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Arabitol Metabolism of *Corynebacterium glutamicum* and Its Regulation by AtlR

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Expression profiling of *Corynebacterium glutamicum* in comparison to a derivative deficient in the transcriptional regulator AtlR (previously known as SucR or MtlR) revealed eight genes showing more than 4-fold higher mRNA levels in the mutant. Four of these genes are located in the direct vicinity of the *atlR* gene, i.e., *xylB*, *rbtT*, *mtlD*, and *sixA*, annotated as encoding xylulokinase, the ribitol transporter, mannitol 2-dehydrogenase, and phosphohistidine phosphatase, respectively. Transcriptional analysis indicated that *atlR* and the four genes are organized as *atlR-xylB* and *rbtT-mtlD-sixA* operons. Growth experiments with *C. glutamicum* and *C. glutamicum* Δ*atlR*, Δ*xylB*, Δ*rbtT*, Δ*mtlD*, and Δ*sixA* derivatives with sugar alcohols revealed that (i) wild-type *C. glutamicum* grows on D-arabitol but not on other sugar alcohols, (ii) growth in the presence of D-arabitol allows subsequent growth on D-mannitol, (iii) D-arabitol is cometabolized with glucose and preferentially utilized over D-mannitol, (iv) RbtT and XylB are involved in D-arabitol but not in D-mannitol metabolism, (v) MtlD is required for D-arabitol and D-mannitol metabolism, and (vi) SixA is not required for growth on any of the substrates tested. Furthermore, we show that MtlD confers D-arabitol and D-mannitol dehydrogenase activities, that the levels of these and also xylulokinase activities are generally high in the *C. glutamicum* Δ*atlR* mutant, whereas in the parental strain, they were high when cells were grown in the presence of D-arabitol and very low when cells were grown in its absence. Our results show that the XylB, RbtT, and MtlD proteins allow the growth of *C. glutamicum* on D-arabitol and that D-arabitol metabolism is subject to arabitol-dependent derepression by AtlR.

Corynebacterium glutamicum is a Gram-positive soil bacterium that since its discovery has been employed for the industrial production of amino acids, in particular L-glutamate and L-lysine (1, 51, 70). More recently, *C. glutamicum* has also been successfully engineered for the production of other compounds, e.g., putrescine and cadaverine (40, 66), 2-ketoisovalerate (46), ethanol (37), isobutanol (12, 68), and organic acids (58, 59). The organism can use a variety of sugars (e.g., glucose, fructose, sucrose, ribose, or maltose) and organic acids (acetate, propionate, pyruvate, lactate, or citrate) as single or combined carbon and energy sources for growth and also for amino acid production. *C. glutamicum* is also able to use ethanol as a sole carbon and energy source, using alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) for NAD-dependent oxidations to acetate, which is then activated to acetyl coenzyme A (acetyl-CoA) and channeled into the citric acid and glyoxylate cycles (2, 4, 5, 44). The ADH gene (*adhA*) of *C. glutamicum* has been shown to be subject to complex, carbon source-dependent regulation by the global transcriptional regulators RamA, RamB, and GlxR (2, 6, 43). In the course of further studies on the transcriptional regulation of the *adhA* gene, we recently identified the DeoR-type transcriptional regulator Cg0146 as the fourth regulator involved in the expression control of *adhA* (7).

The Cg0146 protein was isolated for the first time by DNA affinity chromatography with the promoter region of the lactate dehydrogenase (LDH) gene *ldhA* (22). However, the specific LDH activity of a Cg0146-deficient mutant was not different from that of the parental wild-type (WT) strain, and thus, the physiological role of Cg0146 in *ldhA* expression remained unclear (22). In 2010, Cg0146 was reported to be a novel regulator (designated SucR), which binds to the succinyl-CoA synthetase operon *sucCD* and slightly represses its expression when cells are grown on acetate (17). Very recently, we found that Cg0146 also negatively controls the expressions of *adhA* (see above) and of its own gene (7). The tight negative autoregulation of Cg0146 was shown previously to be mediated by binding to one of the four binding sites (consensus sequence YYAACAWMAW, where Y is C or T, W is A or T, and M is A or C) identified in the cg0146 promoter region (7).

As shown in Fig. 1, the cg0146 gene forms a two-gene cluster with *xylB*, encoding a xylulokinase (XylK), and upstream of cg0146, there is also a gene cluster consisting of *rbtT*, *mtlD*, and *sixA*, putatively encoding a ribitol transporter, a mannitol dehydrogenase (ManDH), and a phosphohistidine phosphatase, respectively (38). Due to this genomic organization of cg0146, we already speculated that the Cg0146 regulator might be involved in the control of these genes and, thus, in the control of the utilization of xylulose, mannitol, ribitol, or other polyols (7). In fact, Peng et al. (60) then corroborated the autoregulation of Cg0146 (designated MtlR by those authors) and provided quantitative reverse transcriptase PCR (qRT-PCR) evidence for the Cg0146-mediated repression of *rbtT* (designated *mtlT* by those authors) and of *mtlD* in *C. glutamicum* strain R. Those authors also showed that Cg0146 as well as the *rbtT* and *mtlD* gene products are involved in mannitol utilization, i.e., that a Cg0146-deficient mu-

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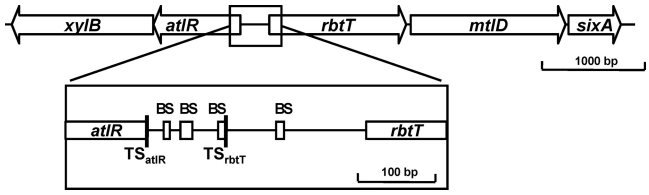

FIG 1 Genomic locus of the *atlR* (cg0146)-*xylB* and *rbtT*-*mtlD*-*sixA* gene clusters in *C. glutamicum*. The arrows represent the computer-predicted coding regions of the two gene clusters and the adjacent genes. The white boxes indicate AtlR binding sites (BS). The transcriptional start sites are indicated as TS_{atlR} and TS_{rbtT}, respectively.

tant but not the parental strain (strain R) grows on mannitol and that *rbtT* and *mtlD* are required for the mannitol metabolism of the mutant. However, *C. glutamicum* strain R lacked significant ManDH activity and was unable to utilize mannitol. Only the Cg0146-deficient mutant showed ManDH activity and was able to use mannitol as a sole or additional carbon and energy source (60). Those observations led those authors to conclude that Cg0146 constitutively represses the transcription of *rbtT* and *mtlD* in *C. glutamicum*.

In the present study, we identified the regulon of Cg0146 by employing DNA microarrays, using total RNA of WT *C. glutamicum* and its Cg0146-deficient *C. glutamicum* Δcg0146 derivative. Based on the results of these studies, we tested for growth on different sugar alcohols and found that WT *C. glutamicum* efficiently metabolizes and grows on arabitol as a sole carbon and energy source and as an additional carbon and energy source to glucose. By construction and growth analyses of single and double mutants and by analyses of arabitol dehydrogenase (AraDH), ManDH, and XylK activities, we tested for the functions of Cg0146 and of the *xylB*, *rbtT*, *mtlD*, and *sixA* gene products in arabitol metabolism by *C. glutamicum*. Due to our finding that Cg0146 tightly controls the genes encoding enzymes necessary for arabitol metabolism (see Results), we designated Cg0146 regulator for arabitol metabolism, AtlR, and the respective gene *atlR*.

MATERIALS AND METHODS

Bacterial strains, plasmids, oligonucleotides, and culture conditions. The bacterial strains and plasmids used in this study are given in Table 1 (for oligonucleotides, see Table S1 in the supplemental material). The minimal medium used for *C. glutamicum* was described previously (28) and contained D-glucose, D-fructose, D-xylulose, D-arabitol, D-mannitol, D-sorbitol, D-ribitol, D-xylitol, or mixtures of these substrates in the concentrations indicated in Results. TY medium (2×) (63) was used as a complex medium for *C. glutamicum* and *Escherichia coli*. When appropriate, kanamycin (50 μg ml^{-1}) was added to the medium. *C. glutamicum* cells were grown aerobically at 30°C, and *E. coli* cells were grown at 37°C as 50-ml cultures in 500-ml baffled Erlenmeyer flasks on a rotary shaker at 120 rpm. The growth experiments with D-xylulose as a substrate were performed with 20-ml tubes with 5 ml of culture. Growth was monitored by measuring the optical density at 600 nm (OD_{600}). The cell mass (dry weight) was calculated from the OD_{600} by using a ratio of 0.3 g of cells (dry weight) (CDW) liter^{-1} per OD_{600} unit (11).

DNA preparation and transformation. Restriction enzymes, T4 DNA ligase, calf intestinal phosphatase, RNase A, and proteinase K were obtained from MBI-Fermentas and were used according to instructions provided by the manufacturer. The isolation of plasmids from *E. coli* was performed as described previously (9). Plasmid DNA transfer into *C. glutamicum* was carried out by electroporation, and the recombinant strains were selected on brain heart infusion (BHI) agar plates containing 0.5 M sorbitol and kanamycin (50 μg ml^{-1}) (74). The electroporation of *E. coli* cells was performed according to a method described previously by Dower et al. (27). The isolation of chromosomal DNA from *C. glutamicum* was performed as described previously (28).

