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# NOTES

## D-Allose Catabolism of *Escherichia coli*: Involvement of *alsI* and Regulation of *als* Regulon Expression by Allose and Ribose

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**Genes involved in allose utilization of *Escherichia coli* K-12 are organized in at least two operons, *alsRBACE* and *alsI*, located next to each other on the chromosome but divergently transcribed. Mutants defective in *alsI* (allose 6-phosphate isomerase gene) and *alsE* (allulose 6-phosphate epimerase gene) were Als$^-$. Transcription of the two allose operons, measured as β-galactosidase activity specified by *alsI-lacZ*$^+$ or *alsE-lacZ*$^+$ operon fusions, was induced by allose. Ribose also caused derepression of expression of the regulon under conditions in which ribose phosphate catabolism was impaired.**

Conversion of the all *cis*-hexose allose to fructose 6-phosphate in *Aerobacter aerogenes* requires the activity of three enzymes—allokinase, allose 6-phosphate isomerase, and allulose 6-phosphate epimerase (7). This pathway appears to operate also in *Escherichia coli*. Thus, five contiguous genes (*alsRBACE*), expressed as one operon, encode a periplasmic binding protein-mediated transport system (*alsB*, *alsA*, and *alsC*), a putative hexose phosphate epimerase (*alsE*), and a regulatory protein for allose utilization (*alsR*). A potential sixth member of the operon (*alsK*) has been postulated to encode allokinase (9). Ribose utilization requires among other enzymes ribose 5-phosphate isomerase. In *E. coli*, two ribose phosphate isomerases, A and B, have been identified biochemically (5, 6) and genetically (18, 20). Ribose phosphate isomerase A, encoded by *rpiA*, is synthesized constitutively, whereas the synthesis of ribose phosphate isomerase B, encoded by *rpiB*, appears to be increased following growth of cells in ribose-containing medium. A repressor protein, encoded by the *rpiR* gene, is involved in regulation of *rpiB* gene expression. Thus, *rpiR* strains contain elevated activity of ribose phosphate isomerase B (6, 20). The *rpiB* and *rpiR* loci are located next to each other at 92.8 min on the linkage map, but are divergently transcribed, with *rpiB* transcribed clockwise (20).

*alsR* and *rpiR* are the same gene (9). In the present work, we show that the *rpiB* gene product is also involved in allose catabolism, and presumably *rpiB* encodes allose 6-phosphate isomerase. Hence *rpiB* will be redesignated *alsI*. Although nucleotide sequence analysis implies *alsK* is the distal cistron of an *alsRBACEK* operon, our results showed that the *alsK* cistron was neither necessary for allose utilization nor coordinately expressed with the remaining *alsRBACE* cistrons. Consequently, we have designated *alsK* as *yjcT* (15).

**Methods.** The *E. coli* K-12 strains used in this study are listed in Table 1. Growth media (NZY broth or phosphate-buffered AB minimal medium) were described before (8). The carbon sources used were glucose, ribose, xylose, and glycerol at 0.2% each or allose at 0.05 or 0.1%. The growth of cell cultures was monitored in an Eppendorf PCP6121 photometer as optical density at 436 nm. Bacteriophage P1-mediated transduction (13), transformation with plasmid DNA (10), techniques for the growth of bacteriophage λ (16), and lysogenization by recombinant phage (17) have been previously described, as well as methods for the isolation of plasmid DNA (2) and chromosomal DNA (16). Restriction and ligation of DNA were performed as described by the suppliers of restriction endonucleases (Amersham, Promega, and New England Biolabs) and T4 DNA ligase (Amersham). PCR was performed with chromosomal or plasmid DNA as a template by standard procedures with DynaZyme II DNA polymerase (Finzymes, Oy, Finland). For enzyme assays, exponentially growing cells were harvested by centrifugation and disrupted by sonication for 60 s at 0°C and then centrifuged to remove cell debris. The assay of β-galactosidase activity at 30°C (13), or allokinase activity at 37°C (7) and determination of protein content (19) were performed as previously described.

