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Modulation of Glucose Transport Causes Preferential Utilization of Aromatic Compounds in *Pseudomonas putida* CSV86[▽]

Aditya Basu,[1] Rahul Shrivastava,[1] Bhakti Basu,[2] Shree K. Apte,[2] and Prashant S. Phale[1]*

Biotechnology Group, School of Biosciences and Bioengineering, Indian Institute of Technology—Bombay, Powai, Mumbai 400 076, India,[1] and Molecular Biology Division, Bhabha Atomic Research Center, Trombay, Mumbai 400 085, India[2]

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***Pseudomonas putida* CSV86 utilizes aromatic compounds in preference to glucose and coutilizes aromatics and organic acids. Protein analysis of cells grown on different carbon sources, either alone or in combination, revealed that a 43-kDa periplasmic-space protein was induced by glucose and repressed by aromatics and succinate. Two-dimensional gel electrophoresis and liquid chromatography-tandem mass spectrometry analysis identified this protein as closely resembling the sugar ABC transporter of *Pseudomonas putida* KT2440. A partially purified 43-kDa protein showed glucose binding activity and was specific for glucose. The results demonstrate that the aromatic- and organic acid-mediated repression of a periplasmic-space glucose binding protein and consequent inhibition of glucose transport are responsible for this strain's ability to utilize aromatics and organic acids in preference to glucose.**

It is well documented that when grown on a mixture of carbon sources, enterobacteria first utilize sugars like glucose, followed by the more complex carbon sources, like polysaccharides, polypeptides, or aromatic compounds, resulting in a diauxic growth response reflecting sequential utilization. In enteric bacteria, such as *Escherichia coli*, glucose is the most preferred carbon source and inhibits the synthesis of enzymes required for the metabolism of other carbon sources, e.g., repression of lactose utilizing enzymes by glucose (13, 15, 33, 40). This phenomenon, referred to as catabolite repression control in *E. coli*, is mediated by levels of cyclic AMP (cAMP) together with cAMP receptor protein (15, 25). In *E. coli*, glucose transport by the phosphoenolpyruvate phosphotransferase-dependent transport system drives a protein phosphorylation cascade which affects cAMP levels by modulating the adenylate cyclase activity and the catabolite activator protein (5, 29).

Catabolite repression control in nonenteric bacteria, like pseudomonads, is not clearly understood. In these bacteria, irrespective of the carbon source, the intracellular cAMP levels and adenylate cyclase activity remain constant and external addition of cAMP does not alter the repression and the sequence of carbon source utilization (28, 36). In *Pseudomonas*, the phosphoenolpyruvate phosphotransferase system for the transport of sugar has been reported only for fructose (8, 27, 32), while glucose is transported by active transport (5, 27) which is inhibited by sodium azide (20). The components involved in the glucose uptake are (i) an outer membrane protein, OprB (9, 34, 42); (ii) a periplasmic glucose binding protein (37, 39); and (iii) an inner membrane glucose transport protein (1).

In pseudomonads, glucose has been reported to exhibit repression at the transcriptional level for the enzymes involved in methyl phenol (22) metabolism. It also represses the enzymes involved in the degradation of benzyl alcohol in *Pseudomonas putida* (10) and delays the induction of the phenylacetic acid transport system in *P. putida* U (35). Moreover, organic acids have also been found to suppress the uptake and the catabolizing enzymes of glucose (12, 23, 24, 31, 41), amide (38), histidine (28), protocatechuate (43), xylene (7), chlorocatechol (19), and methyl phenol (22). Repression of phenanthrene degradation in *P. putida* by plant root extract and exudates containing glucose, acetate, and amino acids has also been reported (30).

Pseudomonas putida CSV86 utilizes aromatic compounds, like naphthalene, methylnaphthalene, benzyl alcohol, salicylate, and benzoate, as the sole carbon source (3, 18). The uptake of glucose in CSV86 is by an active transport, and its metabolism is via an inducible intracellular phosphorylative pathway (4). The strain utilizes aromatic compounds or organic acids in preference to glucose and coutilizes aromatics and organic acids (2). We have previously reported that the preferential utilization of aromatic compounds over glucose in CSV86 involves the suppression of glucose 6-phosphate dehydrogenase (ZWF) by aromatics and the inability of glucose to suppress the aromatic degrading enzymes (2). Here, we show that the aromatic compounds and organic acids suppress the glucose transport protein present in the periplasmic space and in turn the utilization of glucose. Thus, besides regulation of ZWF activity (4), repression of the periplasmic-space glucose binding protein might be responsible for the preferential utilization of aromatics over glucose in this strain.

MATERIALS AND METHODS

Growth conditions and growth profile. *Pseudomonas putida* CSV86 was grown on 150 ml minimal salt medium (MSM [3]) in 500-ml-capacity baffled Erlenmeyer flasks at 30°C on a rotary shaker (200 rpm). The medium was supplemented aseptically with the appropriate aromatics (0.1%), glucose (0.25%), or organic acids (0.25%) either alone or in combination as the source of carbon and

* Corresponding author. Mailing address: Biotechnology Group, School of Biosciences and Bioengineering, Indian Institute of Technology—Bombay, Powai, Mumbai 400 076, India. Phone: 91-22-2576 7836. Fax: 91-22-2572 3480. E-mail: pphale@iitb.ac.in.