TABLE 1 Strains and plasmids used in this study

Strain or plasmid	Relevant characteristic(s)	Reference or source
Strains		
E. coli DH5α	*supE44 hsdR17 recA1 endA1 gyrA96 thi-1 relA1 gyrA96* Δ*lacU169* (ϕ80*lacZ*ΔM15)	33
E. coli DH5α(pK19*mobsacB* Δ*rbtT*)	*E. coli* DH5α carrying plasmid pK19*mobsacB* Δ*rbtT*	This work
E. coli DH5α(pK19*mobsacB* Δ*mtlD*)	*E. coli* DH5α carrying plasmid pK19*mobsacB* Δ*mtlD*	This work
E. coli DH5α(pK19*mobsacB* Δ*sixA*)	*E. coli* DH5α carrying plasmid pK19*mobsacB* Δ*sixA*	This work
E. coli DH5α(pK19*mobsacB* Δ*xylB*)	*E. coli* DH5α carrying plasmid pK19*mobsacB* Δ*xylB*	This work
C. glutamicum WT	Wild-type *C. glutamicum* (ATCC 13032)	American Type Culture Collection
C. glutamicum RES167	Restriction-deficient derivative of WT *C. glutamicum*	73
C. glutamicum Δ*atlR*	*C. glutamicum* RES167 with a truncated AtlR gene (cg0146)	7
C. glutamicum Δ*rbtT*	*C. glutamicum* RES167 with a truncated RbtT gene (cg0144)	This work
C. glutamicum Δ*mtlD*	*C. glutamicum* RES167 with a truncated MtlD gene (cg0143)	This work
C. glutamicum Δ*sixA*	*C. glutamicum* RES167 with a truncated SixA gene (cg0142)	This work
C. glutamicum Δ*xylB*	*C. glutamicum* RES167 with a truncated XylB gene (cg0147)	This work
C. glutamicum Δ*atlR*-Δ*rbtT*	*C. glutamicum* RES167 with truncated AtlR (cg0146) and RbtT (cg0144) genes	This work
C. glutamicum Δ*atlR*-Δ*mtlD*	*C. glutamicum* RES167 with truncated AtlR (cg0146) and MtlD (cg0143) genes	This work
C. glutamicum Δ*atlR*-Δ*sixA*	*C. glutamicum* RES167 with truncated AtlR (cg0146) and SixA (cg0142) genes	This work
C. glutamicum Δ*atlR*-Δ*xylB*	*C. glutamicum* RES167 with truncated AtrlR (cg0146) and XylB (cg0147) genes	This work
Plasmids		
pK19*mobsacB*	Km^r; mobilizable (*oriT*); *oriV*	65
pK19*mobsacB* Δ*rbtT*	pK19*mobsacB* with a truncated *rbtT* gene (shortened by 1,173 bp)	This work
pK19*mobsacB* Δ*mtlD*	pK19*mobsacB* with a truncated *mtlD* gene (shortened by 1,359 bp)	This work
pK19*mobsacB* Δ*sixA*	pK19*mobsacB* with a truncated *sixA* gene (shortened by 324 bp)	This work
pK19*mobsacB* Δ*xylB*	pK19*mobsacB* with a truncated *xylB* gene (shortened by 1,325 bp)	This work

DNA microarray experiments. Cells of WT *C. glutamicum* and the *C. glutamicum* Δ*atlR* strain were grown aerobically in minimal medium containing 2% (wt/vol) glucose at 30°C as 250-ml cultures in a parallel fermentation system (Fedbatch-pro system; Dasgip). The pH was maintained at 7.0, and the dissolved oxygen was adjusted to 30% saturation. Exponentially growing cells were harvested at an OD_{600} of about 9. One milliliter (approximately 1×10^9 *C. glutamicum* cells) was centrifuged for 15 s at 16,000 × *g*, the supernatant was decanted, and the cell pellet was frozen in liquid nitrogen. Cell samples for total RNA preparation were taken from two independently grown *C. glutamicum* cultures of each strain. The isolation of total RNA was carried out as described previously (6, 36). The respective RNA preparations were used in two independent DNA microarray hybridization assays by applying label swapping. The labeling of probes and DNA microarray hybridization were carried out as described previously (6, 36). To minimize the number of false-positive signals, the data were stringently filtered to obtain genes with at least six statistically significant values out of eight technical replicates present on the two microarrays along with an error probability of less than 5% for the Student *t* test. Normalization of the hybridization data by the Lowess function and *t* test statistics was accomplished by use of the EMMA 2.2 software package (25, 26). Genes with a statistical significance of a *P* value of ≤0.05, a changed transcript abundance of ±2 ($M \geq 1$ or $M \leq -1$), and a minimal signal intensity of an *A* value of ≥9 (negative controls) were regarded as differentially expressed.

Real-time RT-PCR. Total RNA from *C. glutamicum* was prepared as described previously (6, 36), and the quantification of purified RNA samples was performed with a NanoDrop ND-1000 spectrophotometer (Peqlab Biotechnology). The primers for real-time reverse transcriptase PCR (RT-PCR) were constructed to amplify intragenic regions with a length of the genes to be analyzed of about 180 bp. The primers (see Table S1 in the supplemental material) were designed by using Sci Ed Central 7.1.1.0 software (Scientific & Educational Software). All real-time RT-PCR experiments were performed by using a LightCycler instrument (Roche) with the SensiMix SYBR one-step RT-PCR kit (Bioline). PCR mixes were set up, and PCR was performed as described previously by Koch et al. (42). All measurements were performed for three biological replicates per condition tested and with two technical replicates per biological replicate. The amounts of the mRNAs of the genes of the cluster were normalized to the amount of total RNA, and the relative change in the transcription rate was determined as $2^{-\Delta CP}$, with ΔCP being equal to the difference of the measured crossing points for the test and the control conditions.

Determination of the *rbtT* TS site and analysis of transcriptional organizations of the *atlR-xylB* and *rbtT-mtlD-sixA* gene clusters. For transcriptional analyses, *C. glutamicum* RES167 and the *C. glutamicum* Δ*atlR* strain were grown on 0.8% (wt/vol) arabitol or on 1% (wt/vol) glucose, respectively, and harvested at an OD_{600} of about 4.5, and total RNA was isolated from aliquots of 2 ml (about 1×10^9 cells) as described previously (6).

The determination of the transcriptional start (TS) site of the *rbtT* gene was performed in duplicate by using RNA from the *C. glutamicum* Δ*atlR* strain, the primers listed in Table S1 in the supplemental material, and the 2nd Generation 5′/3′ rapid amplification of cDNA ends (RACE) kit (Roche Diagnostics). Primer RACE-rbtT.rev was used for cDNA synthesis, and primers rbtT2.rev and RACE-rbtT were used for PCRs. By use of the CloneJet PCR cloning kit (Fermentas), PCR products were ligated into blunt-end-restricted plasmid pJET and transformed into *E. coli* DH5α cells. Plasmids were isolated from transformants and sequenced with the pJET1.2 forward sequencing primer (Fermentas).

For the analysis of the transcriptional organizations of the *atlR-xylB* and *rbtT-mtlD-sixA* gene clusters, total RNA from *C. glutamicum* RES167 or from the *C. glutamicum* Δ*atlR* strain was employed for RT-PCR. Reverse transcription (cDNA synthesis) was performed with Moloney murine leukemia virus (M-MuLV) reverse transcriptase (Fermentas), and PCR was performed with the HotStar HiFidelity polymerase kit (Qiagen). Analysis of *atlR-xylB* was done with primers annealing in the 3′ region of *atlR* and in the 5′ region of *xylB*, and analysis of *rbtT-mtlD-sixA* was done with primers annealing in the 3′ region of *rbtT* and the 5′ region of *mtlD* and annealing in the 3′ region of *mtlD* and the 5′ region of *sixA* (see Table S1 in the supplemental material). RT-PCR was performed by using the RT-PCR kit from Qiagen according to the instructions provided by the manufacturer. All RNA samples were checked for DNA contamination by PCR without prior reverse transcription. Since no amplicons were obtained without RT, DNA contamination could be excluded.

Construction of *C. glutamicum* single and double mutants. The inactivation of the chromosomal *rbtT* (cg0144), *mtlD* (cg0143), *sixA* (cg0142), and *xylB* (cg0147) genes in *C. glutamicum* RES167 and in the *C. glutamicum* Δ*atlR* strain was performed by using crossover PCR and suicide vector pK19*mobsacB*. DNA fragments covering the 5′ end (fragment A) and the 3′ end (fragment B) of the respective genes were generated by using primer pairs A1-x/A2-x and B1-x/B2-x (with x representing the respective gene) (see Table S1 in the supplemental material). The two fragments were purified, mixed in equal amounts, and subjected to crossover PCR using the respective primers A1-x and B2-x. The resulting fusion products were ligated into SalI-EcoRI-restricted plasmid pK19*mobsacB* and transformed into *E. coli* cells The recombinant plasmids were isolated from *E. coli* and electroporated into cells of *C. glutamicum* RES167 and the *C. glutamicum* Δ*atlR* strain. By the application of a method described previously by Schäfer et al. (65), the intact chromosomal gene in *C. glutamicum* was replaced by the truncated version via homologous recombination (double crossover). The screening of the deletion mutants was done on 2× TY agar plates (63) containing 10% (wt/vol) sucrose. The successful deletion of *rbtT*, *mtlD*, *sixA*, and *xylB* was verified by PCR using the appropriate primers (see Table S1 in the supplemental material).

DNA binding assays with a His-tagged AtlR fusion protein. The binding of a His-tagged AtlR fusion protein to the *atlR-rbtT* intergenic promoter region was tested by electrophoretic mobility shift assays (EMSAs) exactly as previously described (7). When indicated, the assay mixture was incubated with arabitol, mannitol, xylulose, or xylulose-5-phosphate (50 mM each) for 20 min at room temperature.

Analytics. For the quantification of substrate consumption and product formation, 1-ml samples were taken from the cultures and centrifuged at 13,000 rpm (10 min at 4°C), and the supernatant was used for determinations of concentrations of glucose, arabitol, mannitol, xylulose, ribulose, and organic acids by reversed-phase high-pressure liquid chromatography (HPLC) using an LC 1100/1200 system (Agilent Technologies). The separation of sugars, sugar alcohols, and organic acids was performed with 100 mM H_2SO_4 as the mobile phase (flow rate of 0.5 ml/min). An organic acid precolumn (40 by 8 mm) and an organic acid main column (300 by 8 mm) (both from Chromatographie Service GmbH) were used at a temperature of 70°C for separation. The detection and quantification of the analytes were carried out by use of an Agilent 1100 variable-wavelength detector at 215 nm (D-xylulose and organic acids) or an Agilent HP G1362A refractive index detector (other sugars and sugar alcohols).

Enzyme assays. For determinations of AraDH, ManDH, and XylK activities in cell extracts, *C. glutamicum* cells were grown in minimal medium containing the respective carbon source(s), harvested in the exponential growth phase (OD_{600} of about 6), and washed twice in 20 ml 50 mM Tris-HCl at pH 8.0 (AraDH and ManDH) or pH 7.5 (XylK). The cell pellets were suspended in 1 ml of the same buffer and transferred into 2-ml screw-cap vials together with 250 mg of glass beads (diameter, 0.1 mm; Roth). Cell disruption was performed four times for 30 s at a speed of 6.5 with a RiboLyser (Thermo Hybaid GmbH) at 4°C with intermittent cooling on ice for 5 min. After cell disruption, the glass beads and cellular debris were removed by two consecutive centrifugation steps (13,000 × *g* at 4°C for 20 min and 45,000 × *g* at 4°C for 90 min), and the supernatants were used for the enzyme assays. The Pierce bicinchoninic acid (BCA)

protein assay with bovine serum albumin as a standard was used to determine protein concentrations (Thermo Fisher Scientific).

The specific AraDH and ManDH activities were determined photometrically at 340 nm. The assays (1-ml mixtures) were carried out at 30°C with assay mixtures containing 50 mM Tris-HCl (pH 8.0), 4 mM NAD^+, and cell extract and were started with 50 mM D-arabitol and 100 mM D-mannitol, respectively. One unit of activity is defined as 1 μmol of NADH formed per min.

