Proteome Analysis of *Paenibacillus polymyxa* E681 Affected by Barley

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Abstract *Paenibacillus polymyxa* E681 is known to be able to suppress plant diseases by producing antimicrobial compounds and to promote plant growth by producing phytohormones, and secreting diverse degrading enzymes. In spite of these capabilities, little is known regarding the flow of information from the bacterial strain to the barley roots. In an attempt to determine the flow of information from the bacterial strain to barley roots, the strain was grown in the presence and absence of barley, and two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and MALDI-TOF mass spectrometry were used. 2D-PAGE detected approximately 1,000 spots in the cell and 1,100 spots in the supernatant at a pH 4–10 gradient. Interestingly, about 80 spots from each sample showed quantitative variations. Fifty-three spots from these were analyzed by MALDI-TOF mass spectrometry and 28 proteins were identified. Most of the cytosolic proteins expressed at higher levels were found in *P. polymyxa* E681 cells grown in the presence of barley rather than in the absence of barley. Proteins detected at a lower level in the supernatant of *P. polymyxa* E681 cells grown in the presence of barley were lipoprotein, glucose-6-phosphate 1-dehydrogenase, heat-shock protein HtpG, spermidine synthase, OrfZ, ribonuclease PH, and coenzyme PQQ synthesis protein, and flagellar hook-associated protein 2 whereas proteins detected at a higher level in the supernatant of *P. polymyxa* E681 cells grown in the presence of barley included D-alanyl-D-alanine ligase A, isopentenyl-diphosphate delta-isomerase, ABC transporter ATP-binding protein Uup, lipase. Many of the proteins belonging to plant-induced stimulons are associated with biosynthetic metabolism and metabolites of proteins and transport. Some of these proteins would be expected to be induced by environmental changes resulting from the accumulation of plant-secreted substances.

Keywords: *Paenibacillus polymyxa* E681, barley, bacteria-plant interaction, 2D PAGE, proteome

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A number of plant-growth promoting rhizobacteria (PGPR) have been found in rhizospheres of various plants and are widely used as seed inoculants to promote plant growth [17, 18, 31]. There have also been many reports on the beneficial effects of PGPR on plant growth, including antagonizing and repressing soilborne pathogens (by the production of HCN, siderophores [5, 15, 20, 21, 22], or antibiotics [14, 19, 26, 29, 35]), fixing atmospheric nitrogen [3, 12], solubilizing soil phosphors [27, 28], producing hydrolytic enzymes [2, 7, 16] and phytohormones (auxin [10], cytokinin [36], or gibberellins [32]), and promoting an increase in soil porosity [9]. Among Gram-positive spore-forming bacteria, *Paenibacillus polymyxa* (previously known as *Bacillus polymyxa*, [1]) showed effective activities when associated with plant roots. Moreover, *P. polymyxa* strains are shown to effectively stimulate the growth of various plants, leading to significant increases of yields for many consecutive cropping seasons, especially in the cold regions of the world [30]. Although *P. polymyxa* has been applied to a variety of different crops, our knowledge on the gene expression related to the specificity of plant-bacteria interactions is not fully understood.

The genus *Paenibacillus*, which contains more than 30 species, is a facultative anaerobic and endospore-forming low-G+C Gram-positive bacillus. Species in the genus are able to fix nitrogen, produce antimicrobial compounds, and secrete a variety of hydrolyzing enzymes [13].

The *P. polymyxa* E681 used in the present experiment was isolated from the rhizosphere of winter barley grown in Chonnam Province, Korea [30]. The full genome of *P. polymyxa* E681 has recently been sequenced by the Genome Research Center at the Korea Research Institute of Bioscience and Biotechnology [13, 25]. This opens up new opportunities for studying global responses to interactions of *P. polymyxa* E681 with barley. The genome of *P. polymyxa* E681 is about 5 Mb in size. It is known to form endospores, suppress plant diseases, produce antimicrobial

compounds, secrete diverse degrading enzymes, and to produce phytohormones.

Although *P. polymyxa* E681 has these capabilities, little is known regarding the flow of information from the bacterial strain to barley root. In this study, a proteomic analyzis was employed to better understand the interactions between *P. polymyxa* E681 and barley. Thus, *P. polymyxa* E681 was grown in the presence or absence of barley. Proteins from the bacterial cytosol and supernatant were analysed by 2D-PAGE. Proteomic analysis is concerned with global changes in protein expression, and typically involves a combination of 2D-PAGE for protein separation and visualization and mass spectrometry (MS) for protein identification. However, not all cellular proteins can be visualized on one single gel. Proteomic analysis has contributed greatly to our understanding of gene function in the post-genomic era through protein chemistry. Two-dimensional gel electrophoresis databases are the core of the bioinformatics for proteome research [11, 24].

The proteome of *P. polymyxa* E681 grown in the presence or absence of barley was examined in order to provide the basis for the application of microbial physiology to interactions of *P. polymyxa* E681 with plants.

MATERIALS AND METHODS

Chemicals

Urea, thiourea, CHAPS (3-[(3-Cholamidopropyl)dimethyl-ammonio]-1-propanesulfonate), DTT, benzamidine, Bradford solution, acrylamide, iodoacetamide, bis-acrylamide, SDS (sodium dodecyl sulfate), acetonitrile, trifluoroacetic acid, and α-cyano-4-hydroxycinnamic acid were purchased from Sigma-Aldrich (electrophoresis grade or ACS reagents, Ultrapure, St. Louis, U.S.A.), Pharmalyte (pH 3–10) was from Amersham Biosciences (Uppsala, Sweden), and IPG DryStrips (pH 4–10, 24 cm) were from Genomine Inc. (Pohang, Korea). Modified porcine trypsin (sequencing grade) was from Promega (Madison, U.S.A.).