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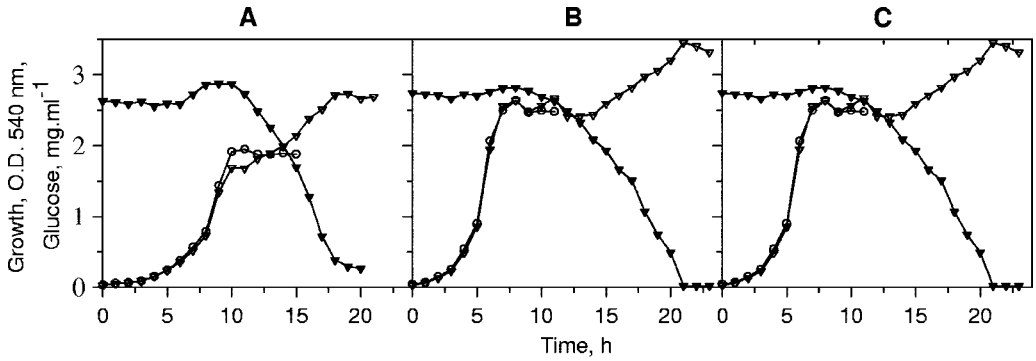

FIG. 1. Growth profiles of *Pseudomonas putida* CSV86 on different carbon sources. The growth profiles of CSV86 (▽) on naphthalene (0.1%) plus glucose (0.25%) (A), benzyl alcohol (0.1%) plus glucose (0.25%) (B), and succinate (0.25%) plus glucose (0.25%) (C) are shown. The growth profile for a single carbon source, such as naphthalene (0.1%), benzyl alcohol (0.1%), or succinate (0.25%), is represented by circles. The decreasing levels of glucose in the spent medium for panels A, B, and C are shown by inverted triangles.

energy. Growth was monitored by measuring the increase in optical density at 540 nm (OD_{540}), using sterile MSM as the reference.

Chemical estimations. Protein was estimated by the method of Bradford (6), using bovine serum albumin as the standard. Glucose was estimated as described by Miller (21), using glucose as the standard.

[^{14}C]glucose uptake by whole cells. [^{14}C]glucose uptake by whole cells of *P. putida* CSV86, grown on either a single or double carbon source, was measured as described earlier (4). The cells were harvested, washed, suspended in MSM to an OD_{540} of 0.2, and kept on a shaking water bath at 30°C for 10 min. [^{14}C]glucose (500 nM; specific activity, 140 mCi/mmol) was added to the prewarmed cell suspension (10 ml) and incubated at 30°C in a shaking water bath. Samples (100 μl) were drawn at various time points and filtered through premoistened 0.45-μm cellulose ester filters (Pall Life Sciences). The filters were immediately washed twice with sterile MSM (1 ml per wash), air dried, and vigorously mixed in a scintillation cocktail {0.4% PPO [2,5-diphenyloxazole] and 0.025% POPOP [1,4-bis(5-phenyloxazolyl)benzene] in toluene}. Radioactivity was measured using a liquid scintillation counter (LKB). Cells incubated with formaldehyde (25 mM) or sodium azide (30 mM) for 15 min were used as a control. Glucose uptake is reported as pmol of [^{14}C]glucose accumulated.

Extraction of periplasmic-space proteins. Periplasmic-space proteins from *P. putida* CSV86 were isolated by the cold-shock method as described by Hoshino and Kageyama (11). Cells grown on either a single or double carbon source were harvested and washed twice with Tris-HCl buffer (50 mM, pH 7.5, 100 ml). Cells (600 mg, wet weight) were resuspended in 3 ml of Tris-HCl buffer (50 mM, pH 7.5) containing $MgCl_2$ (0.2 M). The cell suspension was incubated at 35°C for 10 min in a shaking water bath and then immediately placed on ice and incubated for 15 min. This process was repeated twice, and the cell suspension was centrifuged at 20,000 × *g* for 20 min. The clear supernatant contained periplasmic-space proteins. The proteins were resolved on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel (10%) (17) and visualized by staining with Coomassie brilliant blue (0.025%).

[^{14}C]glucose binding assay. [^{14}C]glucose binding activity in the periplasmic fraction of *P. putida* CSV86 was measured as described by Sly and coworkers (37). The periplasmic-space protein fractions were dialyzed against binding buffer (Tris-HCl [10 mM, pH 7.5], $MgCl_2$ [1 mM]) for 5 h. Glucose binding assays (1 ml) contained periplasmic-space protein (100 μg) and [^{14}C]glucose (500 nM; specific activity, 140 mCi/mmol) in binding buffer. The mixture was incubated for 5 min at 30°C and rapidly filtered through premoistened 0.45-μm polyvinylidene difluoride (PVDF) membranes (Pall Life Sciences). The filters were washed twice with binding buffer (1 ml per wash) and air dried. Radioactivity was measured as described above. Glucose binding activity was expressed as pmol [^{14}C]glucose bound mg^{-1} of protein.

Second carbon source spiking assay. *P. putida* CSV86 cells were grown until mid-log phase (15 h) on glucose (0.25%) and spiked with either naphthalene (0.1%) or succinate (0.25%) under aseptic conditions. After being spiked, cells were harvested at different time points and periplasmic-space protein fractions were prepared as described above. [^{14}C]glucose binding activity was measured as described above and expressed as percent [^{14}C] glucose binding activity with respect to that obtained with nonspiked-glucose-grown cells at 15 h (i.e., 0 h).

Partial purification of glucose binding periplasmic-space protein. Periplasmic-space proteins extracted from *P. putida* CSV86 were fractionated by Sephacryl S-200-HR (Sigma-Aldrich) gel filtration chromatography (column, 90 by 1.0 cm; bed volume, 71 ml; void volume, 33 ml; flow rate, 4 ml h^{-1}; fraction size, 1 ml) using binding buffer at 4°C. Fractions were analyzed for protein OD_{280}, and [^{14}C]glucose binding activity was measured as described above and expressed as pmol [^{14}C]glucose bound mg^{-1} of protein.

To study the substrate specificity, the assay mixture (1 ml) contained partially purified periplasmic-space glucose binding protein (5 μg), [^{14}C]glucose (500 nM), and a 50-fold excess (25 μM) of unlabeled sugars, organic acids, or aromatic compounds. Naphthalene was prepared by dissolving it in dimethyl sulfoxide, while all other compounds were prepared in the binding buffer. Control reaction mixtures contained [^{14}C]glucose, protein, binding buffer, and the appropriate amount of dimethyl sulfoxide. The reaction mixture was incubated for 5 min at 30°C and rapidly filtered through premoistened 0.45-μm PVDF membranes (Pall Life Sciences). Radioactivity was measured as described above and expressed as percent inhibition of [^{14}C]glucose binding.