AraDH activities in the reverse direction were also determined, i.e., as arabitol formation from D-xylulose. The assays (250-μl mixtures) were carried out at 30°C with assay mixtures containing 50 mM Tris (pH 7.5), 2 mM $MgCl_2$, 0.2 mM NADH, and cell extract and were started with 8.5 mM D-xylulose. One unit of activity is defined as 1 μmol of NADH consumed per min.

XylK (xylulokinase) (EC 2.7.1.17) activity and ribulokinase (ribulose kinase) (EC 2.7.1.15) activities were determined photometrically at 340 nm in a coupled test with pyruvate kinase and LDH essentially as described previously by Eliasson et al. (29). The assay (250-μl mixture) was carried out at 30°C with an assay mixture containing 50 mM Tris (pH 7.5), 2 mM $MgCl_2$, 0.2 mM NADH, 0.2 mM phosphoenolpyruvate, 2 mM ATP, 4 U pyruvate kinase, 5 U LDH, and cell extract and was started with 8.5 mM D-xylulose and 8.5 mM D-ribulose, respectively. Since the chosen assay system also allows the NADH-dependent conversion of D-xylulose to arabitol by the reverse reaction of AraDH (which is present in extracts of *C. glutamicum* cells grown in arabitol), this activity was determined (see above) and subtracted from the values obtained with the XylK assay. One unit of XylK activity is defined as 1 μmol of NADH consumed per min.

RESULTS

Expression profile of the *C. glutamicum* Δ*atlR* strain in comparison to WT *C. glutamicum*. To identify AtlR-regulated genes in *C. glutamicum*, the transcriptome of the AtlR-deficient *C. glutamicum* Δ*atlR* mutant (in-frame deletion mutant) was compared to that of WT *C. glutamicum* by DNA microarray hybridization. The strains were cultivated in a parallel fermentation system and grown to the mid-exponential growth phase in minimal medium with glucose. Cell sampling, RNA preparation, labeling of probes, DNA microarray hybridization and filtering, as well as the normalization of the hybridization data were carried out as described in Materials and Methods. Table S2 in the supplemental material shows all genes whose mRNA levels were changed ≥2-fold in the *atlR* mutant. When the transcriptome of the *C. glutamicum* Δ*atlR* strain was compared with that of the WT strain, 28 genes showed higher and 70 showed lower mRNA levels in the *C. glutamicum* Δ*atlR* strain. Thus, the expressions of 98 genes are influenced by the absence of AtlR, either directly or indirectly.

Table 2 shows all genes whose mRNA levels were changed by a factor of ≥4 in the *atlR* mutant. The genes showing the highest mRNA ratio (>10) are those located in the close genomic vicinity of the *atlR* (cg0146) gene itself, i.e., cg0147, cg0144, cg0143, and cg0142, annotated as the XylK gene *xylB*, the ribitol transporter gene *rbtT*, the ManDH gene *mtlD*, and the phosphohistidine phosphatase gene *sixA*, respectively (8, 38). All other genes listed in Table 2 and showing mRNA ratios of 4 to 7.6 or showing more than 4-fold lower mRNA levels (i.e., ratios of <0.25) in the *atlR* mutant (compared to the WT strain) are genes predicted to code for either glycosyltransferases or putative and hypothetical proteins.

To validate the DNA microarray results for *xylB*, *rbtT*, *mtlD*, and *sixA*, real-time RT-PCR of these genes was performed with total RNA from WT *C. glutamicum* and *C. glutamicum* Δ*atlR* strain cells grown to the mid-exponential growth phase in minimal medium with glucose. As shown in Fig. 2A, the mRNA levels of all four genes were much higher in the *C. glutamicum* Δ*atlR* strain, corroborating their strongly induced (or derepressed) expression in the mutant.

TABLE 2 Genes with altered expression (upregulated or downregulated >4.0-fold[c]) in the *C. glutamicum* Δ*atlR* strain compared to WT *C. glutamicum* on glucose

Locus tag[a]	Gene	Annotation[b]	mRNA ratio
cg0144	*rbtT*	Putative ribitol transporter, MFS type	26.35
cg0143	*mtlD*	Mannitol 2-dehydrogenase	23.43
cg0147	*xylB*	Xylulose kinase	11.88
cg0142	*sixA*	Putative phosphohistidine phosphatase	10.70
cg0756	*cstA*	Putative carbon starvation protein A	7.62
cg1182		Putative membrane protein	6.02
cg1181		Glycosyltransferase, probably involved in cell wall biogenesis	4.72
cg1180		Glycosyltransferase, probably involved in cell wall biogenesis	4.17
cg0768		ABC-type putative iron-siderophore transporter, ATPase subunit	0.24
cg1109		Hypothetical protein	0.24
cg2030		Hypothetical protein	0.17
cg1998	*cglIIR*	Putative restriction endonuclease	0.15
cg0096		Conserved hypothetical protein	0.14
cg2031		Conserved hypothetical protein	0.14
cg2071	*int2′*	Putative phage integrase (N-terminal fragment)	0.12
NCgl1806		Integrase	0.10
cg0693	*′groEL*	60-kDa chaperonin, putative pseudogene (C-terminal fragment)	0.07
cg2034		Hypothetical protein	0.04
cg2026		Hypothetical protein	0.04
cg2025		Hypothetical protein	0.03

[a] Gene identity according to Kalinowski et al. (38).
[b] Annotation according to Meyer et al. (55) and Baumbach et al. (8).
[c] $P < 0.05$, determined by Student's *t* test.

The DNA microarray and the real-time RT-PCR data shown here together with data from previous transcriptional analyses of *atlR* itself (7) suggest that AtlR strongly activates a variety of genes coding for so far functionally unknown proteins and that it strongly represses the *atlR*, *xylB*, *rbtT*, *mtlD*, and *sixA* genes in *C. glutamicum* cells grown in glucose.

Transcriptional organization of the *atlR-xylB* and *rbtT-mtlD-sixA* operons. The genomic organization of the *atlR*, *xylB*, *rbtT*, *mtlD*, and *sixA* genes is shown in Fig. 1. The *atlR* gene is separated from the *xylB* gene by 1 bp, and the distances between *rbtT* and *mtlD* and between *mtlD* and *sixA* are 43 and 24 bp, respectively. This finding and the results of the DNA microarray experiments suggested that the two clusters represent operons, expressed from promoters located in the 278-bp intergenic region between *atlR* and *rbtT* (Fig. 1). For WT *C. glutamicum* (7) and also for *C. glutamicum* strain R (60), the TS site of *atlR* was identified

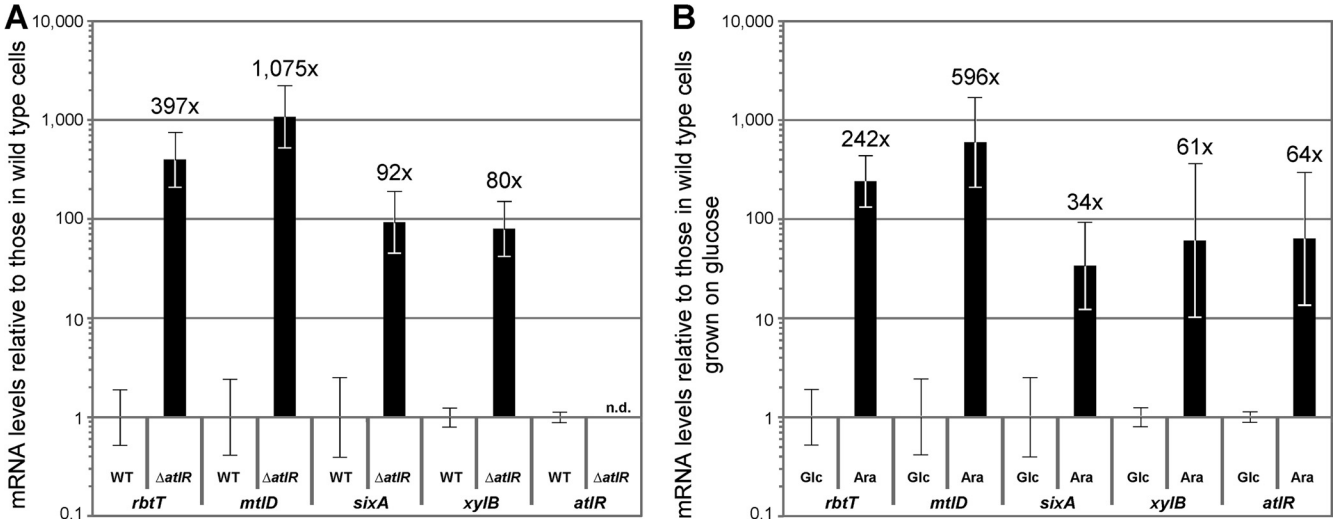

FIG 2 Change of the mRNA levels of the *rbtT*, *mtlD*, *sixA*, *xylB*, and *atlR* genes in the *C. glutamicum* Δ*atlR* strain compared to the wild type, both grown with glucose as the sole carbon source (A) and change of the mRNA levels of the same genes in wild-type cells grown with arabitol compared to wild-type cells grown with glucose as the sole carbon source (B). The relative mRNA levels were compared by using real-time RT-PCR. The mRNA level of wild-type cells grown on glucose was set to 1. All measurements were performed for three biological replicates per condition tested and with two technical replicates per biological replicate. As the primers used to determine the *atlR* mRNA amounts are located within the region deleted in the Δ*atlR* mutant, no data could be obtained for this strain. n.d., not detectable.

and shown to be identical to the A residue of the annotated start codon. The TS site of the *rbtT* gene in *C. glutamicum* strain R was determined to be located 179 bp upstream of the annotated translational start codon (60). Using 5′ RACE with total RNA of WT *C. glutamicum* and two different primers annealing within the 5′ part of *rbtT*, we also confirmed this TS site for our *C. glutamicum* strain (data not shown).

To analyze the operon structures of the *C. glutamicum atlR-xylB* and *rbtT-mtlD-sixA* gene clusters, RT-PCR experiments using RNA from *C. glutamicum* RES167 and primers covering the 3′ ends and the 5′ ends of the respective genes were performed. The RT-PCRs resulted in amplification products of about 450 bp (*atlR-xylB* region), about 380 bp (*rbtT-mtlD*), and about 580 bp (*mtlD-sixA*), being in complete agreement with the expected sizes of common transcripts (461 bp, 372 bp, and 571 bp, respectively). These results indicate that the five genes are transcribed at least in part as *atlR-xylB* and *rbtT-mtlD-sixA* operons.

Taken together, the transcriptional organization, the common transcriptional response to the deletion of *atlR*, and our previous results on the autoregulation of AtlR (7) led us to conclude that *atlR* and *xylB* on the one hand and *rbtT*, *mtlD*, and *sixA* on the other hand represent two operons which are strongly repressed in *C. glutamicum* cells grown on glucose.