Plant and Cultural Conditions

Barley was provided by the Kyungbook National Agricutural Research Institute, Daegu, Korea. Barley seeds were soaked in flowing tap water for 2 days. When they began to bud, they were sterilized with 1.2% NaClO for 40 min, and rinsed 3 times with sterile distilled water. The sterilized seeds were planted in pots filled with the sterile artificial soil and distilled water and cultured for 4 days (14 h of light at 20°C and 10 h of darkness at 16°C; humidity: 65%). They were cultured until their roots had grown to a length of 3–4 cm.

Bacterial Strains and Culture Conditions

The bacterial strain used in these experiments was *P. polymyxa* E681, isolated from the rhizosphere of winter barley grown in Chonnam Province, Korea. To analyze proteins induced by *P. polymyxa* E681 grown in the presence or absence of barley, cells were precultured overnight under vigorous agitation (230 rpm) at 30°C in TSB media (Tryptic soy broth 30 g/l). Forty ml of the precultured starter ($OD_{436\,nm}$ 0.8) was inoculated into fresh TSB medium diluted with 400 ml of fresh TSB medium (Tryptic soy broth 6 g/l). One was only a *P. polymyxa* E681 culture and the other was a *P. polymyxa* E681 culture grown in the presence of barley precultured to 3–4 cm of their rooty length in the fresh diluted TSB medium. Both samples were simultaneously cultured for 6 h at room temperature.

Protein Sample Preparation

For the preparation of soluble cytosolic proteins, *P. polymyxa* E681 cell pellets prepared under both culture conditions were washed twice with ice-cold PBS (phosphate-buffered saline: 0.8% NaCl, 0.02% KCl, 0.144% Na_2HPO_4) and disrupted under liquid nitrogen in sample buffer, composed of 7 M urea, 2 M thiourea containing 4% (w/v) 3-[(3-cholamidopropy)dimethyammonio]-1-propanesulfonate (CHAPS), 1% (w/v) dithiothreitol (DTT), 2% (v/v) pharmalyte, and 1 mM benzamidine, by a motor-driven homogenizer (PowerGen 125, Fisher Scientific, U.S.A.), and cell debris were removed [6]. The supernatant of the *P. polymyxa* E681 culture was incubated overnight with 10% trichloroacetic acid to precipitate proteins in the cold chamber. The precipitates were then lysed in the above sample buffer. Proteins were extracted for 1 h at room temperature by vortexing. After centrifugation at 15,000 ×g for 1 h at 15°C, insoluble material was discarded, and the soluble fraction was used for 2D-PAGE. Protein loading was normalized after measuring the concentration by the Bradford assay [4].

Two-dimensional Polyacrylamide Gel Electrophoresis

IPG dry strips were equilibrated for 12–16 h with 7 M urea, 2 M thiourea containing 2% 3-[(3-cholamidopropy) dimethyammonio]-1-propanesulfonate (CHAPS), 1% dithiothreitol (DTT), and 1% pharmalyte, and were loaded with 200 μg of sample. Isoelectric focusing (IEF) was performed at 20°C using a Multiphor II electrophoresis unit and an EPS 3500 XL power supply (Amersham Biosciences, Uppsala, Sweden) following the manufacturer's instructions. For IEF, the voltage was linearly increased from 150 to 3,500 V during 3 h for sample entry, followed by a constant 3,500 V, with focusing complete after 96 kVh. Prior to the second dimension, the strips were incubated for 10 min in equilibration buffer (50 mM Tris-Cl, pH 6.8, containing 6 M urea, 2% SDS, and 30% glycerol), first with 1% DTT and second with 2.5% iodoacetamide. The equilibrated strips were then inserted onto SDS-PAGE gels (20×24 cm, 10–16%). SDS-PAGE was performed using a Hoefer DALT

2D system (Amersham Biosciences, Uppsala, Sweden) following the manufacturer's instruction. 2D gels were run at 20°C for 1.7 kVh, and the gels were silver stained as described by Oakley *et al.* [23], but the fixing and sensitization steps with glutaraldehyde were omitted.

Image Analysis

Quantitative analysis of digitized images was carried out using the PDQuest software (version 7.0, Bio-Rad, Hercules, U.S.A.) according to the protocols provided by the

manufacturer. The quantity of each spot was normalized by the total valid spot intensity. Protein spots showing a significant expression variation deviating by two-folds in expression level, compared with control or normal samples, were selected.