2-D gel electrophoresis, LC-MS/MS analysis, and NH_2-terminal sequencing. Two-dimensional (2-D) gel electrophoresis was performed with periplasmic-space proteins extracted from *P. putida* CSV86 as described by O'Farrell (26). In the first dimension, the isoelectric focusing was performed using ampholine (pH range of 3.5 to 10; Sigma) for 10,000 V hours. In the second dimension, discontinuous SDS-PAGE (12%) was used as described by Laemmli (17). Other details of 2-D protocols were as described earlier (14). The gels were stained overnight with Coomassie brilliant blue, destained, dried, and visualized. The spots of interest were excised and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis (ESI-TRAP, trypsin digest; peptide mass tolerance, ±2 Da; fragment mass tolerance, 0.8 Da). The data were analyzed by the Mascot protein identification system (Matrix Science, London, United Kingdom) with the database from The Institute of Genomic Research (TIGR; http:/www.tigr.org/). The criterion for reliable identification was a significant Mascot score, >47 ($P < 0.05$). For N-terminal sequencing, the 43-kDa protein from SDS-PAGE (12%) was electroblotted onto a PVDF membrane (0.45 μm; Pall) in CAPS [3-(cyclohexylamino)-1-propanesulphonic acid] buffer (10 mM, pH 11) at 200 V for 8 h, stained with Coomassie brilliant blue R250, and subjected to automated Edman degradation (Applied Biosystems 470).

All experiments described were performed at least three times. The trends observed in SDS-PAGE profiles were identical; the best profiles are shown. [^{14}C]glucose binding assays were performed in duplicate for each experiment, and the observed standard deviation was ±5%.

RESULTS

Growth profiles and whole-cell [^{14}C]glucose uptake. *Pseudomonas putida* CSV86 showed a diauxic growth profile when grown on a double carbon source, such as aromatics (naphthalene or benzyl alcohol) plus glucose (Fig. 1A and B) or succinate plus glucose (Fig. 1C) with glucose utilization in the second log phase. [^{14}C]glucose uptake by whole cells of *P. putida* CSV86 is depicted in Fig. 2. Cells grown on naphthalene

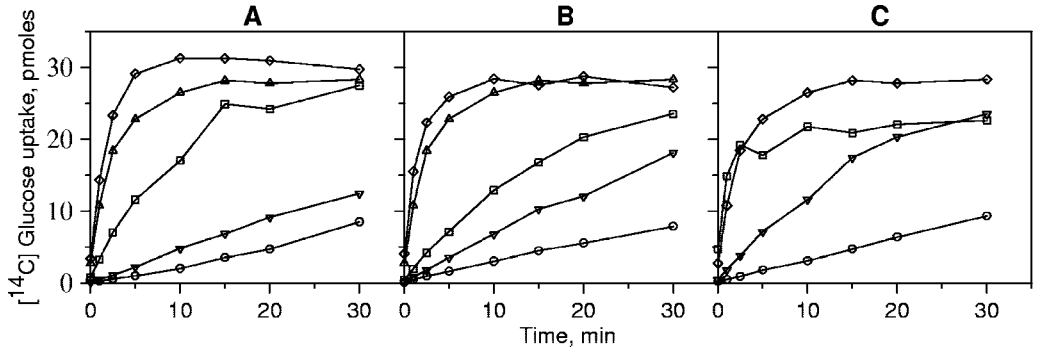

FIG. 2. [^{14}C]glucose uptake by whole cells of *Pseudomonas putida* CSV86. Cells were grown on naphthalene for 8 h (○), glucose for 22 h (◇), and naphthalene plus glucose for 4 h (▽), 8 h (□), or 22 h (△) (A); benzyl alcohol for 8 h (○), glucose for 22 h (◇), and benzyl alcohol plus glucose for 4 h (▽), 8 h (□), or 22 h (△) (B); and succinate for 7 h (○), glucose for 22 h (◇), and succinate plus glucose for 7 h (▽) and 22 h (□) (C).

(Fig. 2A) or benzyl alcohol (Fig. 2B) alone showed significantly low [^{14}C]glucose uptake compared to cells grown on glucose. Early-log (4 h)- and mid-log (8 h)-phase cells grown on naphthalene plus glucose (Fig. 2A) or benzyl alcohol plus glucose (Fig. 2B) showed gradual increases in glucose uptake, which reached maximum levels during the second log phase (22 h) of the diauxic growth profile. A similar trend was observed with succinate-plus-glucose-grown cells (Fig. 2C). The result showed that glucose uptake by the whole cells was suppressed in the first log phase when aromatics were being utilized. Once the cells entered the second log phase, glucose utilization commenced, as marked by glucose uptake by whole cells.

On a single carbon source, like glucose, the strain showed a short lag phase of 7 to 8 h and entered the stationary phase within 20 to 22 h, with glucose-grown cells as an inoculum (Fig. 3A). However, when naphthalene- or succinate-grown cells were used as the inoculum, the culture showed a significantly long lag phase of ~24 to 26 h on glucose and entered the stationary phase at ~42 to 44 h. Under all these conditions, the specific growth rate (μ; h^{-1}) on glucose was found to be 0.2 (Fig. 3A).