Growth of *C. glutamicum* and its Δ*atlR* derivative on glucose and different sugar alcohols. The original annotation of *xylB*, *rbtT*, *mtlD*, and *sixA* and the relatively high mRNA levels of these genes in the *atlR* mutant of *C. glutamicum* suggested an involvement of the respective gene products in sugar alcohol utilization and a regulatory function of AtlR in the expression control of the these genes in *C. glutamicum*. Therefore, we tested *C. glutamicum* RES167 and its AtlR-deficient *C. glutamicum* Δ*atlR* derivative for their growths and substrate consumptions in minimal medium containing 0.8% (wt/vol) glucose, 0.8% (wt/vol) fructose, or 0.8% (wt/vol) various sugar alcohols, i.e., ribitol, xylitol, and arabitol (all C_5) as well as mannitol and sorbitol (both C_6). The growth data for the relevant growth experiments are summarized in Table 3. On glucose and on fructose, both strains grew with about the same growth rate (μ) of 0.35 to 0.36 h^{-1} to approximately the same final OD_{600} of about 14. Neither strain showed growth on ribitol, xylitol, or sorbitol (data not shown). In arabitol-containing medium, the parental strain consumed this carbon source within 8 h and grew with a μ of 0.35 h^{-1} and a biomass yield of 0.3 g dry weight (g arabitol)$^{-1}$ to a final OD_{600} of about 11.5 (Fig. 3A). In contrast, the *atlR* mutant showed (almost) no growth on arabitol. However, although the cells of the *atlR* mutant did not grow, they consumed a significant portion of the provided substrate (Fig. 3A and Table 3), indicating that the cells can metabolize arabitol without being able to use it for growth. With mannitol-containing medium, we observed efficient growth and concomitant substrate consumption with the *atlR* mutant but no growth and no (or marginal) substrate consumption with parental strain RES167 (Table 3). The latter observations are in agreement with results recently obtained by Peng et al. (60) with *C. glutamicum* strain R and a derivative with a disrupted *atlR* gene (see also the introduction). Also in agreement with the results obtained previously by Peng et al. was the observation that during growth on mannitol, the *atlR* mutant transiently accumulated fructose (up to 12 mM) in the medium.

The results of the growth experiments indicate that *C. glutamicum* can take up and efficiently utilize arabitol as a sole carbon and energy source and that AtlR is necessary for growth on arabitol. In addition, these results corroborate the results described by Peng et al. (60) for *C. glutamicum* strain R; i.e., they show that the organism in principle possesses the enzymatic equipment for the transport and utilization of mannitol and that the presence of AtlR in the WT strain of *C. glutamicum* prevents the utilization of this sugar alcohol when it is the only substrate and when the cells are not pregrown in the presence of arabitol (see below).

Since arabitol in bacteria is generally metabolized via xylulose and xylulose-5-phosphate, we also tested *C. glutamicum* RES167

TABLE 3 Maximal growth rates, final OD_{600} values, substrate consumption rates, and other characteristics of different *C. glutamicum* strains grown on different carbon sources[a]

C. glutamicum strain and genotype	Carbon source(s) (concn [%, wt/vol])	Mean maximal growth rate (h^{-1}) ± SD	Mean final OD_{600} ± SD	Substrate consumption rate (mmol g CDW^{-1} h^{-1}) ± SD	Description
RES167	Glucose (0.8)	0.36 ± 0.03	13.7 ± 0.3	4.5 ± 0.4	
Δ*atlR*	Glucose (0.8)	0.35 ± 0.04	13.7 ± 1.5	4.8 ± 1.4	
Δ*rbtT*	Glucose (0.8)	0.39 ± 0.04	13.2 ± 0.2	4.9 ± 0.9	
Δ*atlR*-Δ*rbtT*	Glucose (0.8)	0.35 ± 0.03	12.5 ± 1.8	5.0 ± 0.8	
Δ*mtlD*	Glucose (0.8)	0.34 ± 0.04	13.3 ± 0.1	4.5 ± 1.1	
Δ*atlR*-Δ*mtlD*	Glucose (0.8)	0.34 ± 0.01	13.1 ± 1.8	4.9 ± 1.2	
Δ*xylB*	Glucose (0.8)	0.34 ± 0.02	13.4 ± 0.8	4.0 ± 0.9	
Δ*atlR*-Δ*xylB*	Glucose (0.8)	0.34 ± 0.03	12.6 ± 1.1	4.2 ± 1.2	
RES167	Arabitol (0.8)	0.35 ± 0.03	11.5 ± 0.9	4.6 ± 0.2	
Δ*atlR*	Arabitol (0.8)	NG	NG	3.3 ± 0.3	
Δ*rbtT*	Arabitol (0.8)	0.13 ± 0.02	7.6 ± 0.7	1.9 ± 0.6	Lag phase of about 4 h
Δ*atlR*-Δ*rbtT*	Arabitol (0.8)	NG	NG	2.3 ± 0.5	
Δ*mtlD*	Arabitol (0.8)	NG	NG	<0.2	
Δ*atlR*-Δ*mtlD*	Arabitol (0.8)	NG	NG	<0.2	
Δ*xylB*	Arabitol (0.8)	NG	NG	0.4 ± 0.2	Xylulose formation (up to 3 mM)
Δ*atlR*-Δ*xylB*	Arabitol (0.8)	NG	NG	3.2 ± 0.3	Xylulose formation (up to 9 mM)
RES167	Mannitol (0.8)	NG	NG	0.4 ± 0.2	
Δ*atlR*	Mannitol (0.8)	0.35 ± 0.04	11.4 ± 0.5	4.7 ± 0.4	Fructose formation (up to 12 mM)
Δ*rbtT*	Mannitol (0.8)	NG	NG	<0.2	
Δ*atlR*-Δ*rbtT*	Mannitol (0.8)	0.32 ± 0.02	11.9 ± 0.5	5.3 ± 0.6	
Δ*mtlD*	Mannitol (0.8)	NG	NG	<0.2	
Δ*atlR*-Δ*mtlD*	Mannitol (0.8)	NG	NG	<0.2	
Δ*xylB*	Mannitol (0.8)	NG	NG	0.5 ± 0.2	
Δ*atlR*-Δ*xylB*	Mannitol (0.8)	0.35 ± 0.03	11.6 ± 0.5	4.4 ± 0.5	Fructose formation (up to 12 mM)
RES167	Arabitol (0.3) + mannitol (0.5)	0.24 ± 0.01	10.2 ± 1.4	Arabitol, 4.7 ± 0.5; mannitol, 3.1 ± 0.5	Biphasic growth, first on arabitol and then on mannitol
Δ*atlR*	Arabitol (0.3) + mannitol (0.5)	0.12 ± 0.01	7.0 ± 1.1	Arabitol, 2.7 ± 0.5; mannitol, 1.4 ± 0.4	Growth on mannitol only when arabitol was completely consumed
Δ*xylB*	Arabitol (0.3) + mannitol (0.5)	NG	NG	Arabitol, 0.6 ± 0.1; Mannitol, <0.2	Xylulose formation (up to 1.5 mM)
Δ*atlR*-Δ*xylB*	Arabitol (0.3) + mannitol (0.5)	NG	NG	Arabitol, 1.8 ± 0.3; mannitol, <0.2	Xylulose formation (up to 5 mM)
RES167	Glucose (0.3) + mannitol (0.5)	0.35 ± 0.04	4.5 ± 0.4	Glucose, 5.8 ± 1.0; mannitol, <0.2	No consumption of mannitol
Δ*atlR*	Glucose (0.3) + mannitol (0.5)	0.34 ± 0.01	11.7 ± 0.4	Glucose, 1.4 ± 0.2; mannitol, 2.8 ± 0.3	Parallel consumption of both substrates
RES167	Glucose (0.3) + arabitol (0.5)	0.36 ± 0.02	10.4 ± 0.7	Glucose, 2.2 ± 0.2; arabitol, 4.9 ± 0.4	Parallel consumption of both substrates
Δ*atlR*	Glucose (0.3) + arabitol (0.5)	NG	NG	Glucose, <0.2; arabitol, 2.6 ± 0.4	No consumption of glucose
Δ*mtlD*	Glucose (0.3) + arabitol (0.5)	0.15 ± 0.01	4 ± 0.3	Glucose, 2.7 ± 0.2; arabitol, <0.2	No consumption of arabitol and decreased growth rate
Δ*xylB*	Glucose (0.3) + arabitol (0.5)	0.27 ± 0.04	4.2 ± 0.3	Glucose, 3.7 ± 0.2; arabitol, 1.4 ± 0.4	Xylulose formation (up to 8.5 mM)
Δ*atlR*-Δ*xylB*	Glucose (0.3) + arabitol (0.5)	0.25 ± 0.03	4.2 ± 0.2	Glucose, 3.9 ± 0.5; arabitol, 3.7 ± 1.5	Xylulose formation (up to 12 mM)

[a] NG, not grown.

for its growth and substrate consumption in minimal medium containing D-xylulose (0.5%, wt/vol). After a lag phase of about 4 h, the cells grew with a μ of 0.18 h^{-1} to an OD_{600} of about 6.5, consuming xylulose with a rate of 3.3 mmol (g CDW^{-1} h^{-1}). These results indicate that *C. glutamicum* is able to use xylulose as a carbon and energy source for growth.

Growth of *C. glutamicum* and its Δ*atlR* derivative on mixtures of arabitol, mannitol, and/or glucose. To further study arabitol and mannitol metabolism, the growths of *C. glutamicum* RES167 and the *C. glutamicum* Δ*atlR* strain in minimal medium containing mixtures of arabitol, mannitol, and/or glucose were analyzed, and the relevant growth data are summarized in Table 3.