Enzymatic Digestion of Protein In-Gel

Protein spots were enzymatically digested in-gel in a manner similar to that previously described by Shevchenko *et al.* [34] using modified porcine trypsin. The gel pieces
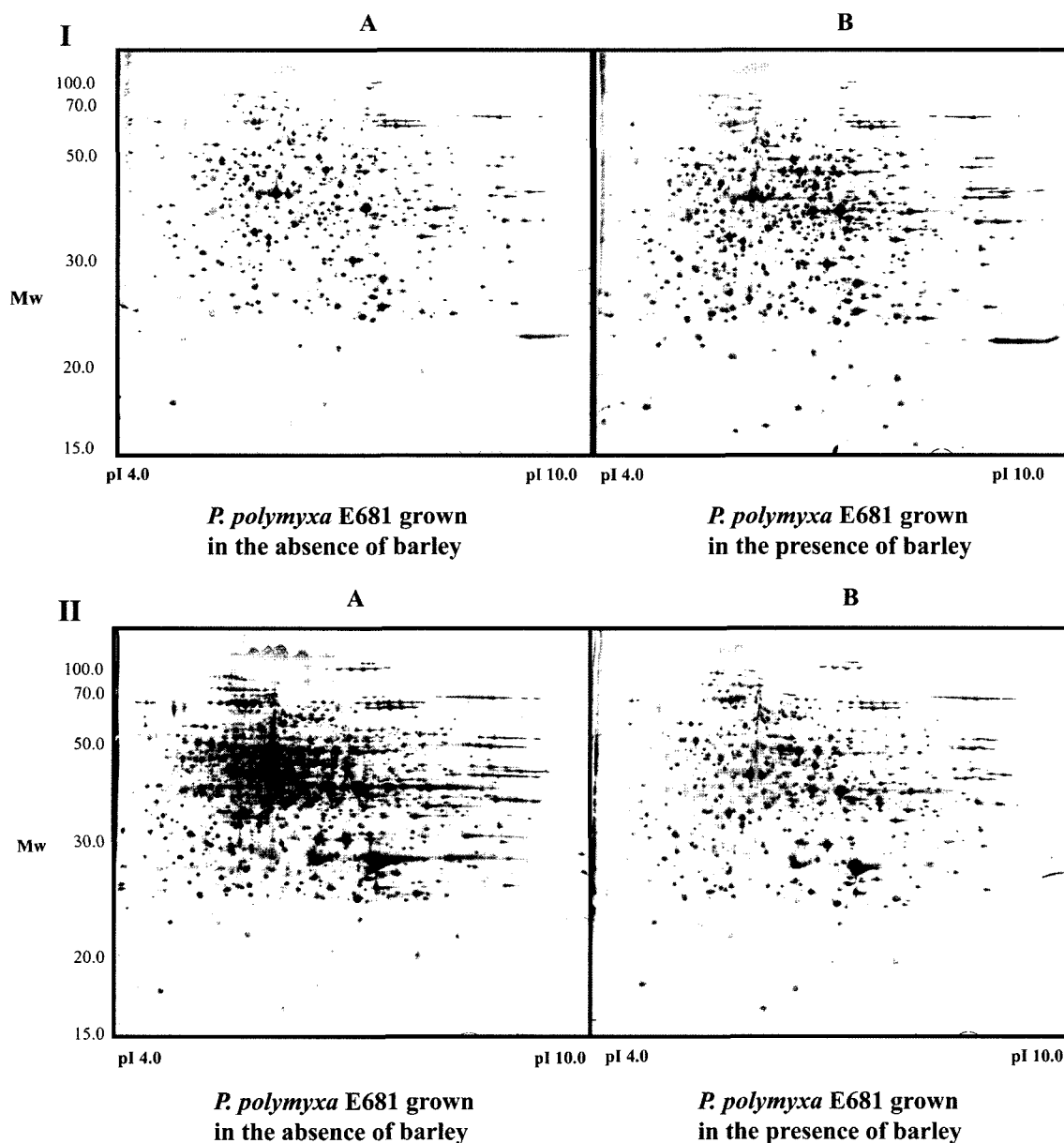
Fig. 1. Representative 2-DE maps of *P. polymyxa* E681 grown in the absence or presence of barley.

Proteins from each sample were extracted and separated by 2-DE. In IEF, proteins were loaded onto pH 4–10 IPG strips (20 cm, linear). SDS-PAGE was performed with 12% gels. Spots were visualized by silver staining. **A.** 2-DE maps of *P. polymyxa* E681 grown in the absence of barley. **B.** 2-DE maps of *P. polymyxa* E681 grown in the presence of barley. **I:** 2-DE maps of cytosolic proteins from *P. polymyxa* E681 grown in the absence of barley or in the presence of barley. **II:** 2-DE maps of proteins from supernatant of *P. polymyxa* E681 grown in the absence or presence of barley.

were washed with 50% acetonitrile to remove SDS, salt, and stain, dried to remove solvent, and then rehydrated with a trypsin solution (8–10 ng/μl) by incubation for 8–10 h at 37°C. The proteolytic reaction was terminated by the addition of 5 μl of 0.5% trifluoroacetic acid. Tryptic peptides were recovered by combining the aqueous phase