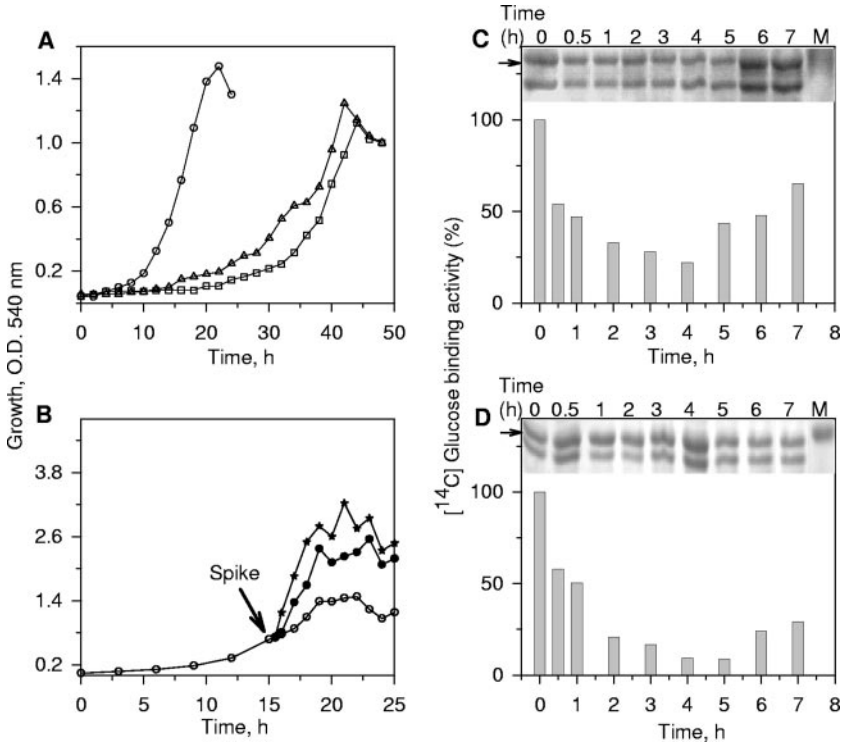

FIG. 3. Growth profiles and glucose binding activities of the periplasmic-space protein from glucose-grown cells of *Pseudomonas putida* CSV86 spiked with the second carbon source. (A) Growth profile of CSV86 precultured on either glucose (○), naphthalene (□), or succinate (△) and then transferred onto glucose (0.25%) as the sole carbon source. (B) Growth profile of glucose-grown CSV86 spiked (indicated by an arrow) at 15 h (mid-log-phase culture) with either naphthalene (●; 0.1%) or succinate (⋆; 0.25%). (C and D) [^{14}C]glucose binding activity and SDS-PAGE profile (inset) of the periplasmic-space protein of glucose-grown cells spiked with naphthalene (C) or succinate (D). The [^{14}C]glucose binding activity of 15-h glucose-grown cells was taken as 100%. The probable glucose-binding protein is indicated by an arrow. Lane M, standard molecular mass marker protein (43 kDa).

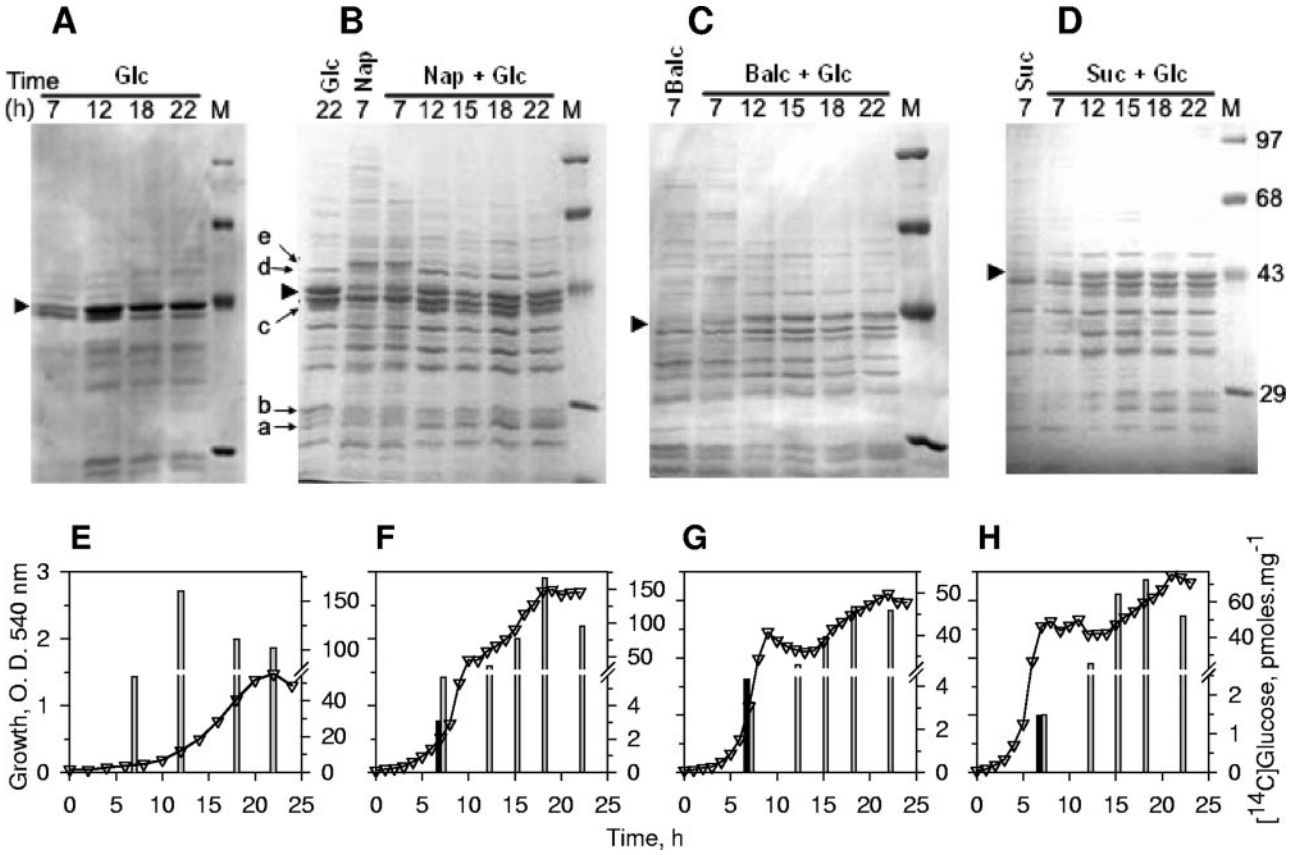

FIG. 4. (A to D) SDS-PAGE (10%) analysis of periplasmic-space proteins from *Pseudomonas putida* CSV86. Cells were grown on glucose (Glc), naphthalene (Nap), benzyl alcohol (Balc), or succinate (Suc) as well as on dual carbon sources for the durations indicated. Each lane contains 15 μg protein. Lane M contains standard molecular mass markers. Thick arrowheads denote the probable glucose binding periplasmic protein (43 kDa). Thin arrows represent significant changes observed in the levels of other proteins. (E to H) [^{14}C]glucose binding activity of the periplasmic-space protein fractions obtained from CSV86 cells grown on either naphthalene, benzyl alcohol, or succinate are represented by black bars (F to H, respectively), while binding activity from cells grown on glucose, naphthalene plus glucose, benzyl alcohol plus glucose, or succinate plus glucose are represented by gray bars (E to H, respectively). Growth profiles for respective double carbon sources are shown (▽).