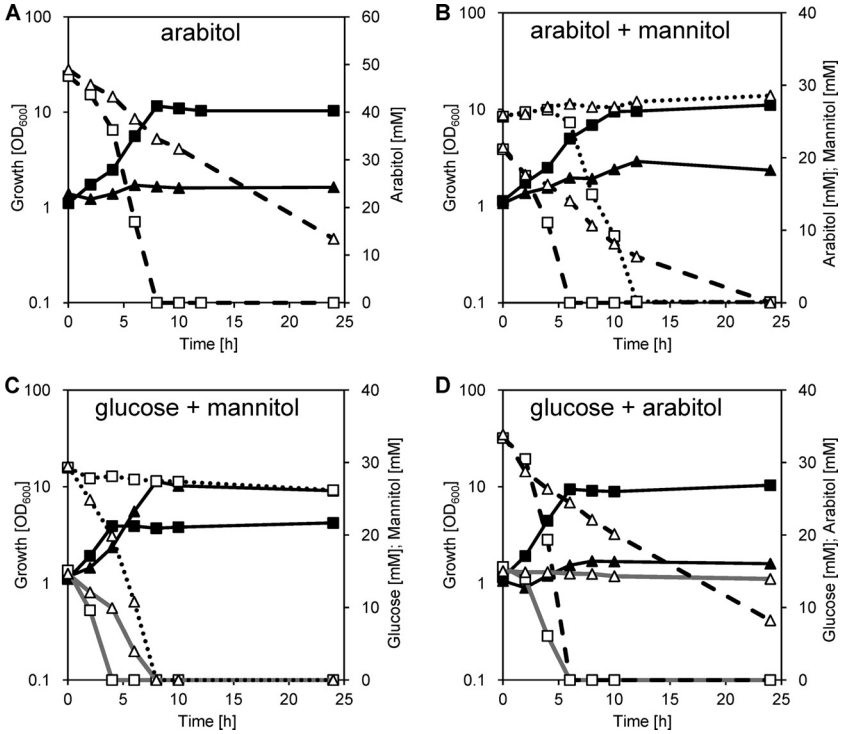

FIG 3 Growth and substrate consumption of *C. glutamicum* RES167 (squares) and the *C. glutamicum* Δ*atlR* strain (triangles) in minimal medium containing 0.8% arabitol (A), 0.3% arabitol plus 0.5% mannitol (B), 0.3% glucose plus 0.5% mannitol (C), or 0.3% glucose plus 0.5% arabitol (D). Dashed lines, arabitol; dotted lines, mannitol; gray lines, glucose. The figure shows one representative data set of at least three independent experiments, all three showing comparable results.

Surprisingly, the growth of the parental strain with a mixture of arabitol and mannitol was biphasic, with arabitol consumption in the first growth phase and mannitol consumption in the second growth phase (Fig. 3B). These results indicate (i) that growth in the presence of arabitol enables *C. glutamicum* RES167 to grow subsequently on mannitol and (ii) that the utilization of mannitol is prevented as long as arabitol is present (i.e., that arabitol is utilized preferentially over mannitol). Whereas the *atlR* mutant did show mannitol consumption and growth on mannitol as the sole carbon source (see above), it showed no mannitol consumption and only very slow growth with the arabitol-mannitol mixture (Fig. 3B). However, when the consumption of arabitol was completed after about 25 h, the Δ*atlR* mutant cells started to consume mannitol and to grow. These results also indicate that in the absence of AtlR, the presence and/or utilization of arabitol efficiently prevents the utilization of mannitol.

In minimal medium containing glucose and mannitol, *C. glutamicum* RES167 consumed only glucose and grew as long as glucose was present in the medium up to an OD_{600} of about 4.5 (Fig. 3C and Table 3). In contrast, the *C. glutamicum* Δ*atlR* strain consumed both substrates simultaneously and grew to an OD_{600} of about 11 (Fig. 3C), corroborating the finding that an AtlR deficiency enables *C. glutamicum* to utilize mannitol (see above). In minimal medium containing glucose and arabitol, the parental strain consumed both substrates simultaneously and grew to an OD_{600} of about 10.5 (Fig. 3D and Table 3). In contrast, the Δ*atlR* mutant hardly consumed any glucose and did not grow; however, the cells consumed a significant portion of arabitol (Fig. 3D), as they did with arabitol as the only carbon source (see above and Fig. 3A). Since the *C. glutamicum* Δ*atlR* strain did show growth on glucose as the sole carbon source (but not on glucose plus arabitol [see above]), the results indicate that in the absence of AtlR, the presence and/or utilization of arabitol has an inhibitory effect on the growth of *C. glutamicum* on glucose.

We also tested the growth and substrate consumption of *C. glutamicum* RES167 in minimal medium containing mannitol (0.5%, wt/vol) plus xylulose (0.3%, wt/vol). With this mixture, *C. glutamicum* RES167 cells grew without a lag phase with a μ of about 0.21 h^{-1} to an OD_{600} of about 12. In the first 10 h, the cells consumed mannitol and xylulose with consumption rates of about 0.5 mmol (g CDW^{-1} h^{-1}) and about 1.1 mmol (g CDW^{-1} h^{-1}), respectively. After the depletion of xylulose, the mannitol consumption rate increased to about 3 mmol (g CDW^{-1} h^{-1}). These results show that the presence of xylulose in the growth medium enables *C. glutamicum* to metabolize mannitol and suggest that ManDH activity is induced under these conditions. Moreover, these results indicate that the utilization of mannitol is restricted as long as xylulose is present (i.e., that xylulose is utilized preferentially over mannitol). However, it might well be that that the latter effect is due to the intracellular formation of arabitol from xylulose by the reversible AraDH reaction of the *mtlD* gene product and thus reflects the prevention of mannitol utilization by arabitol (see above).

Growths of *C. glutamicum* Δ*rbtT*, Δ*mtlD*, Δ*sixA*, Δ*xylB* single mutants and of Δ*atlR*-Δ*rbtT*, Δ*atlR*-Δ*mtlD*, Δ*atlR*-Δ*sixA*, and Δ*atlR*-Δ*xylB* double mutants. In order to test for an involvement of the *rbtT*, *mtlD*, *sixA*, and *xylB* genes in the glucose, arabitol, and mannitol metabolism of *C. glutamicum* and also to further

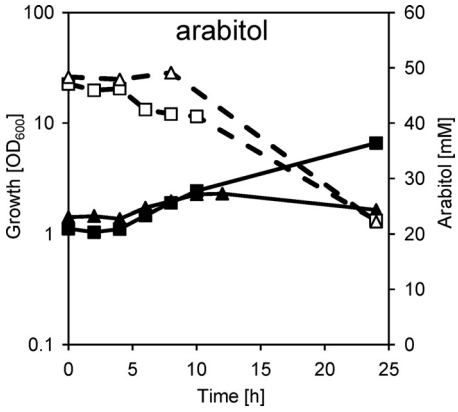

FIG 4 Growth and substrate consumption of the *C. glutamicum* Δ*rbtT* (squares) and *C. glutamicum* Δ*atlR*-Δ*rbtT* (triangles) strains in minimal medium containing 0.8% arabitol (dashed lines). The figure shows one representative data set of three independent experiments, all three showing comparable results.

clarify the function of AtlR, we analyzed the growths of *C. glutamicum* Δ*rbtT*, Δ*mtlD*, Δ*sixA*, Δ*xylB* single mutants and of Δ*atlR*-Δ*rbtT*, Δ*atlR*-Δ*mtlD*, Δ*atlR*-Δ*sixA*, and Δ*atlR*-Δ*xylB* double mutants in minimal medium containing glucose, arabitol, mannitol, or mixtures thereof. The growth data for the relevant growth experiments are summarized in Table 3.

When the growths of the *rbtT*, *mtlD*, *sixA*, and *xylB* deletion mutants of *C. glutamicum* RES167 and of the C. *glutamicum* Δ*atlR* strain in glucose minimal medium were compared with each other and with the growths of the respective parental strains, all strains showed about the same growth rates and glucose consumption rates (Table 3). These results indicate that none of the four genes or gene products is involved in the glucose metabolism of *C. glutamicum*. This is in accordance with the finding that the expression levels of all four genes are relatively low when WT cells are grown in glucose minimal medium (see above).

The growths of the *C. glutamicum* Δ*rbtT* and *C. glutamicum* Δ*atlR*-Δ*rbtT* strains in minimal medium containing arabitol are shown in Fig. 4. Whereas parental strain *C. glutamicum* RES167 grew fast and completely consumed 0.8% arabitol after 8 h (Fig. 3A), the *rbtT* mutant showed a lag phase of about 4 h and then very slow growth ($\mu = 0.13\ h^{-1}$) and a much slower arabitol consumption rate (Fig. 4 and Table 3). When the arabitol concentration was decreased to 0.4% or 0.2% (wt/vol) arabitol, the *C. glutamicum* Δ*rbtT* strain did not grow at all (data not shown). The Δ*atlR*-Δ*rbtT* double mutant showed a growth behavior very similar to that of the *C. glutamicum* Δ*atlR* strain, i.e., no or only slow growth and slow arabitol consumption (Fig. 4). The growth and substrate consumption of both *C. glutamicum* Δ*rbtT* and *C. glutamicum* Δ*atlR*-Δ*rbtT* strain cells in minimal medium containing mannitol were nearly identical to the growth and mannitol consumption rates of parental strain RES167 and the parental Δ*atlR* mutant (see above), respectively; i.e., the *rbtT* mutant did not grow, and the *atlR-rbtT* double mutant grew and consumed the substrate as fast as the C. *glutamicum* Δ*atlR* strain (Table 3). Taken together, the results indicate that the *rbtT* gene product is somehow involved in the arabitol but not in the mannitol utilization of *C. glutamicum*. In view of the annotation of *rbtT* as a transporter gene and in view of the fact that the *rbtT* gene product is most similar to the arabitol transporter DalT from *Klebsiella pneumoniae* (see Discussion), we propose that the RbtT protein represents most probably an arabitol transporter.

The *C. glutamicum* Δ*mtlD* and *C. glutamicum* Δ*atlR*-Δ*mtlD* strains did not show any growth or substrate consumption when inoculated into minimal medium containing arabitol, mannitol, or mixtures thereof (Table 3). These results indicate that the *mtlD* gene product is essential for the arabitol and mannitol metabolism of *C. glutamicum*. In minimal medium containing glucose and arabitol, the *C. glutamicum* Δ*mtlD* strain consumed only glucose and grew within 8 h to a final OD_{600} of about 4. However, the growth rate (about 0.15 h^{-1}) of the *C. glutamicum* Δ*mtlD* strain was considerably lower than that of parental strain *C. glutamicum* RES167 (0.36 h^{-1}), indicating that arabitol somehow inhibits glucose metabolism in the absence of the *mtlD* gene product.

The *C. glutamicum* Δ*sixA* and *C. glutamicum* Δ*atlR*-Δ*sixA* strains did not show any phenotype on any carbon source tested compared to the respective parental strains; i.e., the *sixA* mutant grew on and consumed arabitol, and the *atlR-sixA* double mutant grew on and consumed mannitol (data not shown). These results indicate that under the conditions tested, the *sixA* gene product does not have any function in the arabitol and mannitol metabolism of *C. glutamicum*.

The growths of the *C. glutamicum* Δ*xylB* and *C. glutamicum* Δ*atlR*-Δ*xylB* strains in minimal medium containing arabitol, arabitol plus mannitol, and glucose plus arabitol are shown in Fig. 5. Whereas *C. glutamicum* RES167 showed fast growth on and consumption of arabitol (Fig. 3A), the *xylB* mutant showed no growth and very low levels of arabitol consumption (Fig. 5A). In mannitol medium, the *C. glutamicum* Δ*atlR*-Δ*xylB* strain showed the same growth behavior as that of the parental *C. glutamicum* Δ*atlR* strain, i.e., fast growth and fast substrate consumption (Table 3). These results indicate an involvement of the *xylB* gene product in the arabitol but not in the mannitol metabolism of *C. glutamicum*. As expected from the growth experiment with medium containing arabitol as the sole carbon source (Fig. 5A), the deletion of *xylB* also had a severely negative effect on the growth and substrate consumption of *C. glutamicum* RES167 in the mixture of arabitol and mannitol (compare Fig. 3B and 5B).