from several extractions of gel pieces with 50% aqueous acetonitrile. After concentration, the peptide mixture was desalted using C_{18}ZipTips (Millipore, Bedford, U.S.A.), and then eluted with 1–5 μl of acetonitrile. An aliquot of this solution was mixed with an equal volume of a saturated solution of α-cyano-4-hydroxycinnamic acid in
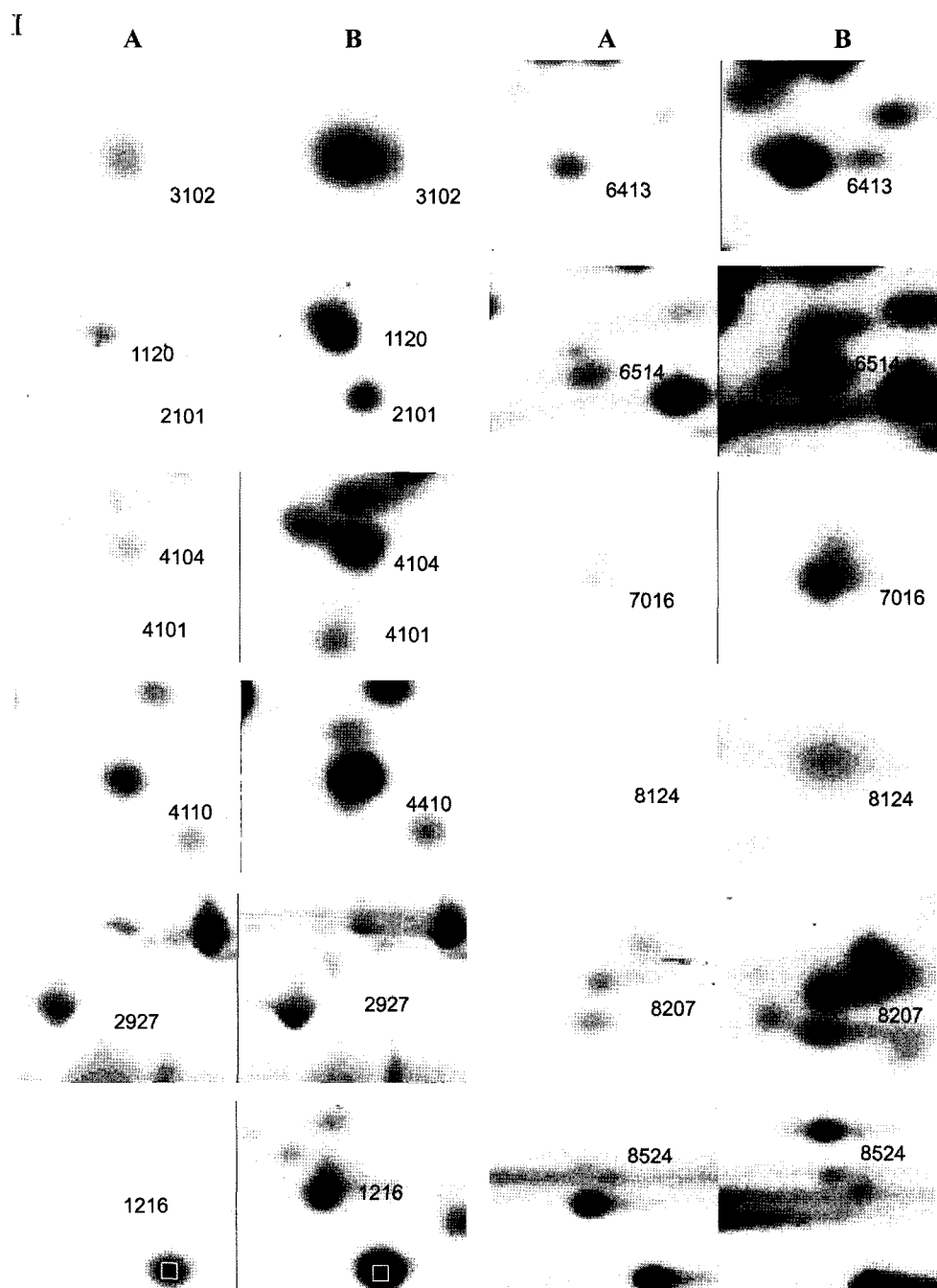
Fig. 2. The enlarged pictures of down- and upregulated protein spots. A and B correspond to the regions I and II framed in Fig. 1.
I. Cytosolic proteins of *P. polymyxa* E681 upregulated in the presence of barley, compared with that in the absence of barley. II. Upregulated proteins from the supernatant of *P. polymyxa* E681 grown in the presence of barley as compared with that in the absence of barley. III. Downregulated proteins from the supernatant of *P. polymyxa* E681 grown in the presence of barley as compared with that in the absence of barley.

50% aqueous acetonitrile, and 1 μl of the mixture was spotted on a target plate.

MALDI-TOF Analysis and Database Search

Protein analysis was performed using an Ettan MALDI-TOF spectrometer (Amersham Biosciences, Uppsala, Sweden). The peptides were evaporated with a N_2 laser at 337 nm. They were accelerated with a 20 Kv injection pulse for the time-of-flight analysis. Each spectrum represents the cumulative average of 300 laser shots. The search program ProFound, developed by Rockefeller University (http://129.85.19.192/profound_bin/WebProFound.exe), was used for protein identification by peptide mass fingerprinting. Spectra were calibrated with the trypsin autodigestion ion peak m/z (842.510, 2211.1046) as an internal standard. Proteins were identified from the DNA database of *P. polymyxa* E681 (http://www.gem.re.kr). The identified proteins were searched on http://www.sanger.ac.uk/software/pfam/ during 2005–2006.

RESULTS AND DISCUSSION

General Description of the Interaction Between *P. polymyxa* E681 and Barley on the 2D Map

P. polymyxa E681 is a plant growth-promoting rhizobacterium (PGPR) and endophytic bacterium. Understanding the interaction of the bacteria with plants as well as the physiology of the PGPR and endophytic bacteria is important for a variety of reasons.

Herein we presented data, obtained *via* proteomic studies, based on bacteria-plant interactions. *P. polymyxa* E681 cells grown in the presence of barley induced the expression of a stimulon consisting of proteins whose involvement in interactions with plants has not previously been demonstrated. Furthermore, we were able to identify several plant-induced posttranslational modifications, thus emphasizing the importance of proteome studies in the field of *P. polymyxa* E681. Two-dimensional gel electrophoresis of protein extracts from *P. polymyxa* E681 permitted the separation and analysis of about 963 individual protein spots in *P. polymyxa* E681 cells grown in the absence of barley, 1,078 protein spots in *P. polymyxa* E681 cells grown in the presence of barley, 1,209 spots from the supernatant of *P. polymyxa* E681 grown in the absence of barley, and 1,082 spots from the supernatant of *P. polymyxa* E681 grown in the presence of barley. These proteins are soluble cytoplasmic external proteins, since the method used to obtain the proteome excluded most of the hydrophobic, membrane-bound proteins.