When glucose-grown mid-log-phase culture (15 h) was challenged/spiked with either naphthalene (0.1%) or succinate (0.25%) as a second carbon source, cells showed significant and immediate increases in specific growth rate, from 0.20 on glucose to 0.52 on naphthalene and 0.80 on succinate (Fig. 3B). These growth rates were comparable to those reported earlier (4). The time-dependent [^{14}C]glucose binding activities of the periplasmic-space protein extract showed concomitant, immediate decreases in glucose binding activity until 4 h for naphthalene-spiked cells (Fig. 3C) and 5 h for succinate-spiked cells (Fig. 3D). As the spiked carbon source was exhausted, the [^{14}C]glucose binding activity again increased (6- and 7-h time points) (Fig. 3C and D). However, the protein profile showed no significant changes in the level of the 43-kDa protein (Fig. 3C and D, insets). These results strongly suggest that aromatics and organic acid trigger immediate suppression of periplasmic glucose binding activity.

Periplasmic-space protein profile and glucose binding studies. Periplasmic-space protein fractions prepared from cells grown on either a single or double carbon source at different time intervals and resolved by SDS-PAGE are shown in Fig. 4, upper panels. Periplasmic fractions from glucose-grown cells showed a prominent protein band with a molecular mass of 43 kDa (Fig. 4A and B) compared to periplasmic fractions from cells grown on naphthalene, benzyl alcohol, or succinate alone (Fig. 4B to D). Besides the 43-kDa protein, other significant differences observed (Fig. 4B) in the periplasmic fractions are 25 kDa (a), 29 kDa (b), 39.5 kDa (c), 48 kDa (d), and 54 kDa (e). Time-dependent analysis of periplasmic fractions from glucose-grown cells showed higher levels of the 43-kDa protein in the early-log phase of the culture (~12 h) (Fig. 4A). Cells grown on a double carbon source showed low intensities of the 43-kDa protein in the first log phase, which increased significantly in the second log phase between 12 and 18 h (Fig. 4B to D) when cells were using glucose as the carbon source.

The periplasmic-space protein fractions were further analyzed for glucose binding activity, using [^{14}C]glucose as the substrate (Fig. 4, lower panels). Periplasmic fractions from glucose-grown cells showed high glucose binding activities, reaching maximum levels at 12 h (Fig. 4E). Compared to glucose-grown cells, aromatic- or succinate-grown cells showed very low glucose binding activities (Fig. 4F to H). However, cells grown on aromatic plus glucose or organic acid plus glucose showed low [^{14}C]glucose binding activities in the first

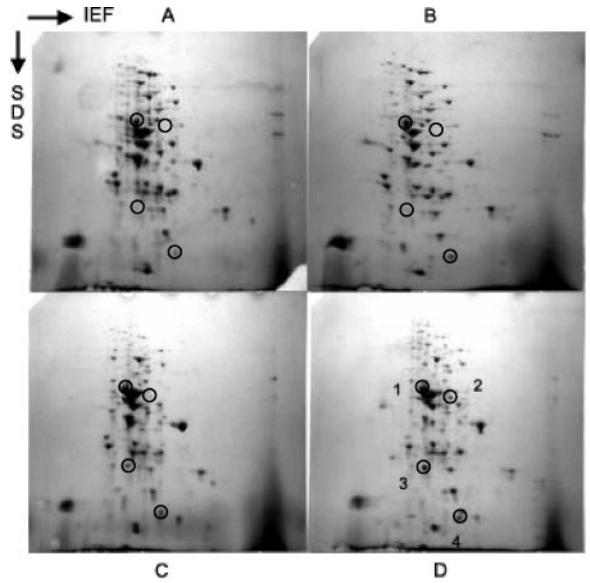

FIG. 5. 2-D gel electrophoretic analysis of the periplasmic-space protein fraction of *Pseudomonas putida* CSV86. Cells were grown on naphthalene (0.1%) (A), on naphthalene (0.1%) plus glucose (0.25%) for 7 h (B) and 15 h (C), and on glucose (0.25%) (D). Spots marked with circles were excised after visual comparison of profiles and analyzed further by LC-MS/MS as described in Materials and Methods. IEF, isoelectric focusing.

log phase, which increased significantly as cells entered the second log phase, reaching maximum levels at 18 h of diauxic growth (Fig. 4F to H). Periplasmic protein analysis by SDS-PAGE (Fig. 4A to D) and [^{14}C]glucose binding activity profiles (Fig. 4E to H) together indicate that even in the presence of glucose, the 43-kDa periplasmic protein was suppressed by aromatics and succinate in the first log phase and induced in the second log phase of the diauxic growth profile.

2-D gel electrophoresis and identification of the 43-kDa protein. 2-D-gel electrophoretic resolution showed significant differences in the protein profiles (Fig. 5). Compared to naphthalene-grown cells (Fig. 5A), glucose-grown cells (Fig. 5D) showed high levels of at least four proteins (circled and labeled 1 to 4). In cells grown on naphthalene plus glucose (Fig. 5B and C), the levels increased with time. The circled spots were excised, purified, and analyzed by LC-MS/MS (Table 1). Due to the block, the N-terminal sequence of spot 1 could not be obtained but internal peptide fragments of spot 1 corresponded to the sugar ABC transporter protein from *Pseudomonas putida* KT2440. The molecular mass (43 kDa) and isoelectric point (pI ~6.5) of spot 1, as measured from 2-D gels, also resembled to those of the sugar ABC transporter protein from *P. putida* KT2440 (45.4 kDa and pI 6.15, respectively). Spots 2, 3, and 4 corresponded to ornithine carbomyl transferase, plant type ferredoxin, and integrase-like protein, respectively, and seemed to be unrelated to glucose transport or metabolism (Table 1).