**Isolation and characterization of *als* and *yjcT* mutants.** Transposon technology was used to generate one plasmid-harbored *alsR*::Tn*phoA′-1* mutation, four *alsE*::Tn*phoA′-1* mutations, and four *yjcT*::Tn*phoA′-1* mutations (Fig. 1). To avoid effects of a high copy number in an analysis of the regulation of *als* and *yjcT* gene expression, allele replacement by homologous recombination was conducted with each of the plasmid-borne *als* or *yjcT* mutations. This recombination resulted in the production of strains harboring chromosomally located *als* or *yjcT* mutations. The Tn*phoA′-1* insertions generated polar mutations. For each mutation, a nonpolar version was constructed (Fig. 1). We also constructed an operon fusion allele to the *alsI* gene [Φ(*alsI-lacZ*$^+$)*139*] (Fig. 2). A map of the 10 insertions is shown in Fig. 1A. The nucleotide sequences of the fusion points of the transposon-generated fusions are shown in Fig. 1B. The growth of the *als* strains, polar as well as nonpolar, on allose was analyzed. The *alsR* and *alsE* strains containing polar mutations were Als$^-$, whereas the *yjcT* strains were Als$^+$. The strains containing nonpolar *alsE* mutations were also Als$^-$. In contrast, the strains containing nonpolar *alsR21* or *yjcT8* mutations were Als$^+$. Furthermore, a strain harboring a mutation in the *alsI* gene (HO1973) was Als$^-$. These results indicated

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TABLE 1. Bacterial strains

| Strain | Relevant genotype[a] | Source, reference, or construction |
|---|---|---|
| BW18524 | —[b] | B. Wanner, Purdue University |
| CC118 | Δ*(lac)χ74 recA1* | 11 |
| DPB271 | *recD*::miniTet | 1 |
| HO340 | —[c] | 14 |
| HO890 | Δ*(rpiA)103*::Tet$^{rc}$ | 8 |
| HO1048 | Δ*(rpiA-serA)101*::Kan$^{r}$ *alsR128*$^{c}$ | 20 |
| HO1446 | *alsR128*$^{c}$ | Ser$^{+}$ derivative of HO1048 |
| HO1458 | *alsI137*::Kan$^{rc}$ | 20 |
| HO1686 | Δ*(rpiA)103*::Tet$^{r}$/λYYC205[Φ*(alsI-lacZ$^{+}$)139*]$^{c}$ | HO890, lysogenization with λYYC205 |
| HO1693 | Δ*(rpiA)103*::Tet$^{r}$ *alsI137*::Kan$^{r}$/λYYC205[Φ*(alsI-lacZ$^{+}$)139*]$^{c}$ | P1(HO1458) × HO1686, Kan$^{r}$ |
| HO1868 | *alsI137*::Kan$^{r}$/λYYC205[Φ*(alsI-lacZ$^{+}$)139*]$^{c}$ | P1(HO1458) × YYC1060, Kan$^{r}$ |
| HO1973 | *alsI137*::Kan$^{rb}$ | P1(HO1458) × BW18524, Kan$^{r}$ |
| HO2190 | *yjcT8*::Tn*phoA′-2*/λYYC205[Φ*(alsI-lacZ$^{+}$)139*]$^{c}$ | P1(TP2058) × YYC1060, Kan$^{r}$ |
| HO2376 | Δ*(rpiA)103*::Tet$^{r}$ *alsE11*::Tn*phoA′-9*$^{c}$ | P1(TP2086) × HO890, Kan$^{r}$ |
| P90C | *ara* Δ*(lac-pro) thi* | 17 |
| TP1904 | *recD*::miniTet$^{b}$ | P1(DPB271) × BW18524, Tet$^{r}$ |
| TP2058 | *yjcT8*::Tn*phoA′-2*$^{b}$ | BW18524, recombination of pTP908 (TP1904) followed by recombinational switching with λTn*phoA-132* (Tet$^{r}$) |
| TP2061 | *alsE11*::Tn*phoA′-2*$^{b}$ | BW18524, recombination of pTP911 (TP1904) followed by recombinational switching with λTn*phoA-132* (Tet$^{r}$) |
| TP2083 | *yjcT8*::Tn*phoA′-9*$^{b}$ | TP2058, recombinational switching with λTn*5-112* (Kan$^{r}$) |
| TP2086 | *alsE11*::Tn*phoA′-9*$^{b}$ | TP2061, recombinational switching with λTn*5-112* (Kan$^{r}$) |
| TP2110 | *alsR21*::Tn*phoA′-2*$^{b}$ | BW18524, recombination of pTP925 (TP1904) followed by recombinational switching with λTn*phoA-132* (Tet$^{r}$) |
| TP2115 | *alsR21*::Tn*phoA′-9*$^{b}$ | TP2110, recombinational switching with λTn*5-112* (Kan$^{r}$) |
| YYC1060 | —[c]/λYYC205[Φ*(alsI-lacZ$^{+}$)139*] | HO340, lysogenization with λYYC205 |
| YYC1062 | *alsR128*/λYYC205[Φ*(alsI-lacZ$^{+}$)139*]$^{c}$ | HO1446, lysogenization with λYYC205 |