The protein synthesis patterns of cells grown in the presence or absence of barley were matched and analyzed by high-resolution 2D-PAGE using PDQuest. A representative set of two-dimensional gels showed differential protein
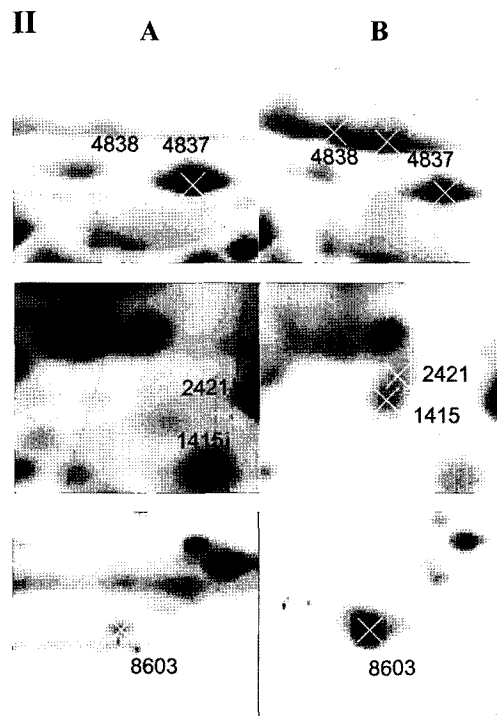

Fig. 2. Continued.

synthesis patterns in *P. polymyxa* E681 when grown in the presence or absence of barley, visualized by alkaline silver stain (Fig. 1). Proteins in the gels were analyzed using an IPG strip with a pH range of 4 to 10. Interestingly, about 80 spots from each sample showed quantitative variations. In preliminary investigations of proteins by a simple visual interpretation of the two-dimensional gels, we selected and separated 53 proteins that showed quantitative variations after alkaline silver staining (Fig. 2 and Fig. 3). Twenty-eight spots were analyzed by MALDI-TOF mass spectrometry and database searching (Table 1 and Table 2).

Proteome Analysis of the Cytosolic Proteins from *P. polymyxa* E681 Grown in the Presence or Absence of Barley

In order to examine interactive proteins between *P. polymyxa* E681 cells and barley, we cultured *P. polymyxa* E681 in the presence and absence of barley, as described above. Using 2D gels to separate the proteins, the cytosolic proteins of *P. polymyxa* E681 that were cultured in the presence of barley were compared with the cytosolic proteins of *P. polymyxa* E681 that were cultured in the absence of barley. Both samples were incubated in TSB medium. In an overlay of the two images, new proteins that were induced by culture of *P. polymyxa* E681 in the presence or absence of barley were visualized by PDQuest. Proteins that were synthesized under both conditions are shown as black spots and indicate the expression of proteins under the two conditions (Fig. 1-I). Fig. 2-I and Fig. 3-I show the relative
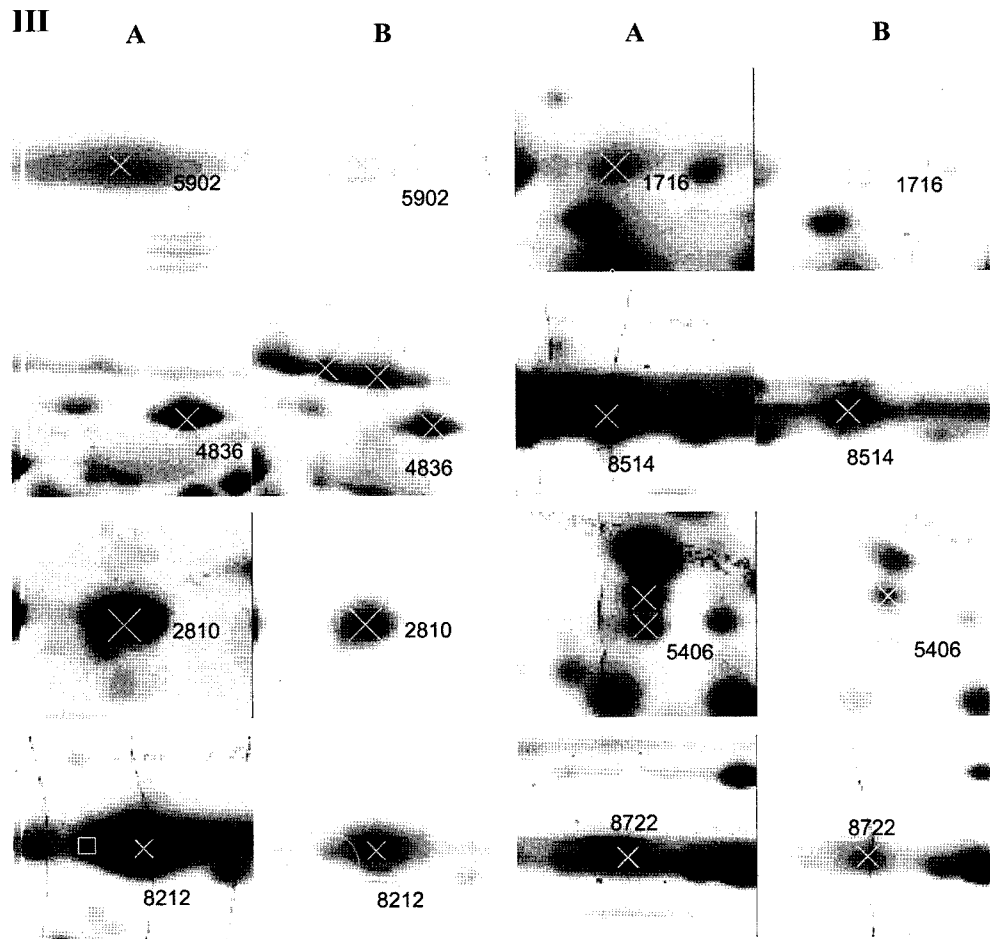
Fig. 2. Continued.