Partial purification of periplasmic-space glucose binding protein and its specificity. The gel filtration elution profile (Sephacryl S-200HR) and [^{14}C]glucose binding activities of the fractions from the periplasmic-space proteins from glucose-grown cells are shown in Fig. 6. High [^{14}C]glucose binding activity was found to be associated with fraction 39, which contained predominantly the 43-kDa protein (Fig. 6A, inset). A partially purified 43-kDa glucose binding protein (fraction 39; yield, 0.7 mg g^{-1} cells) was further analyzed for its sugar binding specificity by chasing the bound [^{14}C]glucose by using various compounds (Fig. 6B). In the presence of a 50-fold-higher concentration of unlabeled sugars, like fructose, xylose, arabinose, mannitol, ribose, gluconate, mannose, glycerol, and galactose, only 10 to 18% of [^{14}C]glucose could be displaced. Similarly, organic acids, like succinate or pyruvate, and aromatic compounds, like salicylate, benzyl alcohol, or naphthalene, showed only 1 to 13% displacement of [^{14}C]glucose (Fig. 6B). These results suggest that the periplasmic-space protein was active as a monomer of 43 kDa, specifically bound to glucose, and those aromatics and organic acids did not inhibit the glucose binding activity of the purified 43-kDa protein by direct interaction.

DISCUSSION

The ability of an organism to utilize glucose as the carbon source can be regulated at the level of either (i) glucose binding and transport, (ii) induction/repression of intracellular glucose metabolizing enzymes, or (iii) both. The glucose transport system has not been studied in detail for *Pseudomonas*. Based on the few reports, in *Pseudomonas aeruginosa* and *Pseudomonas putida* the probable components involved in the glucose transport are (i) an outer membrane protein, OprB, which is

TABLE 1. Proteomic analysis of periplasmic proteins from *Pseudomonas putida* CSV86[a]

Spot	Protein identified[b]	Accession no.	
		TIGR	SP
1	Sugar ABC transporter, periplasmic sugar-binding protein; *Pseudomonas putida* (strain KT2440)[c]	PP1015	Q88P38_PSEPK
2	Ornithine carbamoyltransferase, catabolic (EC 2.1.3.3) (OTCase); *Pseudomonas putida* (strain KT2440)	PP1000	OTCC_PSEPK
3	Probable plant-type ferredoxin; *Pseudomonas* sp. strain S-47		Q9ANS9_9PSED
4	Integrase-like protein (fragment); *Xanthomonas maltophilia* (*Pseudomonas maltophilia*) (*Stenotrophomonas maltophilia*)		Q6UQH2_XANMA

[a] Abbreviations: TIGR, The Institute of Genomic Research; SP, Swiss-Prot/TrEMBL.
[b] Identification based on the obtained annotated genes.
[c] A Mascot score of 179 indicated significant similarity/identity with this protein. Two peptide fragments obtained by LC-MS/MS with the amino acid were K.DGFIWK.D and K.AGMQIMGDWAK.S.

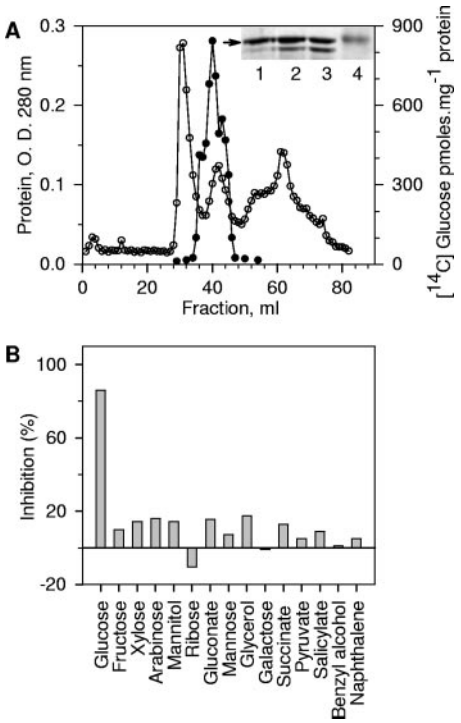

FIG. 6. Partial purification and substrate specificity of the periplasmic-space glucose binding protein from *Pseudomonas putida* CSV86. (A) Gel filtration protein elution profile (Sephacryl S-200HR) of periplasmic-space proteins (○) from glucose-grown cells of CSV86 and their [^{14}C]glucose binding activities (●). The gel filtration column was calibrated by using β-amylase (200 kDa), alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), and carbonic anhydrase (29 kDa). Inset: SDS-PAGE analysis (with Coomassie staining) of the fractions showing the highest [^{14}C]glucose binding activities: lane 1, fraction 39 (maximum [^{14}C]glucose binding activity); lane 2, fraction 40; lane 3, fraction 41; and lane 4, molecular mass marker for Ovalbumin (43 kDa). The arrow shows the 43-kDa protein. (B) Specificity of the 43-kDa glucose binding protein. See Materials and Methods for details.

an inducible protein with a molecular mass of 43 kDa (9, 34, 42); (ii) a 44.5-kDa periplasmic-space glucose binding protein (37, 39); and (iii) a 29-kDa glucose transporter protein present in the inner membrane (1). Earlier, we demonstrated that, in *P. putida* CSV86, glucose induces ZWF, an intracellular glucose metabolizing enzyme, while succinate and aromatic compounds repress it (4). In addition to intracellular regulation, the present study demonstrates another upstream regulatory event at the level of modulation of glucose binding in the periplasm.