[a] All of the strains are F$^{-}$. *phoA′* specifies β-galactosidase. Mutations generated by Tn*phoA-132*, Tn*phoA′-1*, or Tn*phoA′-2* are polar and are transposition proficient. Mutations generated by Tn*phoA-112* or Tn*phoA′-9* are nonpolar and are transposition deficient.
[b] Also contains Δ*(lac)χ74* Δ*phoA532 phnCp*Δ*(phnCDE)59*.
[c] Also contains *araC*(Am) *araD* Δ*(lac)U169 trp*(Am) *mal*(Am) *rpsL relA thi supF*.

that the *alsI* and *alsE* gene products are essential for allose utilization, whereas the repressor, encoded by *alsR*, is dispensable for allose utilization.

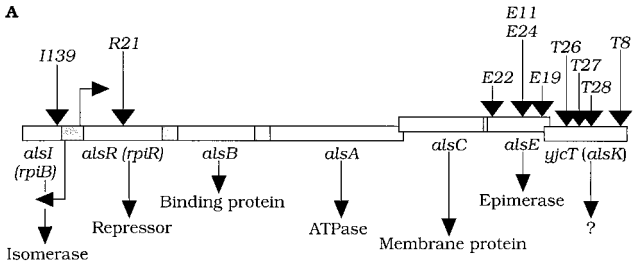
The addition of allose (0.05%) appeared to potently inhibit the growth with glycerol as the carbon source (0.2%) of strains harboring mutations in *alsI* (HO1973) or *alsE* (TP2086), encoding allose 6-phosphate isomerase and allulose 6-phosphate epimerase, respectively. In contrast, the growth of the remaining strains, i.e., those defective in the regulatory protein (TP2115 [*alsR*]) or YjcT (TP2083) were not inhibited by allose. The lack of growth of the *alsE* and *alsI* strains in the presence of allose indicated that a compound, which accumulated in these strains, caused inhibition. It is likely that this compound is allose 6-phosphate in the *alsI* strain and allose 6-phosphate, allulose 6-phosphate, or both in the *alsE* strain.

We previously showed that the *alsI* (*rpiB*)-encoded enzyme is able to isomerase ribose 5-phosphate and ribulose 5-phosphate. Thus, this enzyme appears to have substrate specificity

TABLE 2. Regulation of *als* regulon expression by allose

| Strain | *als* allele(s) or plasmid[a] | β-Galactosidase activity (nmol min$^{-1}$ mg of protein$^{-1}$) in medium supplemented with[b]: | | Activity ratio[c] |
|---|---|---|---|---|
| | | Glycerol | Glycerol + allose | |
| YYC1060 | Φ*(alsI-lacZ$^{+}$)139* | 438 | 25,300 | 58 |
| TP2115 | *alsR21*::Tn*phoA′-9* | 20,300 | 26,900 | 1.3 |
| TP2086 | *alsE11*::Tn*phoA′-9* | 201 | 8,630 | 43 |
| TP2083 | *yjcT8*::Tn*phoA′-9* | 48.0 | 50.1 | 1.0 |
| BW18524 | *als*$^{+}$ | 2.0 | 2.0 | 1.0 |
| HO2190 | Φ*(alsI-lacZ$^{+}$)139 yjcT8* | 451 | 14,800 | 33 |
| TP2083 | *yjcT8*/pHO390 (*yjcT*$^{+}$) | 42.0 | 34.0 | 0.8 |
| TP2083 | *yjcT8*/pBR322 | 32.0 | 44.0 | 1.4 |

[a] Tn*phoA′* fusions specify β-galactosidase activity.
[b] Cells were grown without or with allose (0.05%), and β-galactosidase activity was assayed as described in the text.
[c] Ratio of β-galactosidase activity in the presence of allose to β-galactosidase activity in the absence of allose.