quantity of proteins expressed under the two conditions. Whereas the levels of most proteins remained unchanged, the protein pattern induced by exposure was not consistent. Thirty-three proteins showed quantitative variations between pI 4 and pI 8, compared with the control. Fifteen cytoplasmic proteins from bacterial cells were identified (Table 1). This 2D gel analysis was useful for separating water-soluble cytoplasmic proteins. Very few membrane proteins can be detected, since they are highly hydrophobic and are only sparingly soluble in the rehydration solution.

The expression of almost most of the cytoplasmic proteins was induced to a greater level in *P. polymyxa* E681 cells grown in the presence of barley than in bacterial cells grown in the absence of barley. Of these, 15 cytosolic proteins were identified: methanol dehydrogenase activator protein, triosephosphate isomerase, guanylate kinase, alpha-glucosidase III, protease synthase, sporulation negative regulatory protein PAI 1, DNA-binding response regulator PhoP, stage II sporulation protein R, oxidoreductase, the protein YicC homolog, chorismate synthase, transcriptional regulator, hypothetical cytosolic protein, methyltransferase, and periplasmic ribose binding protein.

Methanol dehydrogenase (MDH) is a bacterial periplasmic quinoprotein that oxidizes methanol to formaldehyde. Triosephosphate isomerase (TIM) is the glycolytic enzyme that catalyzes the reversible interconversion of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. TIM plays an important role in several metabolic pathways and is essential for efficient energy production. Guanylate kinase (GK) catalyzes the ATP-dependent phosphorylation of GMP into GDP, and is essential for recycling GMP and indirectly cGMP. Oxidoreductase causes many chemical changes including anabolism and catabolism in living organisms and transports electrons. Members of the YicC protein family have been reported to play a role in stationary-phase survival. Chorismate synthase catalyzes the last of the seven steps in the shikimate pathway, which is used in prokaryotes, fungi, and plants for the biosynthesis of aromatic amino acids. It catalyzes the 1,4-transelimination of the phosphate group from 5-enolpyruvylshikimate-3-phosphate (EPSP) to form chorismate, which can then be used in the biosynthesis of phenylalanine, tyrosine, or tryptophan. Methyltransferase may be involved in antibiotic production. Several of the bacterial periplasmic binding proteins are
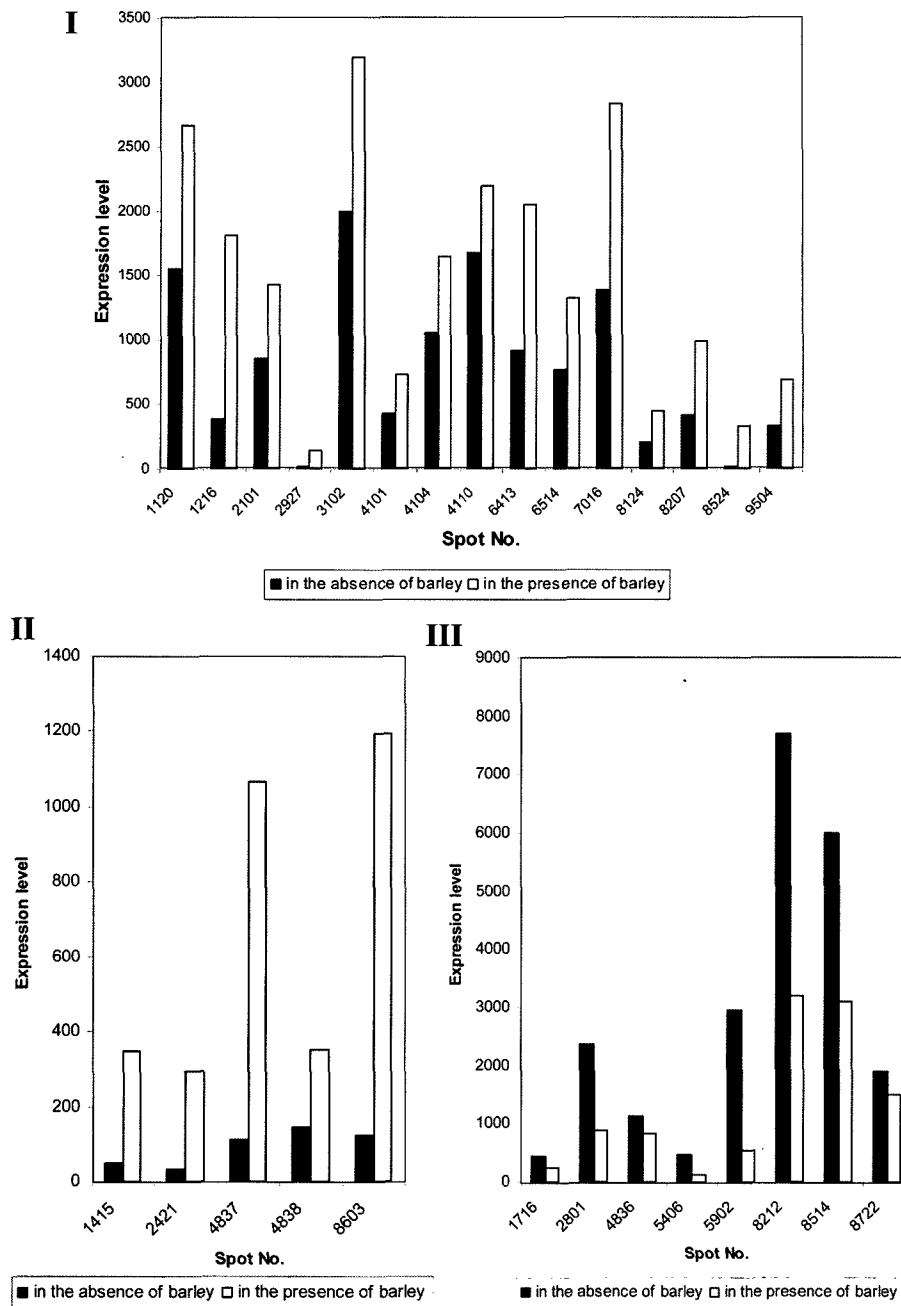
Fig. 3. Comparison of the abundance in the differentially accumulated proteins from *P. polymyxa* E681 grown in the absence or presence of barley.

