Supplementary Experimental Procedures

Soil and chemical properties

Soil samples were collected from three different regions, a soybean field at a private organic farm in Saitama, Japan (soybean soil sample), a maize field at the National Agricultural Research Center for the Kyushu Okinawa Region in Miyazaki, Japan (maize soil sample), and a sub-boreal forest at Tomakomai Experimental Forest at Hokkaido University in Hokkaido, Japan (forest soil sample). The soil samples were collected from the top 10 cm of each field. Plant material was carefully removed by hand. The soil samples were stored at 4°C until use.

The soil chemical properties measured were pH, total carbon content (C%), total nitrogen content (N%), carbon and nitrogen ratio (C/N), NH^{4+}, NO^{3-}, and PO_4^{3-}. Soil pH (H_2O) was measured with a soil-water ratio of 1:2.5. Total C and N levels were determined using an automatic, highly sensitive N-C analyzer (MT-700, Yanaco New Sci., Kyoto, Japan), equipped with an MTA-600 autosampler. The NH^{4+}, NO^{3-}, and PO_4^{3-} concentrations in the soil samples were determined using reflectoquant tests with a RQflex reflectometer (Merck, Darmstadt, Germany), following the manual provided.

Soil culture conditions of bacterial cells

P. putida F1 purchased from the American Type Culture Collection was grown at 30°C for 16 h in mineral salt (MS) medium [18.3 mM $Na_2HPO_4 \cdot 12H_2O$, 11.2 mM KH_2PO_4, 4.8 mM $(NH_4)_2SO_4$, 0.8 mM $MgSO_4 \cdot 7H_2O$, and 0.3 mM $CaCl_2$] (Muňoz et al., 2007) supplemented with trace elements (14.9 μM EDTA·2Na, 7.2 μM $FeSO_4 \cdot 7H_2O$, 0.35 μM $ZnSO_4 \cdot 7\ H_2O$, 0.15 μM $MnCl_2 \cdot 4H_2O$, 4.9 μM H_3BO_3, 0.84 μM $CoCl_2 \cdot 6H_2O$, 0.06 μM $CuCl_2 \cdot 2H_2O$, 0.08 μM $NiCl_2 \cdot 6H_2O$, and 0.14 μM $NaMoO_4 \cdot 2H_2O$) and 0.5% (w/v) glucose as the sole sources of carbon and energy, with vigorous shaking. The cells were harvested by centrifugation, and washed twice using 0.8% NaCl. Finally, the cell pellet was resuspended in 0.8% NaCl and diluted to 1.0×10^9 CFU ml^{-1}. The cell suspension (5 ml) was inoculated into 50 g of each soil containing 0.5% (w/w) glucose in a petri dish. The non-inoculated soil samples were used as references. The inoculated and non-inoculated soil samples were incubated at 30°C for 3 days. The moisture content (approximately 50%) of the soil samples was gravimetrically controlled during

incubation using distilled water (DW). The soil cultures experiments were performed in three independent biological replicates. Soil samples were taken from three random locations in a petri dish and then mixed for soil characterization, viable cell count and proteome analysis.

Viable count of bacterial cells in soil

Viable cell numbers of indigenous bacteria and *P. putida* F1 in soil were determined using the dilution plate method. Soil samples were taken prior to the inoculation of F1cells to count indigenous bacteria and at 1 h (as day 0) and, 1, 2, and 3 days after the inoculation to count F1 cells. Approximately 1 g soil sample was serially diluted. The soil suspensions were inoculated onto a nutrient broth (Beckton Dickinson, Bedford, MA, USA) agar (1.5%) plate for indigenous bacteria and onto an Luria broth ager (LB; 0.5% yeast extract, 1.0% tryptone, 0.5% NaCl and 1.5% agar) plate containing ampicillin at 100 μg ml^{-1} for the F1strain. Viable cells (CFU g^{-1}) were counted in the samples after incubation of indigenous bacteria and the F1 strain at 30°C for 7 days and 24 h, respectively. Five replicate plates were prepared for each sample.

Separation of bacterial cells from soil

Bacterial cells were separated from the soil samples using Nycodenz density gradient centrifugation as previously described (Rickwood et al., 1982, Lindahl and Bakken 1995, Morimoto et al., 2013). The incubated soil samples (12 g, wet weight) were suspended in 24 ml 0.8% NaCl and sonicated for 5 min using a VS-F100 sonicator (AS One, Osaka, Japan). Next, the soil suspension was equally divided into six tubes, and 6 ml suspension was added to an equal volume of Nycodenz (Axis-Shield PoC AS, Oslo, Norway) with a 1.3 g ml^{-1} density, followed by centrifugation at 10,000 × *g* for 40 min at 4°C. The bacterial cell layer was carefully collected from the six tubes using a pipette. The bacteria cells were washed using 0.8% NaCl by centrifugation at 10,000 × *g* for 20 min at 4°C to remove the Nycodenz solution.