B

| Mutation | Nucleotide sequence | Codon | Nucleotide |
|---|---|---|---|
| *alsR21* | CAGCTAGAACAGGTATTGCC↓GACT | Pro-95 | 8356 |
| *alsE22* | AAATCTCCCCCTCGTTAATG↓GACT | Cys-9 | 4102 |
| *alsE11/24* | TAAACCGCTCGACTGTCATC↓GACT | Leu-64 | 3936 |
| *alsE19* | GCACTTCCGGCCTGTTTAAT↓GACT | His-202 | 3523 |
| *yjcT26* | ACCCAGTATAGGGGGACCCT↓GACT | Asp-171 | 2937 |
| *yjcT27* | ACCCTCTATACTGGGTTGTG↓GACT | Cys-176 | 2922 |
| *yjcT28* | ACGGACCTTTGGTTAACGAG↓GACT | Ser-189 | 2881 |
| *yjcT8* | CATTGCCACCAGCATTAATC↓GACT | Leu-236 | 2741 |

FIG. 1. Structure of the *als* operon and location of *als-lacZ*$^+$ and *yjcT-lacZ*$^+$ insertions. Mutagenization of the *alsRBACE* operon and *yjcT* was performed as follows. Strain CC118 (Δ*lac*) harboring pTP680 (*alsR*$^+$*B*$^+$*A*$^+$*C*$^+$*E*$^+$ *yjcT*$^+$) or pHO390 (*yjcT*$^+$) was infected with λ::Tn*phoA′-1* (12). Tn*phoA′-1* contains a promoterless *lacZ*$^+$ allele, and a β-galactosidase-producing mutant has acquired an operon fusion. Mutants were selected as kanamycin resistant. Plasmid DNA was isolated and transformed back into strain CC118 and screened for production of β-galactosidase activity by the presence of 5-bromo-4-chloro-3-indolyl galactoside (40 mg liter$^{-1}$). Insertion of a transposon into the *als* operon was ascertained by restriction endonuclease analysis. Allele replacement of plasmid-harbored *als*::Tn*phoA′-1* or *yjcT*::Tn*phoA′-1* mutations and the chromosomal *als* or *yjcT* genes was performed by homologous recombination. Restriction endonuclease-linearized plasmid DNA was transformed into an *recD* strain (TP1904) by selection for kanamycin resistance. Genetic mapping ensured the location of the inserted DNA at 92.8 min on the linkage map. Recombinational switching among transposons was performed as previously described (21). The insertion of Tn*phoA′-1* generated polar mutations. A recombinational switching, using Tn*phoA-132* (encoding tetracycline resistance) followed by Tn*5-112* (encoding kanamycin resistance), resulted in the isolation of a nonpolar version of each *als* allele or *yjcT8*, essentially by removing a transcription terminator located within the right-hand IS*50* element of the transposon. The plasmids used were pTP680, which contained a wild-type version of the *alsRBACE* operon and *yjcT* in a 7.8-kb DNA fragment of chromosomal origin in pUC19 (22), or pHO390, which contained a PCR-amplified wild-type *yjcT* allele ligated to the *Bam*HI site of pBR322 (4). The inserted *yjcT* sequence was confirmed by sequencing. (A) Boxes indicate open reading frames of the *alsI* and *alsRBACE* operons and *yjcT*. Staggered boxes indicate open reading frames with possible overlapping translation (*alsA* and *alsC*, and *alsE* and *yjcT*). Shaded boxes indicate intercistronic regions. The angled arrows indicate the transcription initiation points before the *alsI* and *alsR* cistrons (20). Vertical arrows above the boxes indicate the positions of insertions of *als-lacZ*$^+$ or *yjcT-lacZ*$^+$ operon fusions. The *alsI-lacZ*$^+$ fusion was generated by in vitro techniques (Fig. 2). The presumed gene product of each cistron is indicated below the bar. The plasmids constructed were pTP908 (*yjcT8*::Tn*phoA′-1*), pTP911 (*alsE11*::Tn*phoA′-1*), pTP919 (*alsE19*::Tn*phoA′-1*), pTP922 (*alsE22*::Tn*phoA′-1*), pTP924 (*alsE24*::Tn*phoA′-1*), and pTP925 (*alsR21*::Tn*phoA′-1*), which were isolated from pTP680; and pTP926 (*yjcT26*::Tn*phoA′-1*), pTP927 (*yjcT27*::Tn*phoA′-1*), and pTP928 (*yjcT28*::Tn*phoA′-1*), which were isolated from pHO390. (B) Nucleotide sequence of the points of insertion of Tn*phoA′-1*. Sequencing was performed at the Botanical Institute, University of Copenhagen, in an Applied Biosystems model 377 sequencer by cycle sequencing with dye terminators (ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit; Perkin-Elmer) and with the oligodeoxyribonucleotide 5′-GCAGTAATTTCCGAGTCCC-3′ as a primer (Hobolth DNA Syntese, Hillerød, Denmark). A vertical arrow indicates an insertion point. Nucleotides to the left of the arrow originate from the *als* or *yjcT* cistrons. Nucleotides to the right of the arrow originate from Tn*phoA′* sequences. The codon where insertion occurred is indicated together with the nucleotide position of insertion. The latter numbers refer to the nucleotide sequence reported in the database sequence under accession no. AE00482 (3).