In minimal medium containing glucose and arabitol, the *C. glutamicum* Δ*xylB* strain grew to an OD_{600} of about 4.2, stopped growing as soon as the glucose was completely consumed, and slowly continued to consume arabitol (Fig. 5C). In contrast, the parental strain of the *xylB* mutant showed higher final OD_{600} values and a simultaneous and fast consumption of both substrates (Fig. 3D). The *C. glutamicum* Δ*atlR*-Δ*xylB* double mutant showed the same growth behavior and the same glucose consumption as those of the *xylB* single mutant; however, arabitol consumption was significantly faster (Fig. 5C and Table 3). In contrast, the *C. glutamicum* Δ*atlR* strain showed no growth, no glucose consumption, and slow arabitol consumption (Fig. 3D). Taken together, the results obtained with the Δ*xylB* mutants allow the following conclusions: (i) the *xylB* gene product is essential for growth on arabitol, but it is not essential for arabitol consumption; (ii) the *xylB* gene product is not essential for growth on and metabolism of mannitol; and (iii) a deficiency of the *xylB* gene product relieves the growth phenotype of the *C. glutamicum* Δ*atlR* strain on glucose plus arabitol (or, in other words, the *xylB* gene product is responsible for the inhibition of growth on glucose by arabitol).

In the course of the HPLC analyses of the substrates, we ob-

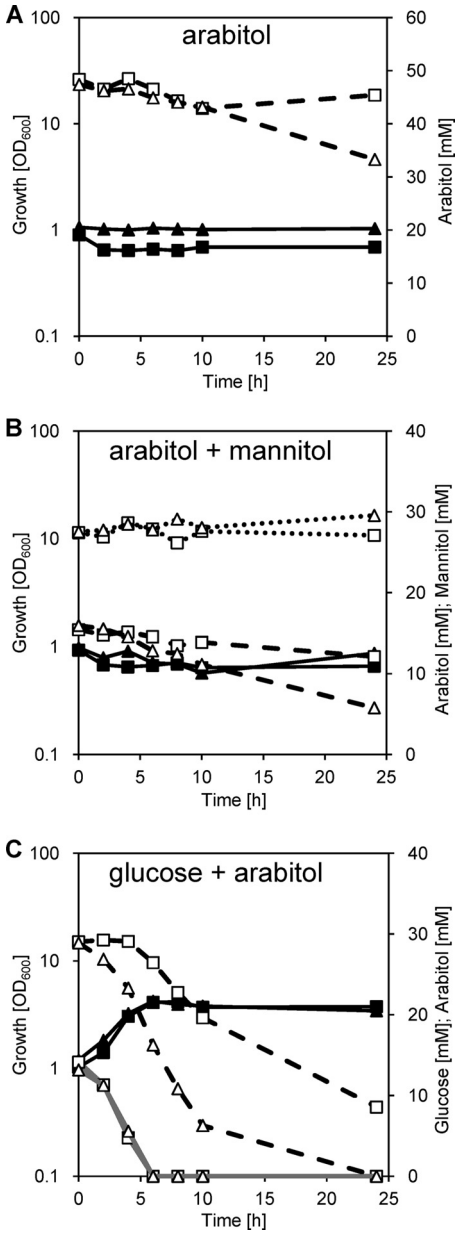

FIG 5 Growth and substrate consumption of the *C. glutamicum* Δ*xylB* (squares) and *C. glutamicum* Δ*atlR*-Δ*xylB* (triangles) strains in minimal medium containing 0.8% arabitol (A), 0.3% arabitol plus 0.5% mannitol (B), or 0.3% glucose plus 0.5% arabitol (C). Dashed line, arabitol; dotted line, mannitol; gray line, glucose. The figure shows one representative data set of at least three independent experiments, all three showing comparable results.

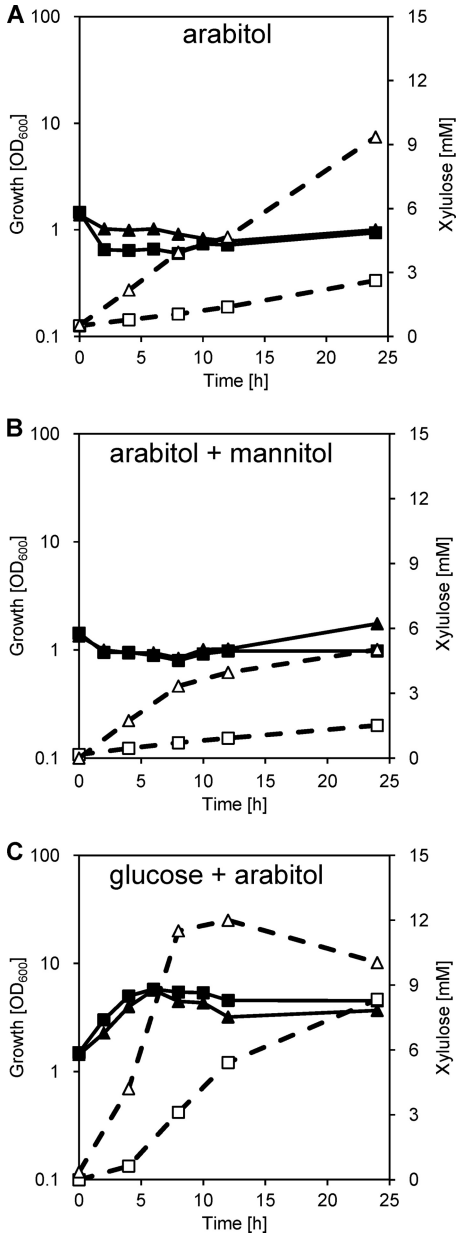

FIG 6 Growth (solid lines) and xylulose formation (dashed lines) of the *C. glutamicum* Δ*xylB* (squares) and *C. glutamicum* Δ*atlR*-Δ*xylB* (triangles) strains in minimal medium containing 0.8% arabitol (A), 0.3% arabitol plus 0.5% mannitol (B), or 0.3% glucose plus 0.5% arabitol (C). The figure shows one representative data set of two independent experiments, both showing comparable results.

served an unknown peak for the samples taken from the cultures of all *C. glutamicum* Δ*xylB* and Δ*atlR*-Δ*xylB* strains when grown in the presence of arabitol but not when grown with glucose or mannitol as a substrate(s). Spike experiments with D-xylulose and D-ribulose standard solutions unequivocally identified D-xylulose as the substance represented by the peak. As shown in Fig. 6, the xylulose concentrations increased in the course of the cultivations and roughly correlated to the decrease in arabitol concentrations. These results indicate that those strains unable to phosphorylate and, thus, unable to further metabolize xylulose secrete this compound into the medium and suggest that xylulose is a physiological intermediate of arabitol metabolism.

AraDH, XylK, and ManDH activities of *C. glutamicum* RES167 and its Δ*atlR* derivative. The growth of *C. glutamicum* RES167 on arabitol and the formation of xylulose by the *xylB* mutants suggested that *C. glutamicum* possesses the AraDH and XylK activities necessary to convert arabitol in two steps via xylulose to xylulose-5-phosphate, an intermediate of the pentose phosphate pathway.

As shown in Table 4, extracts of cells of *C. glutamicum* RES167 grown in glucose showed no AraDH activity. In contrast, extracts

TABLE 4 Specific activities of arabitol dehydrogenase and mannitol dehydrogenase in cell extracts of *C. glutamicum* RES167 and the Δ*atlR* and Δ*atlR*-Δ*mtlD* strains grown in minimal medium containing different carbon sources or mixtures thereof

Strain	Mean sp act (U/mg) ± SD[a]								
	AraDH					ManDH			
	Glucose	Arabitol	Mannitol	Arabitol + mannitol	Glucose + arabitol	Glucose	Arabitol	Mannitol	Arabitol + mannitol
C. glutamicum RES167	0.01 ± 0.00	2.07 ± 0.45	NG	1.50 ± 0.56	1.22 ± 0.09	0.01 ± 0.01	1.19 ± 0.39	NG	0.87 ± 0.32
C. glutamicum Δ*atlR*	3.46 ± 0.46	NG	1.55 ± 0.22	2.08 ± 0.07	NG	2.24 ± 0.44	NG	0.97 ± 0.10	1.17 ± 0.06
C. glutamicum Δ*atlR*-Δ*mtlD*	<0.01	NG	NG	NG	NG	<0.01	NG	NG	NG

[a] NG, not grown.

of cells grown on arabitol, arabitol plus mannitol, or glucose plus arabitol showed relatively high specific AraDH activities. These results show that *C. glutamicum* possesses AraDH and indicate that the expression of the respective gene is induced (or derepressed) when grown in the presence of arabitol. However, the almost-2-fold-lower specific AraDH activity in cells grown on arabitol-glucose than in cells grown on arabitol may indicate a repressive effect of glucose on *mtlD* expression. The *C. glutamicum* Δ*atlR* strain showed the highest specific AraDH activities when grown on glucose and about 50% to 70% lower specific activities when grown on mannitol or on arabitol plus mannitol (Table 4). Thus, the respective gene seems to be constitutively expressed (or derepressed) in the *atlR*-deficient strain, with a slight negative effect of mannitol. The K_m value for arabitol was determined to be 7.91 ± 0.52 mM (see Fig. S1A in the supplemental material), which is comparable to values obtained with AraDHs of other microorganisms (49, 67).

AraDH activity was also determined for *C. glutamicum* RES167 cells grown on xylulose and mannitol plus xylulose. The specific AraDH activity of cell extracts of cells grown in xylulose was 0.49 U/mg protein, and that of cell extracts of cells grown on xylulose plus mannitol was 0.45 U/mg protein.

We also tested AraDH activity in extracts of the *C. glutamicum* Δ*atlR*-Δ*mtlD* double mutant grown on glucose. As shown in Table 4, AraDH activity could not be detected, corroborating that the *mtlD* gene product is responsible for this activity in *C. glutamicum*.

AraDH activity was also determined in the unphysiological, reverse (arabitol-forming) direction with extracts from *C. glutamicum* RES167 cells grown on glucose and on arabitol and with extracts from cells of the *C. glutamicum* Δ*atlR* strain grown on glucose. The specific activities with D-xylulose as a substrate were in all cases about one-third of those determined in the physiological direction (data not shown).