In the bar-graph, ■ shows the abundance of proteins expressed in *P. polymyxa* E681 grown in the absence of barley. □ shows the abundance of proteins expressed in *P. polymyxa* E681 grown in the presence of barley. I: Comparison of cytosolic proteins in the cells of *P. polymyxa* E681 grown in the absence or presence of barley. II and III: Comparisons of external proteins from *P. polymyxa* E681 grown in the absence or presence of barley.

involved in iron transport. Most high-affinity systems for iron uptake in Gram-negative bacteria are thought to employ periplasmic binding protein-dependent transport. The periplasmic binding proteins are the primary receptors for chemotaxis and the transport of many sugar-based solutes. The regulatory proteins such as protease synthase and sporulation negative regulatory protein PAI 1, DNA-

binding response regulator PhoP, and transcriptional regulator were expressed at higher levels. Moreover, hypothetical cytosolic protein, alpha-glucosidase III, and stage II sporulation protein R were also expressed more highly in *P. polymyxa* E681 cells grown in the presence of barley. These results suggest that *P. polymyxa* E681 might interact with barley roots, showing their thriving metabolites. Asparaginase

Table 1. Identification of cytosolic proteins. Proteins were identified from the DNA database of *P. polymyxa* E681.

Cytosolic protein	Spot no.	Protein name	Theoretical pI/MW (kDa)	Identified pI/MW (kDa)
	1120	Methanol dehydrogenase activator protein	4.69/23.49	4.47/22.72
	1216	Triosephosphate isomerase	4.69/28.35	4.78/25.50
	2101	Guanylate kinase	4.71/22.65	4.91/21.45
	2927	Alpha-glucosidase III	4.91/85.10	4.90/86.67
	3102	Protease synthase and sporulation negative regulatory protein PAI 1	4.93/21.98	4.91/21.45
Expressed more in the cell of *P. polymyxa* E681 grown in the presence of barley	4101	DNA-binding response regulator PhoP	5.14/25.34	5.18/26.89
	4104	Stage II sporulation protein R	5.15/26.05	5.09/23.41
	4110	Oxidoreductase	5.25/26.07	5.39/26.56
	6413	Protein YicC homolog	5.73/36.11	5.60/37.00
	6514	Chorismate synthase	5.80/43.62	5.61/43.05
	7016	Transcriptional regulator	6.62/17.99	6.51/15.09
	8124	Hypothetical cytosolic protein	7.44/22.12	7.63/21.35
	8207	Methyltransferase	6.91/27.99	7.16/26.02
	8524	Periplasmic ribose binding protein	7.44/43.82	7.27/40.57
	9504	Asparaginase	7.72/42.59	7.68/40.21

Proteins were identified from the DNA database of *P. polymyxa* E681 (http://129.85.19.192/profound-bin/WebProFound.exe & http://www.gem.re.kr).

catalyzes the deamination of asparagine to yield aspartic acid and ammonium ion, resulting in a depletion of free circulatory asparagine in the plasma. Based on these results, it appears to be highly likely that the interactions between plants and bacteria might deeply affect each other during bacterial as well as plant metabolism.

Proteome Analysis of the External Proteins from *P. polymyxa* E681 Grown in the Presence or Absence of Barley

To analyze and identify proteins in the supernatant of *P. polymyxa* E681 grown in the presence or absence of barley, they were separated from the extracellular preparations

by 2D gel electrophoresis, as described above. Proteins synthesized under both conditions are shown as black spots on the 2D map and indicate the expression of the proteins under the two different conditions (Fig. 1II). One-thousand, two-hundred and nine individual protein spots from the supernatant of *P. polymyxa* E681 grown in the absence of barley, and 1,082 spots from the supernatant of *P. polymyxa* E681 grown in the presence of barley were found. About 80 detectable spots were generated on the gel. Among these, 20 protein spots (pI 4–10) were analyzed and compared with the control (Fig. 2-II, Fig. 2-III, Fig. 3-II, and Fig. 3-III), and 13 proteins were identified (Table 2).

Table 2. Identification of extracellular proteins.