toward both pentose phosphates and hexose phosphates. A similar situation exists for the *Streptococcus mutans* galactose 6-phosphate isomerase (encoded by *lacAB*), which is also able to isomerize ribose 5-phosphate (20).

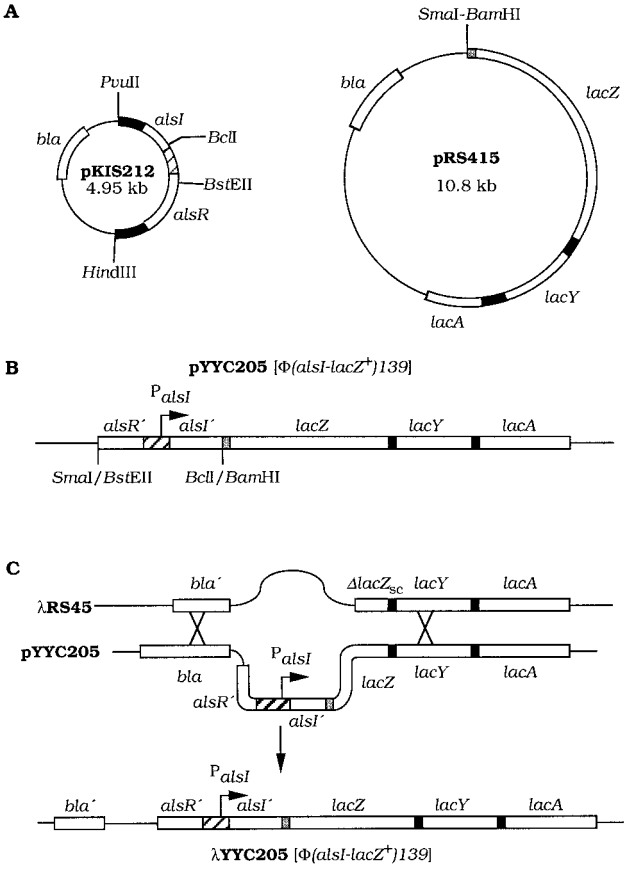



FIG. 2. Construction of an *alsI-lacZ*$^+$ gene fusion. Open reading frames are indicated by open double lines, vector sequences are indicated by thin lines, and flanking DNA sequences or intercistronic regions are shown as black or shaded double lines. Relevant restriction endonuclease recognition sites are included. (A) The plasmid pKIS212 contains the *alsR* and *alsI* genes (20). The plasmid pRS415 contains a promoterless *lac* operon, which includes a wild-type *lacZ* gene with translation initiation sequences. Intercistronic regions, which are not drawn to scale, are shown in black. The shaded region contains part of the *trp* operon as well as the original W205 fusion (17). (B) Construction of a plasmid-borne gene fusion. DNA of pKIS212 was digested with restriction endonuclease *Bst*EII, followed by incubation with the large fragment of *E. coli* DNA polymerase I in the presence of the four deoxyribonucleoside triphosphates and digestion with *Bcl*I. Plasmid pRS415 was digested with endonucleases *Sma*I and *Bam*HI. The two DNA species were ligated. Transformation followed by selection for ampicillin resistance in the presence of 5-bromo-4-chloro-3-indolyl galactoside to screen for β-galactosidase synthesis resulted in the isolation of pYYC205. The insert of pYYC205 contained 129 nucleotides of the N-terminal encoding end of the *alsR* reading frame, the 358 nucleotides of the *alsR-alsI* intercistronic region (cross-hatched), and 28 nucleotides of the N-terminal encoding end of the *alsI* reading frame. $P_{alsI}$ indicates the promoter driving transcription of the *alsI* gene, and an angled arrow indicates the transcription initiation point. *alsR′* and *alsI′* indicate deletion of the C-terminal encoding ends of the *alsR* and *alsI* genes, respectively. The various DNA elements of pYYC205 are not drawn to scale. (C) Isolation of a bacteriophage λ-borne gene fusion by homologous recombination. Bacteriophage λRS45 contains a version of the *lacZ* cistron that is deleted for the