoding amino acid transport components and many degradative enzymes (Magasanik, 1982). Transcription by  $\sigma^{54}$ -holoenzyme appears to be controlled by a common mechanism: use of an activator protein and ATP to catalyze formation of transcriptionally-productive open complex (Popham et al., 1989). Each activator allows  $\sigma^{54}$ -holoenzyme to initiate transcription in response to a distinct physiological signal, such as (i) limitation of combined nitrogen (Magasanik, 1982); (ii) low oxygen tension (Virts et al., 1988); (iii) availability of dicarboxylic acids external to the cell (Ronson et al., 1987); (iv) availability of toluene, xylenes or their alcohol catabolic products (Inouye et al., 1987, Ramos et al., 1987); (v) energy limitation (Eberz et al., 1986); and (vi) presence of formate under anaerobic conditions (Birkmann et al., 1987). Thus, it might be reasonable that *S. macrogoltabida* strain 103 contains two-component signal transduction system in response to PEG stress.

Many protein spots showed homology to transcription regulators from various families and microorganisms as follows: (i) LacI transcription regulator (spot #4 of cytoplasmic fraction) might be a transcription regulator for *pegA* and *pegR* promoters described previously (Charoenpanich et al., 2006; Charoenpanich, 2006), inducible by PEG 4000, (ii) Transcription regulator from spot #7 of cytoplasmic fraction was reported as a regulator for amino acid metabolism which requires ATP/GTP (IEA metabolism), (iii) The LysR regulatory family, found in membrane-bound (spot#1) and signal protein (spot #6) fractions, implicitly represents a very general mechanism for gene regulation (Rothmel et al., 1990). Most of them appear to be positive regulators, and many of them regulate their own expression (Stragier and Patte, 1983; Wek and Hatfield, 1988; Christman et al., 1989; Linduist et al., 1989; Maxon et al., 1989; Schell and Poser, 1989; Schell and Sukordhaman, 1989), (iv) GutR transcription regulator obtained from spot #2 of membrane-bound fraction served as an activator to modulate glucitol induction system (Ye et al., 1994). Moreover, it has been known to be directly or indirectly involved in repression of signal transduction in carbohydrate metabolism (Trumbly, 1988; Mueller et al., 1992). The functions of these regulators in PEG

degradation are to be elucidated.

The other five protein spots such as spot #5, spot #6, spot #8 and spot #10 of cytoplasmic fraction and spot #2 of signal protein fraction showed homology to the proteins that did not seem to be related to PEG metabolism. Galactinol synthase is a key enzyme for the biosynthesis of raffinose oligosaccharides which are the flatulence factors present in soybean seeds and several other legumes (Cunningham et al., 2003). Spot #6 corresponded to the protein that is required for coenzyme pyrroloquinoline quinone (PQQ) biosynthesis (Goosen et al., 1989). PQQ is probably formed by crosslinking a specific glutamate to a specific tyrosine residue and excising these residues from the peptide. Until now, involvement of PQQ for PEG metabolism in Sphingomonads has not been reported. However, many key enzymes for catabolic degradation of xenobiotics require PQQ for their expression (Shimao et al., 1986; Zarnt et al., 2001; Vangnai et al., 2002). Thus, this PEG-induced protein might synthesize PQQ as a cofactor for some enzymes relevant to PEG degradation. Next, translation-initiation factor like protein is expected to be an essential protein for translation of the proteins involved in PEG degradation. ATP sulfurylase is the first enzyme in the sulfate assimilation pathway that catalyzes the formation of adenosine phosphosulfate from ATP and sulfate (Schmidit and Jager, 1992). The functions of this protein as well as a hypothetical protein are still ambiguous.

### CONCLUSION

At present, reports on the nature of the two-dimensional protein patterns for PEG-degradative Sphingomonads are still not available. This work has focused on the PEG-inducible proteins which would provide preliminary data as to response to PEG in *S. macrogoltabida* strain 103. Further transcriptional and translational studies on them would lead to the total resolution of the PEG degradation.

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