Media and culture conditions

MS and LB media

P. putida F1 was cultured in MS or LB medium. The cultures were incubated at 30°C with vigorous shaking (200 rpm) and the growth was monitored through OD600 measurements. The cultures were harvested by centrifugation at 6,000 × *g* when the mid-exponential phase (OD600 = 0.3) and the stationary phase (25 h of incubation) were reached. The pellets were washed twice using 0.8% (w/v) NaCl.

Soil extract medium

Soil extract (SE) media were prepared for each soil by suspending 60 g air-dried soil in 300 ml 3-(N-morpholino)-propanesulfonic acid buffer (10 mM, pH 7) and shaking at 200 rpm for 1 h (Vilain et al., 2006). The soil suspension was centrifuged at 10,000 × *g* for 20 min at 4°C.The extract was filtered sequentially through 3.0-, 0.45-, and 0.2-μm mixed cellulose ester-type membrane filters (Advantec, Tokyo, Japan) to remove soil particles and bacteria cells. The *P. putida* F1 stain was grown at 30°C with vigorous shaking in the SE media supplemented with 0.5% glucose. The bacteria cells were harvested at the mid-exponential phase by centrifugation. The pellets were washed twice using 0.8% (w/v) NaCl.

Extraction of bacterial proteins.

Protein extraction from the soil bacterial pellets was performed using a modified protocol described by Wang *et al* (2006). A soil pellet was washed sequentially using 1 ml 10% trichloroacetic acid/acetone, 0.1 M ammonium acetate/80% methanol, and 80% acetone in a 2-ml microtube. After the sample was dried by evaporation to remove the residual acetone, 0.5 ml SDS buffer [30% sucrose, 2% SDS, 0.1 M Tris-Cl (pH 8.0), and 5% β-mercaptoethanol] and 0.5 ml phenol (pH 8.0) were added and the tube was shaken for 30 min. After centrifugation at 8,000 × *g* for 10 min, the upper phenol phase was transferred to a fresh tube. The SDS-phenol extraction step was repeated twice. To wash the phenol phase (1 ml), an equal volume of 1 mM Tris-HCl (pH 8.0) was added, and the mixture was shaken for 10 min and centrifuged at 8,000 × *g* for 10 min; this washing step was repeated twice. The phenol phase (0.8 ml) was added to one-third volume of 100% ethanol and two volumes of 100% isopropanol. The solution was mixed thoroughly and stored at −20°C overnight to precipitate the proteins. The phenol

solution was centrifuged at 12,000 × *g* for 15 minutes at 4°C. The protein pellet was washed once using 2.0 ml 0.1 M ammonium acetate/methanol and once using 2.0 ml 80% acetone and then air-dried. Finally, the protein was dissolved in a UTC buffer [7 M urea, 2 M tiourea, 2% CHAPS, and 0.1 M Tris-HCl (pH 6.8)]. The F1 cell pellets that were harvested from the liquid cultures were lysed using the ReadyPrep Protein Extraction Kit (Total Protein) (Bio-Rad Laboratories, Hercules, CA, USA).

The protein concentrations of all samples were measured using the Protein Assay Kit (Bio-Rad Laboratories).

Trypsin in-gel proteolysis and nanoLC-MS/MS analysis

Proteome analysis was performed as previously described (Kasahara et al., 2012). Proteins (50 μg) were separated using 12.5% SDS–PAGE and stained using Coomassie brilliant blue. The gel lanes were cut into 60 strips of ~1 mm. The gel strips were completely de-stained using 30% acetonitrile (ACN) in 25 mM NH_4HCO_3, reduced using 10 mM dithiothreitol, and alkylated using 55 mM iodoacetamide. After the gel strips were completely dried, the proteins were digested using 40 μl sequencing-grade modified trypsin (12.5 ng μl^{-1} in 50 mM NH_4HCO_3) at 37°C overnight. The digested peptides were extracted once using 25 mM NH_4HCO_3 in 60% ACN and twice using 5% formic acid in 70% ACN.

Nano-liquid chromatography-electrospray ionization-tandem mass spectrometry (nanoLC–ESI–MS/MS) analysis of the peptide mixtures was performed using an LTQ ion-trap MS (Thermo Fisher Scientific, Yokohama, Japan) coupled with a multidimensional HPLC Paradigm MS2 (AMR Inc., Tokyo, Japan) and a nano-spray electrospray ionization device (Michrom Bioresources Inc., Aubum, CA, USA). The tryptic peptides were loaded onto an L-column2 ODS (Chemicals Evaluation & Research Inst., Tokyo, Japan) packed with C18 modified silica particles (5 μm, 12-nm pore size) and separated by a linear gradient of 15–65% buffer B for 40 min, followed by a gradient of 65–95% buffer B for 1 min (buffer B: 90% methanol and 0.1% formic acid in H_2O) at a flow rate of 1 μl min^{-1}. Peptide spectra were recorded in a mass range of m/z 450–1,800. MS/MS spectra were acquired in a data-dependent scan mode. After completing the full spectrum scan, the MS/MS spectra of the most intense individual

peaks were also collected. The dynamic exclusion features were set as follows: a repeat count of one within 30 s, exclusion duration of 180 s, and an exclusion list size of 50.