promoter-proximal two-thirds ($\Delta lacZ_{SC}$), wild-type versions of the *lacY* and *lacZ* cistrons, and a truncated *bla* gene (*bla′*). Thus, λRS45 forms white plaques, and lysogens of λRS45 form white colonies in the presence of 5-bromo-4-chloro-3-indolyl galactoside. The *lac-bla* sequence of λRS45 is homologous to sequences of pYYC205. Consequently homologous recombination (indicated by X) which occurred among plasmid and bacteriophage replicons within the *bla* sequence and within the *lac* sequence resulted in the formation of a bacteriophage λ genome carrying the gene fusion. Host strain P90C harboring pYYC205 was infected with λRS45 to allow recombination and to generate a lysate. Strain P90C was infected with this lysate and plated on NZY broth containing 5-bromo-4-chloro-3-indolyl galactoside. Blue plaques, which appeared at a frequency of approximately $4 \times 10^{-3}$, were restreaked, and one isolate, λYYC205, was kept for further analysis. Insertion of the prophage at the *att*λ site at 17 min, rather than at *alsI* at 92.8 min on the linkage map, was confirmed by genetic mapping.

TABLE 3. Regulation of *als* regulon expression by pentoses

| Strain | Genotype | β-Galactosidase activity (nmol $min^{-1}$ mg of $protein^{-1}$) in minimal medium supplemented with[a]: | | | | |
|---|---|---|---|---|---|---|
| | | Glucose | Xylose | Ribose | Ribose + xylose | Ribose + glucose |
| YYC1060 | Φ*(alsI-lacZ⁺)139* | 89.6 | 126 | 174 | 132 | 83.8 |
| HO1686 | Φ*(alsI-lacZ⁺)139 rpiA* | − | − | 1,510 | 349 | 62.4 |
| HO1693 | Φ*(alsI-lacZ⁺)139 rpiA alsI* | − | − | − | 1,590 | 63.8 |
| HO1868 | Φ*(alsI-lacZ⁺)139 alsI* | 57.5 | 121 | 132 | 100 | 63.3 |
| YYC1062 | Φ*(alsI-lacZ⁺)139 alsR* | 2,480 | 12,100 | 13,000 | 9,300 | 1,910 |
| HO2376 | *alsE11*::Tn*phoA′-9 rpiA* | − | − | 788 | 221 | 54.0 |

[a] Cells were grown and β-galactosidase activity was assayed as described in the text. −, no growth.

**Regulation of *alsI* operon expression.** The recombinant Φ*(alsI-lacZ⁺)139* fusion-harboring λ phage was used to lysogenize various *E. coli* strains. A 58-fold increase in β-galactosidase activity was observed when cells of strain YYC1060 were grown in the presence of allose and compared to the activity of cells grown in the absence of allose (Table 2).