The kinetics of glucose binding and uptake in CSV86 cells grown on different carbon sources clearly showed that these are repressed in the presence of aromatics, induced by glucose, and progressively induced as aromatics are utilized during growth on double carbon (glucose plus aromatics) sources (Fig. 1 and 2). In particular, a 43-kDa glucose binding periplasmic-space protein is repressed by the aromatics and modulates glucose uptake. This is supported by the following lines of evidence: (i) the high correlation between the levels of the 43-kDa periplasmic-space protein and glucose binding activity (Fig. 1 to 4), (ii) the proteomic identification of the 43-kDa protein as very similar to the sugar ABC transporter periplasmic sugar binding protein of *P. putida* strain KT2440 (16) (Fig. 5 and Table 1), and (iii) the high-specificity binding of the partially purified 43-kDa protein to [^{14}C]glucose (Fig. 6). The inabilities of aromatic compounds or organic acids to inhibit glucose binding to the purified 43-kDa periplasmic-space protein rule out the possibility of direct modulation of its glucose binding activity (Fig. 6). However, periplasmic protein fractions obtained from naphthalene- or succinate-spiked cells showed immediate inhibition of glucose binding activity, with no significant change in the levels of the 43-kDa protein (Fig. 3). This observation suggests that there may be an involvement of a second protein, probably from the periplasmic space, that binds to aromatics or organic acid, which in turn interacts with the 43-kDa protein to inhibit its glucose binding activity. The involvement of an inducible, glucose binding periplasmic-space protein (44.5 kDa, pI 4.7) in the glucose transport has also been reported for *P. aeruginosa* (37, 39).

Based on our earlier data (2, 4) and the results obtained from the present study, the preferential utilization of aromatics or organic acids over glucose by *P. putida* CSV86 seems to be a consequence of (i) induction of aromatic degrading enzymes by respective aromatics even in the presence of glucose, (ii) a lack of repression of aromatic degrading enzymes by glucose, (iii) repression of the glucose metabolizing enzyme ZWF even in the presence of glucose, and (iv) modulation of glucose transport by repression of a 43-kDa periplasmic-space glucose binding protein by aromatics or products of their catabolism, such as organic acids. During growth on a double carbon source, once the aromatics/organic acids have been depleted, glucose triggers the induction of glucose binding protein, leading to its uptake, metabolism, and growth.

The environmental occurrence of aromatics is a serious cause for concern on account of their ill effects on life forms at all levels. Several aromatic degrading microbes have been isolated in the past, but many of them suffer from catabolite repression by glucose. Strain CSV86 is able to perform this novel feat due to preferential utilization of aromatics over glucose mainly, although not exclusively, through repression of a 43-kDa periplasmic-space glucose binding protein. The present study clearly shows that genetic manipulations aimed at constructing aromatic-suppressible glucose transport systems may yield bacterial strains more proficient for environmental degradation of aromatic compounds.

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REFERENCES

1. **Adewoye, L. O., and E. A. Worobec.** 2000. Identification and characterization of the *gltK* gene encoding a membrane-associated glucose transport protein of *Pseudomonas aeruginosa*. Gene **253:**323–330.
2. **Basu, A., S. K. Apte, and P. S. Phale.** 2006. Preferential utilization of aromatic compounds over glucose by *Pseudomonas putida* CSV86. Appl. Environ. Microbiol. **72:**2226–2230.
3. **Basu, A., S. S. Dixit, and P. S. Phale.** 2003. Metabolism of benzyl alcohol via catechol *ortho*-pathway in methylnaphthalene-degrading *Pseudomonas putida* CSV86. Appl. Microbiol. Biotechnol. **62:**579–585.
4. **Basu, A., and P. S. Phale.** 2006. Inducible uptake and metabolism of glucose by the phosphorylative pathway in *Pseudomonas putida* CSV86. FEMS Microbiol. Lett. **259:**311–316.
5. **Boos, W.** 1974. Bacterial transport. Annu. Rev. Biochem. **43:**123–146.
6. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of

microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
7. **Duetz, W. A., S. Marques, C. de Jong, J. L. Ramos, and J. G. van Andel.** 1994. Inducibility of the TOL catabolic pathway in *Pseudomonas putida* (pWW0) growing on succinate in continuous culture: evidence of carbon catabolite repression control. J. Bacteriol. **176:**2354–2361.
8. **Eagon, R. G., and P. V. Phibbs, Jr.** 1971. Kinetics of transport of glucose, fructose, and mannitol by *Pseudomonas aeruginosa*. Can. J. Biochem. **49:** 1031–1041.
9. **Hancock, R. E., and A. M. Carey.** 1980. Protein D1—a glucose-inducible, pore-forming protein from the outer membrane of *Pseudomonas aeruginosa*. FEMS Microbiol. Lett. **8:**105–109.
10. **Holtel, A., S. Marques, I. Mohler, U. Jakubzik, and K. N. Timmis.** 1994. Carbon source-dependent inhibition of *xyl* operon expression of the *Pseudomonas putida* TOL plasmid. J. Bacteriol. **176:**1773–1776.
11. **Hoshino, T., and M. Kageyama.** 1980. Purification and properties of a binding protein for branched-chain amino acids in *Pseudomonas aeruginosa*. J. Bacteriol. **141:**1055–1063.
12. **Hylemon, P. B., and P. V. Phibbs, Jr.** 1972. Independent regulation of hexose catabolizing enzymes and glucose transport activity in *Pseudomonas aeruginosa*. Biochem. Biophys. Res. Commun. **48:**1041–1048.
13. **Inada, T., H. Takahashi, T. Mizuno, and H. Aiba.** 1996. Down regulation of cAMP production by cAMP receptor protein in *Escherichia coli*: an assessment of the contributions of transcriptional and posttranscriptional control of adenylate cyclase. Mol. Gen. Genet. **253:**198–204.
14. **Joshi, B., R. Schmid, K. Altendorf, and S. K. Apte.** 2004. Protein recycling is a major component of postirradiation recovery in *Deinococcus radiodurans* strain R1. Biochem. Biophys. Res. Commun. **320:**1112–1117.
15. **Kimata, K., H. Takahashi, T. Inada, P. Postma, and H. Aiba.** 1997. cAMP receptor protein-cAMP plays a crucial role in glucose-lactose diauxie by activating the major glucose transporter gene in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **94:**12914–12919.
16. **Kurbatov, L., D. Albrecht, H. Herrmann, and L. Petruschka.** 2006. Analysis of the proteome of *Pseudomonas putida* KT2440 grown on different sources of carbon and energy. Environ. Microbiol. **8:**466–478.
17. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
18. **Mahajan, M. C., P. S. Phale, and C. S. Vaidyanathan.** 1994. Evidence for the involvement of multiple pathways in the biodegradation of 1- and 2-methylnaphthalene by *Pseudomonas putida* CSV86. Arch. Microbiol. **161:**425–433.
19. **McFall, S. M., B. Abraham, C. G. Narsolis, and A. M. Chakrabarty.** 1997. A tricarboxylic acid cycle intermediate regulating transcription of a chloroaromatic biodegradative pathway: fumarate-mediated repression of the *clcABD* operon. J. Bacteriol. **179:**6729–6735.
20. **Midgley, M., and E. A. Dawes.** 1973. The regulation of transport of glucose and methyl alpha-glucoside in *Pseudomonas aeruginosa*. Biochem. J. **132:** 141–154.
21. **Miller, G. L.** 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. **31:**426–428.
22. **Müller, C., L. Petruschka, H. Cuypers, G. Burchhardt, and H. Herrmann.** 1996. Carbon catabolite repression of phenol degradation in *Pseudomonas putida* is mediated by the inhibition of the activator protein PhlR. J. Bacteriol. **178:**2030–2036.
23. **Nandadasa, H. G., M. Andreesen, and H. G. Schlegel.** 1974. The utilization of 2-ketogluconate by *Hydrogenomonas eutropha* H 16. Arch. Microbiol. **99:**15–23.
24. **Ng, F. M., and E. A. Dawes.** 1973. Chemostat studies on the regulation of glucose metabolism in *Pseudomonas aeruginosa* by citrate. Biochem. J. **132:** 129–140.
25. **Notley-McRobb, L., A. Death, and T. Ferenci.** 1997. The relationship between external glucose concentration and cAMP levels inside *Escherichia coli*: implications for models of phosphotransferase-mediated regulation of adenylate cyclase. Microbiology **143:**1909–1918.
26. **O'Farrell, P. H.** 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250:**4007–4021.
27. **Phibbs, P. V., Jr., and R. G. Eagon.** 1970. Transport and phosphorylation of glucose, fructose, and mannitol by *Pseudomonas aeruginosa*. Arch. Biochem. Biophys. **138:**470–482.
28. **Phillips, A. T., and L. M. Mulfinger.** 1981. Cyclic adenosine 3′,5′-monophosphate levels in *Pseudomonas putida* and *Pseudomonas aeruginosa* during induction and carbon catabolite repression of histidase synthesis. J. Bacteriol. **145:**1286–1292.
29. **Postma, P. W., J. W. Lengeler, and G. R. Jacobson.** 1993. Phosphoenolpyruvate:carbohydrate phosphotransferase systems of bacteria. Microbiol. Rev. **57:**543–594.
30. **Rentz, J. A., P. J. Alvarez, and J. L. Schnoor.** 2004. Repression of *Pseudomonas putida* phenanthrene-degrading activity by plant root extracts and exudates. Environ. Microbiol. **6:**574–583.
31. **Roberts, B. K., M. Midgley, and E. A. Dawes.** 1973. The metabolism of 2-oxogluconate by *Pseudomonas aeruginosa*. J. Gen. Microbiol. **78:**319–329.
32. **Romano, A. H., S. J. Eberhard, S. L. Dingle, and T. D. McDowell.** 1970. Distribution of the phosphoenolpyruvate: glucose phosphotransferase system in bacteria. J. Bacteriol. **104:**808–813.
33. **Santillan, M., and M. C. Mackey.** 2004. Influence of catabolite repression and inducer exclusion on the bistable behavior of the lac operon. Biophys. J. **86:**1282–1292.
34. **Saravolac, E. G., N. F. Taylor, R. Benz, and R. E. Hancock.** 1991. Purification of glucose-inducible outer membrane protein OprB of *Pseudomonas putida* and reconstitution of glucose-specific pores. J. Bacteriol. **173:**4970–4976.
35. **Schleissner, C., E. R. Olivera, M. Fernandez-Valverde, and J. M. Luengo.** 1994. Aerobic catabolism of phenylacetic acid in *Pseudomonas putida* U: biochemical characterization of a specific phenylacetic acid transport system and formal demonstration that phenylacetyl-coenzyme A is a catabolic intermediate. J. Bacteriol. **176:**7667–7676.
36. **Siegel, L. S., P. B. Hylemon, and P. V. Phibbs, Jr.** 1977. Cyclic adenosine 3′,5′-monophosphate levels and activities of adenylate cyclase and cyclic adenosine 3′,5′-monophosphate phosphodiesterase in *Pseudomonas* and *Bacteroides*. J. Bacteriol. **129:**87–96.
37. **Sly, L. M., E. A. Worobec, R. E. Perkins, and P. V. Phibbs, Jr.** 1993. Reconstitution of glucose uptake and chemotaxis in *Pseudomonas aeruginosa* glucose transport defective mutants. Can. J. Microbiol. **39:**1079–1083.
38. **Smyth, P. F., and P. H. Clarke.** 1975. Catabolite repression of *Pseudomonas aeruginosa* amidase: the effect of carbon source on amidase synthesis. J. Gen. Microbiol. **90:**81–90.
39. **Stinson, M. W., M. A. Cohen, and J. M. Merrick.** 1977. Purification and properties of the periplasmic glucose-binding protein of *Pseudomonas aeruginosa*. J. Bacteriol. **131:**672–681.
40. **Stulke, J., and W. Hillen.** 1999. Carbon catabolite repression in bacteria. Curr. Opin. Microbiol. **2:**195–201.
41. **Tiwari, N. P., and J. J. Campbell.** 1969. Enzymatic control of the metabolic activity of *Pseudomonas aeruginosa* grown in glucose or succinate media. Biochim. Biophys. Acta **192:**395–401.
42. **Wylie, J. L., and E. A. Worobec.** 1995. The OprB porin plays a central role in carbohydrate uptake in *Pseudomonas aeruginosa*. J. Bacteriol. **177:**3021–3026.
43. **Zylstra, G. J., R. H. Olsen, and D. P. Ballou.** 1989. Cloning, expression, and regulation of the *Pseudomonas cepacia* protocatechuate 3,4-dioxygenase genes. J. Bacteriol. **171:**5907–5914.