Protein identification

The MS/MS data obtained were searched against a database using Mascot ver. 2.4 (Matrix Science, London, UK), on an in-house server to identify proteins. The protein databases used were the *P. putida* F1 (NC_009512) sequence and all completed bacterial genomes (ftp://ftp.ncbi.nlm.nih.gov/genomes/Bacteria/all.faa.tar.gz) in NCBI. The search parameters were set as follows: tryptic digest with a maximum of two missed cleavage sites; fixed modifications, carbamidomethyl cysteine; variable modifications, methionine oxidation; peptide masses, monoisotopic, positive charge (+1, +2, +3) of the peptide; and mass tolerance of 1.2 Da for the precursor ion and 0.8 Da for product ions. To assess false-positive identifications, an automatic decoy search was performed against a randomized database with a default significance threshold of $P < 0.05$; the false discovery rate at the identity threshold was below 8.9%. Proteins were identified with more than two unique peptide-filtering criteria.

Response to NO

The NO-releasing compound used was 1-Hydroxy-2-oxo-3-(*N*-ethyl-2-aminoethyl)-3-ethyl-1-triazene (NOC12) (Dojindo, Kumamoto, Japan). Stock solutions (10 mM) were freshly prepared in 0.1 M NaOH. The F1 strain was cultured in MS medium supplemented with the trace elements and 0.5% glucose containing 0.1 mM NOC12. The medium without NOC12 was used as a control. The cultures were incubated at 30°C with vigorous shaking and harvested at the mid-exponential phase (OD600 = 0.3). The pellets were washed using 0.8% NaCl, and lysed using the ReadyPrep Protein Extraction Kit (Total Protein) (Bio-Rad Laboratories).

Expression factor analysis for the cluster of soil-induced genes

Culture condition

The F1 strain was cultured at 30°C with vigorous shaking to the mid-exponential

phase (OD600 = 0.3) and centrifuged at 6,000 × *g* for 5 min. The cell pellet was resuspended and transferred to the original MS medium, modified MS media without $MgSO_4$, $(NH_4)_2SO_4$, SO_4^{2-} [$MgSO_4$ and $(NH_4)_2SO_4$], $CaCl_2$, PO_4^{3-} (KH_2PO_4 and Na_2HPO_4), or trace element, respectively, 0.8% NaCl, and sterile DW. The cell suspensions were incubated for 2 h at 30°C, and the cells were harvested by centrifugation. The cell pellets were used for proteome analysis and reverse transcription PCR (RT-PCR) experiments.

Proteome analysis

After separating the F1 cellular proteins (50 μg) using 12.5% SDS-PAGE and staining with Coomassie, the gel was cut into seven and four strips corresponding to the protein ranges of 20–30 and 40–55 kDa, respectively.

RT–PCR analysis

Total RNA from F1 cells incubated in MS medium or DW was extracted using Isogen II (Nippon Gene Co., Ltd., Tokyo, Japan), with the addition of a DNase treatment step, using 10 U DNase (TaKaRa Bio, Otsu, Japan) for 30 min at 37°C. RT-PCR was performed with the RNA samples using the SuperScript III First-Strand Synthesis System (Life Technologies, Tokyo, Japan) according to the manufacturer's instructions. The following gene sequences were amplified using specific sets of forward and reverse primers: Pput_3040 (160 bp), 5'-TTGGACCAGGCAGGCAGC-3' and 5'-TCAAGGGTTCAGGTGTGC-3'; Pput_3041 (158 bp), 5'-CTGGAGCTGGCTGAACAG-3' and 5'-TCGATGACATGTTCGCGCC-3'; Pput_3042 (156 bp), 5'- GTCAGCCTGGACAGCTAC-3' and 5'-GTGGCCGTACTCCTCTTC-3'; Pput_3043 (150 bp), 5'-GCAGCGTTACACCTACCG-3' and 5'-GCGCGTTCGGCGAACAGC-3' and Pput_3044 (154 bp), 5'-GATGTGCAGCATTACCTG-3' and 5'-GGTTACCCGTGAAACAGC-3'. DNA-directed RNA polymerase subunit beta (*rpoB*, Pput_0480) was used as a control, with the primers 5'-CCGGACGTCATGGATGTG-3' and 5'-CTCCAGGGCAGCATTGCC-3'. The RT-PCR products were separated and visualized using 2.0% agarose gel electrophoresis.

The experiment was repeated three times.

References

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