The β-galactosidase activity specified by the Φ*(alsI-lacZ⁺)139* fusion contained in host strains, which harbored various genetic lesions of ribose catabolism, and grown on different carbon sources is shown in Table 3. In a wild-type strain (YYC1060), only a modest increase (twofold or less) in *alsI* gene expression was observed when cells were grown with pentose as a carbon source (xylose, ribose, or both) compared to growth in the presence of glucose. In contrast, *alsI* gene expression was greatly increased in ribose auxotrophic strains (*rpiA* or *rpiA alsI*), when grown on ribose. Thus, with growth in the presence of ribose, the β-galactosidase activity of an *rpiA* strain (HO1686) increased approximately 25-fold compared to growth in the presence of both ribose and glucose. The increase was less pronounced by growth in the presence of both ribose and xylose: approximately fivefold compared to growth in the presence of both ribose and glucose. Furthermore, *alsI* gene expression increased 25-fold or more in an *rpiA alsI* strain (HO1693) grown in the presence of ribose and xylose, compared to growth in the presence of ribose and glucose. A mutation in the *alsI* gene alone was essentially without effect on *alsI* gene expression, because the *alsI* strain HO1868 responded like the wild-type strain YY1060. The β-galactosidase activity in an *alsR* strain harboring the operon fusion (YYC1062) was increased 20- to 100-fold compared to the activity of the otherwise isogenic *alsR*⁺ strain (YYC1060).

**Regulation of *alsRBACE* operon expression.** Strains harboring a *phoA′-1* (*lacZ*⁺) gene fusion to the *alsR* (TP2115) or *alsE* (TP2086) cistrons were assayed for β-galactosidase activity in extracts of cells grown in the presence or absence of allose (Table 2). In the presence of allose, β-galactosidase activity increased 43-fold compared to the activity in the absence of allose in cells harboring a *lacZ* fusion to *alsE* (TP2086). Thus, expression of the *alsE* cistron appeared to be induced by the presence of allose. Cells harboring an *alsR-lacZ*⁺ gene fusion (TP2115) contained a high, constitutive level of β-galactosidase activity. The β-galactosidase activity of a nonfusion strain (BW18524) was negligible. In addition, the expression of the *alsRBACE* operon was regulated by ribose similarly to that described for the *alsI* operon. Thus, β-galactosidase activity specified by the *alsE11*::Tn*phoA′-9* fusion increased approximately 15-fold in cells grown with ribose or 4-fold in cells grown with ribose and xylose, compared to that in cells grown with ribose and glucose (Table 3).

**Lack of involvement of *yjcT* (*alsK*) in allose utilization.** The open reading frames of the distal cistron *alsE* and the following cistron *yjcT* overlapped by five codons, which may suggest translational coupling of the two cistrons (3). We constructed four independent insertions in *yjcT*, all of which had similar properties. Most importantly, expression of *yjcT* apparently was unaffected by allose (Table 2, strain TP2083). Strains with transposon insertions in *yjcT* were Als⁺. Furthermore, a *yjcT* mutation had no effect on expression of the *alsRBACE* and *alsI* operons: the introduction of *yjcT8* into an *alsI139-lacZ*⁺ strain had little effect on the fold of induction of β-galactosidase synthesis (Table 2, strains YYC1060 and HO2190). Supplying *yjcT* in *trans* had no effect, as shown by the lack of regulation of the *yjcT-lacZ*⁺ fusion strain transformed with pHO390, which contains a wild-type *yjcT* allele (Table 2, strains TP2083, TP2083/pHO390 and TP2083/pBR322). The allokinase activity in extracts of cells harboring pHO390, (i.e., with *yjcT* in multicopy) was identical to the activity in extracts of cells harboring pBR322 (0.5 nmol $min^{-1}$ mg of $protein^{-1}$). Finally, allokinase activities were similar in cells of wild-type and *yjcT* strains grown in glycerol (0.3 nmol $min^{-1}$ mg of $protein^{-1}$). Allose did not cause induction of allokinase synthesis, and *alsR* and *alsR*⁺ strains contained identical activities of allokinase. These results suggest that the kinase responsible for phosphorylation of allose either has a broad substrate specificity, which may not be subject to induction by allose, or it utilizes a phosphoryl donor different from ATP.

**Conclusion.** We have shown that *alsI* is essential for allose catabolism and that expression of both of the operons, *alsI* and *alsRABCE*, is induced by the presence of allose or ribose. In both cases, regulation is dependent on the *alsR* gene product. Thus, the *alsI* and *alsRBACE* operons constitute the *als* regulon. Apparently the *yjcT* gene is not a member of the *als* regulon.

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