The specific XylK activities were determined for extracts of *C. glutamicum* RES167 and *C. glutamicum* Δ*atlR* cells grown in glucose- and in arabitol-containing medium and in extracts of *C. glutamicum* Δ*atlR* and *C. glutamicum* Δ*atlR*-Δ*xylB* strain cells grown in glucose-containing medium. As with the AraDH activities, RES167 cells grown with glucose showed no XylK activity (<0.01 U/mg protein), whereas cells grown with arabitol showed high specific activities of 0.96 ± 0.26 U/mg protein. Extracts of the Δ*atlR* strain even showed 5-fold-higher specific XylK activities after growth on glucose (4.99 ± 1.12 U/mg protein), indicating the complete derepression of the respective gene by the lack of AtlR. Both the *C. glutamicum* Δ*xylB* and *C. glutamicum* Δ*atlR*-Δ*xylB* strains did not show any XylK activities, indicating that *xylB* in fact encodes the XylK enzyme.

We also determined ribulokinase activities in cell extracts of *C. glutamicum* RES167 and *C. glutamicum* Δ*atlR* cells grown on glucose, arabitol, and/or mannitol. However, ribulokinase activities were not observed for any of the extracts, indicating (i) that XylK of *C. glutamicum* is specific for D-xylulose and (ii) that *C. glutamicum* does not possess a ribulokinase enzyme under the conditions tested.

The growth of the *C. glutamicum* Δ*atlR* strain on mannitol and the transient formation of fructose suggested that *C. glutamicum*, aside from its AraDH activity, also possesses ManDH activity, which necessary to metabolize mannitol to fructose, which can be secreted by a so-far-unknown transporter and then taken up by a fructose-specific phosphotransferase system (PTS) (24). Peng et al. (60) recently showed the presence of ManDH activity in an AtlR-deficient derivative of *C. glutamicum* strain R (0.29 U/mg protein), whereas the parental R strain lacked any such activity. In accordance with these results, cell extracts of *C. glutamicum* RES167 showed no ManDH activity when grown on glucose and a specific activity of 0.87 to 1.19 U/mg protein when grown on arabitol or on arabitol plus mannitol (Table 4). The *C. glutamicum* Δ*atlR* strain showed a specific activity of more than 2 U/mg protein when grown on glucose and about half of this specific activity when grown on mannitol or arabitol plus mannitol. In contrast, cells of the *C. glutamicum* Δ*atlR*-Δ*mtlD* double mutant grown on glucose showed no ManDH activity (Table 4), showing that the *mtlD* gene product is responsible for this activity. The K_m value for mannitol was determined to be 6.40 ± 0.63 mM (see Fig. S1B in the supplemental material), which is comparable to values obtained for ManDHs of other microorganisms (49, 67).

ManDH activity was also determined for *C. glutamicum* RES167 cells grown on xylulose and mannitol plus xylulose. The specific ManDH activity of extracts of cells grown on xylulose was 0.31 U/mg protein, and that of extracts of cells grown on xylulose plus mannitol was 0.30 U/mg protein. These results indicate that *mtlD* expression is induced or derepressed in the presence of xylulose in the growth medium.

The relatively similar results obtained upon the determinations of both AraDH and ManDH activities in our strains, the annotation of one of the AtlR-controlled genes as the ManDH gene *mtlD*, the observation that the *mtlD* mutants did not grow on either arabitol or mannitol, and the lack of AraDH and ManDH activities in *C. glutamicum* Δ*atlR*-Δ*mtlD* strain cells grown on glucose indicated that both these activities are due to one protein, i.e., to the *mtlD* gene product.

Expression of the *rbtT-mtlD-sixA* and *atlR-xylB* operons and AtlR binding to the *atlR-rbtT* promoter region. The results for the AraDH and XylK activities of *C. glutamicum* cells grown on

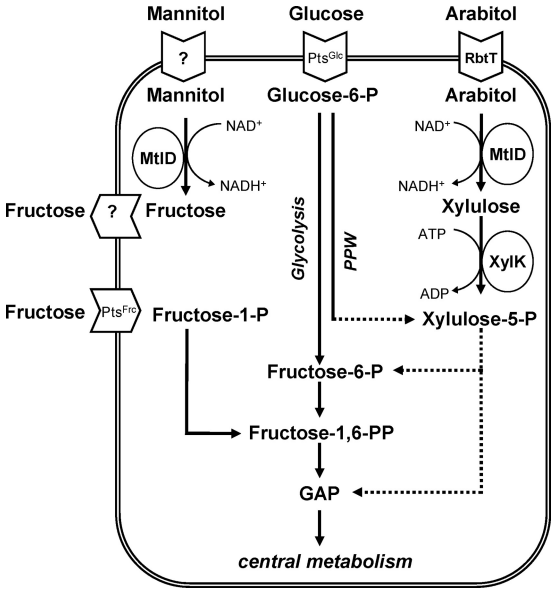

FIG 7 Model of the pathways for arabitol and mannitol (and glucose) uptake and metabolism in *C. glutamicum*. GAP, glyceraldehyde-3-phosphate; MtlD, gene product of the *mtlD* gene, possessing arabitol and mannitol dehydrogenase activities; Pts^{Glc} and Pts^{Frc}, phosphoenolpyruvate phosphotransferase systems specific for glucose and fructose, respectively; RbtT, gene product of *rbtT*, responsible for arabitol transport; XylK (xylulokinase), gene product of *xylB*.

glucose and on arabitol suggested that the respective genes *mtlD* and *xylB*, and, thus, probably the *rbtT-mtlD-sixA* and *atlR-xylB* operons, are subject to transcriptional control (induction or derepression) in response to the carbon source arabitol in the medium. To test this hypothesis, we compared *rbtT*, *mtlD*, *sixA*, *xylB*, and *atlR* gene expressions of arabitol- and glucose-grown cells of WT *C. glutamicum* by real-time RT-PCR. As shown in Fig. 2B, the expression levels of all five genes were much higher in cells grown on arabitol than cells grown on glucose, indicating that these genes were induced or derepressed when *C. glutamicum* cells were grown on arabitol.

In order to test for possible physiological effectors influencing the binding of AtlR to the *atlR-rbtT* promoter region and, thus, possibly affecting the induction or derepression of the two operons, we performed DNA binding assays (EMSAs) with the purified AtlR (His fusion) protein and tested for the effects of arabitol and mannitol and additionally for those of xylulose and xylulose-5-phosphate. As shown in Fig. S2 in the supplemental material, none of these metabolites led to the abolition of AtlR binding to the DNA fragment. These results suggest that none of the metabolites tested is a direct effector of the derepression of the *atlR-xylB* and *rbtT-mtlD-sixA* operons by AtlR.

DISCUSSION

To our knowledge, arabitol has not been reported to be a substrate or a cosubstrate for the growth or amino acid production of *C. glutamicum*. Here we show that this organism is able to efficiently use D-arabitol as the sole carbon and energy source with growth rates of up to 0.35 h^{-1} and biomass yields of about 0.3 g dry weight (g arabitol)$^{-1}$. The pathway of arabitol uptake and metabolism deduced from our studies is depicted in the model shown in Fig. 7.

Arabitol is prevalent in nature and has been found in plants and in many fungi, fulfilling a function in osmoprotection (19, 61) or as carbohydrate storage (21, 52). Growth on arabitol has been described for several microorganisms, e.g., for *Enterobacter aerogenes* (15, 16, 76, 77), *K. pneumoniae* (34, 35), *Rhizobium meliloti* and *Rhizobium trifolii* (53, 62), *E. coli* C strains (64), and *Pseudomonas fluorescens* (13). All these organisms possess D-AraDH activity, mediated by NAD^+-dependent dehydrogenases (either AraDH or ManDH), and oxidize arabitol to D-xylulose, which is then phosphorylated by a XylK protein. The formed xylulose-5-phosphate is further metabolized by reactions of the pentose phosphate pathway. For most of the arabitol-utilizing bacteria, the genes encoding AraDH and XylK and, in some bacteria, a gene also (putatively) encoding sugar transport proteins are transcribed as an operon, which is induced (derepressed) in response to D-arabitol (13, 14, 16, 34, 64, 76). For some of these bacteria, e.g., *E. aerogenes* and *K. pneumoniae*, the genes encoding the respective repressors have also been localized in the close vicinity of the arabitol genes, being transcribed as a monocistronic unit in the opposite direction (16, 34). For arabitol utilization by *C. glutamicum*, here we present evidence for the involvement of a sugar alcohol permease (encoded by *rbtT*), an enzyme with AraDH and ManDH activities (encoded by *mtlD*), XylK (encoded by *xylB*), and AtlR (encoded by *atlR*), which was previously identified as a regulator for some genes encoding enzymes of the central metabolism in *C. glutamicum* (see the introduction). The *rbtT* gene product shows significant identity (29%) to an H^+ symporter (DalT) of *K. pneumoniae*, belonging to the major facilitator superfamily (MFS) of carbohydrate transporters and being responsible for arabitol uptake in this organism (35). The *mtlD* gene product shows a striking level of identity (up to 68%) with bacterial ManDHs, which, at least in some cases, have also been shown to catalyze arabitol oxidation with NAD^+ (e.g., see references 14, 15, and 72). The *xylB* gene product shows up to 65% identity with XylK genes from other bacteria, and the functionality of the *xylB* gene product as XylK was previously shown by Kawaguchi et al. (39). All genes for the enzymes of D-arabitol uptake and metabolism in *C. glutamicum* are organized into two operons, i.e., the *atlR-xylB* operon and the *rbtT-mtlD-sixA* operon (Fig. 1). A similar genomic organization of the *rbtT*, *mtlD*, and *xylB* genes can be found for *Corynebacterium variabile*, *C. ammoniagenes*, and *C. bovis* (data not shown). However, among the species of corynebacteria with annotated genomes (http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi), only *C. glutamicum* and *C. variabile* possess *atlR* homologues, and only *C. glutamicum* and *C. efficiens* possess *sixA* homologues. Thus, the genomic arrangement of the *C. glutamicum* arabitol genes and their expression control seem to be unique for this species.