External protein	Spot no.	Protein name	Theoretical pI/MW (kDa)	Identified pI/MW (kDa)
Induced more in the supernatant of *P. polymyxa* E681 grown in the presence of barley than in the absence of barley	1415	D-Alanyl-D-alanine ligase A	4.73/40.32	4.62/39.96
	2421	Isopentenyl-diphosphate delta-isomerase	4.74/40.61	4.96/38.84
	4837	ABC transporter ATP-binding protein Uup	5.31/79.47	5.08/75.81
	4838	ABC transporter ATP-binding protein Uup	5.28/79.87	5.08/75.81
	8603	Lipase	6.84/52.49	6.66/52.73
Reduced more in the supernatant of *P. polymyxa* E681 grown in the presence of barley than in the absence of barley	1716	Lipoprotein	4.45/57.67	4.94/56.06
	2801	Glucose-6-phosphate 1-dehydrogenase	4.70/60.63	4.90/59.40
	4836	Heat-shock protein HtpG	5.33/75.95	5.07/72.02
	5406	Spermidine synthase	5.37/36.09	5.51/36.39
	5902	OrfZ	5.44/137.64	5.51/135.13
	8212	Ribonuclease PH	7.06/28.92	7.18/27.28
	8514	Coenzyme PQQ synthesis protein	7.14/42.63	7.18/41.74
	8722	Flagellar hook-associated protein 2	7.28/54.04	7.20/52.79

Proteins were identified from the DNA database of *P. polymyxa* E681 (http://129.85.19.192/profound-bin/WebProFound.exe & http://www.gem.re.kr).

Five of the plant-induced proteins from the extracellular preparations were identified; D-alanyl-D-alanine ligase A, isopentenyl-diphosphate delta-isomerase, lipase, ABC transporter ATP-binding protein Uup (pI 5.31/79.47 kDa), and ABC transporter ATP-binding protein Uup (pI 5.28/79.87 kDa) that can be detected at pI 1–9 on 2D gels.

D-Alanine is one of the central molecules of the cross-linking step of peptidoglycan assembly. D-Alanyl-D-alanine ligase A is thought to be involved in substrate binding. Isopentenyl-diphosphate delta-isomerase might be related to synthesizing a cytokinin that induces shoot formation on host plants.

The ABC transporter ATP-binding protein Uup family consists of several bacterial ABC transporter proteins that are homologous to the EcsB protein of *Bacillus subtilis*. EcsB is thought to be a hydrophobic protein with six membrane-spanning helices in a pattern found in other hydrophobic components of ABC transporters. Bacterial high-affinity transport systems are involved in the active transport of solutes across the cytoplasmic membrane. The protein components of these traffic systems include one or two transmembrane protein components, one or two membrane-associated ATP-binding proteins (ABC transporters), and a high-affinity periplasmic solute-binding protein. The latter is thought to bind the substrate in the vicinity of the inner membrane, and to transfer it to a complex of inner membrane proteins for concentration. Lipase is a lipolytic enzyme that hydrolyzes ester linkages of triglycerides. Lipase is widely distributed in plants and prokaryotes.

The proteins with a lower concentration in the supernatant of *P. polymyxa* E681 grown in the presence of barley than that grown in the absence of barley included lipoprotein, glucose-6-phosphate 1-dehydrogenase, heat-shock protein HtpG, spermidine synthase, OrfZ, ribonuclease PH, coenzyme PQQ synthesis protein, and flagella hook-associated protein 2. Lipoprotein is probably involved in evasion of the host immune system by pathogens. Glucose-6-phosphate 1-dehydrogenase is a ubiquitous protein, and catalyzes the first step in the pentose phosphate pathway; *i.e.*, the conversion of glucose-6-phosphate to gluconolactone 6-phosphate in the presence of NADP, producing NADPH. It plays a major role in the production of NADPH for many NADPH-mediated reductive processes in cells. Heat-shock protein HtpG has recently been reported to be required for the biosynthesis of a polyketide-peptide antibiotic. Previous findings have suggested that the heat-shock response is involved in bacteria-host interactions [8, 33, 37]. Biosynthase for spermidine has been suggested to protect plants from stress, OrfZ is the putative immunity protein, and ribonuclease PH hydrolyzes the phosphodiester bonds in RNA and oligoribonucleotides. Coenzyme PQQ synthesis protein is one of many proteins that enhance the expression of extracellular enzymes, such as alkaline protease, neutral protease, and levansucrase. Flagellar hook-associated protein

2 is the capping protein for the flagella and forms the distal end of the flagella. The protein plays a role in mucin-specific adhesion of the bacteria.

It has been suggested that the reduced amount of secreted proteins in the supernatant from *P. polymyxa* E681 cells grown with barley might be resulted from direct or indirect interactions with *P. polymyxa* E681 and barley.

The proteins induced or reduced more by *P. polymyxa* E681 grown in the presence of barley than that grown in the absence of barley are involved in biosynthetic metabolism, lipid metabolism, protein transport, substrate binding in cell wall, flagellum biogenesis, and bacteria-host interaction. The effect of the bacteria on the plant may affect mainly the profile of the membrane proteins of barley. Many of the proteins belonging to the plant-induced stimulon are associated with biosynthetic metabolism, and others are involved in the transport of proteins or metabolites. Some of these proteins are expected to be induced by environmental changes resulting from the accumulation of plant-secreted substances. Following exposure to plants, several proteins were found in more than one spot, and these spots usually had a different pI, but similar molecular masses. This pattern suggests the existence of regulated plant-dependent posttranslational modifications. Such modifications include the addition of charged groups or the cleavage of a charged residue or a peptide.

In conclusion, with all the evidences presented above, it is clear that much is going on in the association between *P. polymyxa* E681 and barley. These proteins are indicative of the significance of protein modifications in interactions of *P. polymyxa* E681 with barley. However, for a better understanding of this issue, each of these proteins needs to be examined further, particularly regarding the underlying mechanisms of how they affect barley and *P. polymyxa* E681 and related interactions.

Acknowledgments

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