In previous studies, Cg0146 (AtlR) was shown to slightly repress the succinyl-CoA synthetase genes *sucCD* in *C. glutamicum* when the cells were grown on acetate as a carbon source, and as such, the protein was designated SucR (17). However, our microarray results show no evidence for a primary function of this protein in *sucCD* regulation, and in light of the other findings in this study, we designated the Cg0146 protein regulator of arabitol metabolism, AtlR, and the respective gene *atlR*. Peng et al. (60) recently found that Cg0146 (AtlR)-deficient mutants of *C. glutamicum* strain R grow on mannitol, although the parental WT strain was unable to utilize this sugar alcohol, a phenotype which is identical to that described in this study for our strains. Based on homology studies and on the results of growth experiments with

the parental R strain and with Δ*cg0146*, Δ*cg0146*-Δ*rbtT*, and Δ*cg0146*-Δ*mtlD* derivatives, Peng et al. (60) concluded that *rbtT* and *mtlD* encode a mannitol transporter and ManDH, respectively; that the two genes (proteins) are necessary for mannitol catabolism; and that, for unknown reasons, Cg0146 (AtlR) constitutively represses the expressions of these genes in *C. glutamicum* strain R. For congruence with the ManDH gene *mtlD*, those authors designated the cg0146 (*atlR*) gene *mtlR* and designated the *rbtT* gene *mtlT* and designated the respective products the mannitol regulator MtlR and the mannitol transporter MtlT, respectively. However, according to the data presented here, this naming is also not justified for the following reasons: (i) our data show that the physiological function of the cg0146 (*atlR*) and *rbtT* gene products is to enable WT *C. glutamicum* to grow on arabitol and not on mannitol; (ii) the growth experiments with the Δ*rbtT* mutant (Fig. 3) show that growth on arabitol rather than that on mannitol is severely affected by the lack of the *rbtT* gene product; (iii) only the presence of arabitol (or of its degradation product, xylulose) in the growth medium and not mannitol is able to relieve the repression of these genes, and thus, mannitol is not the inducer of the genes to be expressed for mannitol metabolism, and mannitol consumption is due only to the fact that MtlD (when the respective gene is derepressed either by the inactivation of AtlR or by the presence of arabitol) can convert not only arabitol to xylulose but also mannitol to fructose (which, after secretion and reuptake, can be channeled via fructose-1-phosphate into glycolysis [Fig. 7]); and (iv) the designation MtlR was previously allocated to repressor proteins controlling mannitol operons in several Gram-negative bacteria (e.g., see references 30, 50, and 71) but showing no significant similarity to AtlR. In our opinion, all these reasons justify the renaming of MtlR (SucR; Cg0146) and the designation of this protein AtlR and the respective gene *atlR*.

Our DNA microarray and real-time RT-PCR data indicate a negative expression control of the *atlR*, *xylB*, *rbtT*, *mtlD*, and *sixA* genes by AtlR and the release of this control by the presence of arabitol (or of xylulose, an intermediate of arabitol metabolism) in the growth medium. Based on this conclusion and on further results of this study, we propose the following regulatory model of arabitol consumption in wild-type *C. glutamicum*. AtlR represses the *atlR-xylB* and *rbtT-mtlD-sixA* operons as long as there is no arabitol (or xylulose) present in the medium. In the presence of arabitol (or xylulose) in the growth medium, AtlR changes its conformation and loses its ability to repress both operons, the enzymes for arabitol metabolism are formed, and the wild-type cells grow on arabitol. However, probably due to the concomitant formation of the AtlR repressor, the derepression of both operons in the presence of arabitol is not complete, as can be seen from the specific AraDH/ManDH and XylK activities, which were much higher in the absence of AtlR (*C. glutamicum* Δ*atlR* strain cells grown on glucose) than in its presence (e.g., *C. glutamicum* RES167 cells grown on arabitol). Thus, the deletion of the *atlR* gene leads to complete derepression and to the highest specific AraDH/ManDH and XylK activities. The quite high XylK specific activity of the AtlR-deficient mutant (about 5 U/mg protein versus about 1 U/mg protein for the parental strain) (see Results) may lead to the formation of a toxic (phosphorylated) intermediate from arabitol (or from an intermediate of arabitol metabolism), and this might explain why the *C. glutamicum* Δ*atlR* strain does not grow on arabitol. This hypothesis is substantiated by the observations that the AtlR-deficient mutant shows normal growth on glucose but (in contrast to the AtlR-positive parental strain) severely impaired growth on glucose plus arabitol. The deletion of the XylK gene in the AtlR-deficient mutant relieved this (negative) growth phenotype, showing that XylK is somehow responsible for the growth inhibition. Thus, at least some AtlR is needed to prevent unrestricted *xylB* expression in the presence of arabitol and, thus, to prevent growth inhibition by XylK activity.

Although the AtlR-deficient *C. glutamicum* mutant and its *C. glutamicum* Δ*atlR*-Δ*rbtT* and Δ*atlR*-Δ*xylB* derivatives did not grow on arabitol, they consumed arabitol at significant rates (Fig. 3A and B, 4, and 5A and Table 3). We do not have an experimentally proven explanation for this phenotype. However, we did not detect pyruvate, acetate, lactate, or succinate in the culture broth for any of these strains, nor did we detect sugars or any sugar alcohol other than the substrate arabitol, except with the Δ*xylB* mutants, which formed significant amounts of xylulose. Therefore, it can be assumed that for the other strains, most of the arabitol is channeled into the pentose phosphate pathway and is then further oxidized in the citric acid cycle to CO_2.

Our growth experiments with *C. glutamicum* RES167 on mixed substrates revealed that arabitol is cometabolized with glucose and that it is consumed with a high preference when mannitol is a cosubstrate (Fig. 3B and D). The simultaneous consumption of two substrates and the monophasic growth of *C. glutamicum* with glucose or other sugars and with glucose and additional organic acids, such as lactate, pyruvate, acetate, and propionate, were shown previously (18, 20, 23, 32, 47, 54, 56, 69, 75). Diauxic growth and the sequential utilization of carbon sources by *C. glutamicum* have been described only for mixtures of glucose and glutamate (45, 48) and for mixtures of glucose and ethanol (3, 4, 44). In both cases, *C. glutamicum* consumes glucose in the first growth phase and consumes the other substrate in the second growth phase, due to the carbon catabolite repression of the glutamate uptake system (*gluABCD* cluster) or of the ADH and ALDH genes (*adhA* and *ald*), respectively, in the presence of glucose (2, 44, 48). The sequential utilization of arabitol and mannitol is not due to a downregulation or a lack of ManDH activity in the first growth phase. According to the similar affinities of the MtlD protein for arabitol and mannitol (K_m values of 7.9 mM and 6.4 mM, respectively) and according to its relatively high specific activity with both arabitol and mannitol as substrates, substrate competition as a reason for the exclusive metabolism of arabitol in the arabitol-mannitol mixture is very unlikely. One possible explanation for this phenotype would be an inhibition of the mannitol transporter by the presence of arabitol or the arabitol-induced repression of the respective gene(s). However, the nature of the mannitol transporter or of its gene has not been identified so far.

With a mixture of 0.3% glucose and 0.5% arabitol, the *C. glutamicum* Δ*mtlD* strain reached the same final OD_{600} as that with 0.3% glucose, but the significantly lower growth rate than those of cultures grown in the presence of glucose as the only carbon source led us to conclude that arabitol exerts a negative effect on the growth of *C. glutamicum* strains lacking a functional AraDH. Scangos and Reiner (64) showed previously that the deletion of the AraDH gene in *E. coli* resulted in the loss of the ability to grow on glucose in the presence of arabitol. Those authors assumed that the deletion of this gene leads to the intracellular accumulation of arabitol, which might then be phosphorylated by a side reaction of XylK. In accordance with this hypothesis, *E. coli* suppressor mu-

tants that regained the ability to grow in the presence of arabitol exhibited mutations in the gene encoding XylK (64). As in the AraDH-deficient *E. coli* strains, XylK activity might be responsible for the slow growth of the *C. glutamicum* Δ*mtlD* strain in the presence of arabitol. Arabitol should act as an inducer for the transcription of *rbtT* and *xylB*, and thus, arabitol is transported into the cells, and the accumulated arabitol might then be phosphorylated by XylK and, in its phosphorylated form, may then have disadvantageous effects on growth. In favor of this hypothesis, we observed that a *xylB* deletion in the *C. glutamicum* Δ*atlR* strain relieved the growth phenotype of this strain on glucose plus arabitol; i.e., it relieved the inhibitory effect of arabitol on growth on glucose (see also above).

Another explanation for the slower growth of the *C. glutamicum* Δ*mtlD* strain on glucose in the presence of arabitol might be the inhibition of glucose uptake or utilization by arabitol. As shown in Table 3, the glucose consumption rate of the *mtlD* mutant (and also that of parental strain RES167) was almost 50% lower in the presence of arabitol than in its absence. Since the *mtlD* mutant on glucose plus arabitol can utilize only glucose for growth, the lower glucose consumption rate may limit growth. The construction and analysis of a *C. glutamicum* Δ*xylB* Δ*mtlD* double mutant would possibly allow the discrimination between the arabitol inhibition of glucose uptake or catabolism (the double mutant should show the same growth as that of the Δ*mtlD* single mutant) and the inhibition of growth by phosphorylated arabitol (faster growth of the double mutant than that of the Δ*mtlD* single mutant).

In contrast to the deletions of *rbtT* and *mtlD*, the deletion of *sixA* had no distinct effect on the growth behavior or on the substrate consumption of *C. glutamicum* on all carbon sources tested. The *sixA* gene product shows 85% identity to the phosphohistidine phosphatase SixA of *E. coli*. In this organism, SixA is involved in the so-called His-Asp phosphorelay between the His-containing phosphotransfer domain of the aerobic respiration control sensor protein ArcB and the DNA binding response regulator for osmoregulation, OmpR (57). However, no proteins with similarity to ArcB of *E. coli* have been identified for *C. glutamicum* (38), although there were some response regulators of two-component systems with similarity to OmpR (41). Thus, it might well be that SixA is involved in the function of one of the two-component systems in *C. glutamicum*. However, the lack of a *sixA* gene or homologue in most corynebacteria and the failure to detect any obvious phenotype of the Δ*sixA* strains indicate that under the conditions tested, SixA is of minor importance.

The AraDH activities observed for cells of *C. glutamicum* RES167 grown on glucose, arabitol, and glucose plus arabitol suggest that in addition to the induction effect of arabitol, there is an approximately 2-fold repressive effect of glucose on *mtlD* expression. With respect to this effect, it is noteworthy that Frunzke et al. (31) found previously that during growth on glucose, a GntR1-GntR2-deficient double mutant of *C. glutamicum* showed 4.5-fold- and 8.1-fold-higher levels of *mtlD* and *rbtT* mRNAs, respectively, than did the parental WT strain. This result might indicate a function of GntR1 and/or GntR2 as a repressor of *mtlD* and *rbtT* expressions. GntR1 and GntR2 are two functionally equivalent transcriptional regulators controlling gluconate catabolism and glucose uptake (31). They repress the genes encoding enzymes involved in gluconate uptake and metabolism, and they activate the expression of *ptsG*, encoding the EII^{Glc} permease of the glucose-specific PTS. However, we found no obvious GntR1/GntR2 binding site in the promoter region of the *rbtT-mtlD-sixA* operon, and thus, it is questionable whether these two regulators are directly responsible for the lower specific AraDH activities during the growth of *C. glutamicum* in the presence of glucose.

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