JOURNAL OF BACTERIOLOGY, Jan. 1991, p. 108–115
0021-9193/91/010108-08$02.00/0

Vol. 173, No. 1

Cloning, Nucleotide Sequence, and Characterization of *mtr*, the Structural Gene for a Tryptophan-Specific Permease of *Escherichia coli* K-12†

VIRGINIA M. HEATWOLE AND RONALD L. SOMERVILLE*

Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907

Received 23 July 1990/Accepted 3 October 1990

The *mtr* gene of *Escherichia coli* K-12 encodes an L-tryptophan-specific permease. This gene was originally identified through the isolation of mutations in the 69-min region of the chromosome, closely linked to *argG*. Cells with lesions in *mtr* display a phenotype of 5-methyltryptophan resistance. The *mtr* gene was cloned by using the mini-Mu system. The amino acid sequence of Mtr (414 codons), deduced by DNA sequence analysis, was found to be 33% identical to that of another single-component transport protein, the tyrosine-specific permease, TyrP. The hydropathy plots of the two permeases were similar. Possible operator sites for the tyrosine and tryptophan repressors are situated within the region of DNA that is likely to be the *mtr* promoter.

In *Escherichia coli*, the aromatic amino acids are concentrated in the cytoplasm mainly via four operationally distinguishable transport systems. These systems are a general aromatic amino acid permease, encoded by *aroP*, and three other transport systems specific for phenylalanine, tyrosine, and tryptophan (6). The general aromatic amino acid permease has strong affinity for the aromatic amino acids (K_m for all three amino acids, 10^{-7} M), whereas the individual transport systems have a lower affinity but a higher specificity for their respective aromatic amino acids (K_m for each, ca. 10^{-6} M). Mutations in the structural genes for each specific permease have been found (6, 19, 39). The genes for the general aromatic amino acid permease, *aroP*, and the tyrosine-specific permease, *tyrP*, have been cloned (10, 14) and sequenced (20, 43). The regulation of transcription from the *tyrP* and *aroP* promoters has been thoroughly investigated (11, 23, 24). AroP and TyrP are hydrophobic proteins that are associated with the cytoplasmic membrane (10, 44).

The tryptophan-specific transport system is encoded by a single gene designated *mtr*. This gene is situated near the 69-min region of the bacterial chromosome (19), and the locus was first identified through the isolation of mutations that impart resistance to the antimetabolite 5-methyltryptophan. By P1 transduction, *mtr* is 60 to 80% linked to *argG* (19, 25). Mutations in *mtr* result in a loss of tryptophan-specific uptake (C. Yanofsky, cited by Oxender [34]). In addition to the *aroP* and *mtr* systems for L-tryptophan uptake, a gene for a low-affinity tryptophan permease (K_m, 10^{-5} M) is situated within the *tna* operon (14).

This report describes the cloning and structural characterization of the *mtr*$^+$ gene. The cloned DNA has structural and genetic properties predicted for a segment from the 69-min region of the chromosome. Several lines of evidence support the notion that the region cloned and sequenced in this study encodes the tryptophan-specific transport protein Mtr. Structural comparisons of Mtr with the two other well-characterized aromatic amino acid permeases of *E. coli* are also presented.

* Corresponding author.

† Journal paper no. 12585 from the Purdue University Agricultural Experiment Station.

MATERIALS AND METHODS

Bacterial strains, plasmids, and phage. The bacterial strains, plasmids, and bacteriophage used are listed in Table 1. The P1 transduction protocol used in strain constructions was previously described by Miller (31).

Media. Minimal medium contained 1.5% Bacto-Agar (Difco Laboratories), 0.2% glucose, vitamin B1 (1 mg/liter), biotin (0.1 mg/liter), and salt mix E of Vogel and Bonner (38). All amino acids were added at concentrations of 40 mg/liter unless otherwise specified. All of the amino acids except arginine were added to minimal medium to make ArgG selection medium. Acid-hydrolyzed casein was added to minimal medium at 0.2%. L broth (31) (with 0.1% glucose) and nutrient agar (31 g/liter) were used as complete media. M13 phage were propagated on yeast extract-tryptone solid medium and 2× yeast extract-tryptone liquid medium (31). Ampicillin and kanamycin were used at 25 mg/liter.

DNA preparations. Plasmid DNA and M13 replicative-form DNA were isolated by the alkaline lysis procedure of Ish-Horowicz and Burke (21). Minipreparations of plasmid and M13 replicative-form DNAs were made by using a scaled-down alkaline lysis procedure. Cells were transformed by the method of Cohen et al. (12). Single-stranded M13 templates were prepared for sequence analysis by the procedure of Sanger et al. (35).

Chemicals and reagents. Restriction endonucleases, T4 DNA ligase, and −20 and −40 oligodeoxynucleotide sequencing primers were purchased from New England BioLabs. Modified T7 DNA polymerase (Sequenase) and the Taquence (*Taq* polymerase) DNA sequencing kit were purchased from U.S. Biochemicals. [α-^{35}S]dATP (1,000 Ci/mmol) was purchased from Amersham. Acid-hydrolyzed casein was purchased from ICN Nutritional Biochemicals. Custom-made oligodeoxynucleotides used for sequencing were synthesized on an Applied Biosystems machine.

Preparation of mini-Mu lysates. Mini-Mu lysates were prepared as described previously (18). MC1040-2 carrying plasmid pEG5005 was used to prepare a mini-Mu lysate. Lysates were stored in $MgSO_4$ (2 mM)–$CaCl_2$ (0.2 mM) at 4°C for no more than 1 week.

Infection with mini-Mu lysate. The protocol for infection of cells with a mini-Mu lysate was based on the procedure of Groisman and Casadaban (18). A 100-μl saturated culture of

TABLE 1. Bacterial strains, phages, and plasmids

Strain, phage, or plasmid	Relevant genotype or description	Reference or source
E. coli strains		
CSH26	*ara* Δ (*lac-pro*) *thi*	31
KB3100	F⁻ *aroP*	6
KL16 argG	Hfr KL16 *thi argG rel-1*	9
KL16 argG Mul⁺	As KL16 *argG*; lysogenic for Mul⁺	This work
KY4124	HfrH *relA mtr24 rpsL140 met*	19
JC7623	*thr-1 leuB6 proA2 sbcB15 hisG4 recB21 recC22 argE3 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 tsx-33* λ⁻ *supE44*	42
JM101	Δ(*lac-pro*) *thi rpsL hsdR4 endA sbcB supE44* F′ *traD36 proA⁺B⁺ laqI^q* Δ(*lacZ*)M15	30
KY404	*trpS5* F′	22
MC1040-2	F⁻ *araD169 araB*::Mu cts Δ*lacX74 galU galK rpsL*	8
W3110	Wild type	1
SP867	*trpC*::Tn*10 cysB metB*	G. P. Zhao
SP1015	As CSH26 but *mtr24* from KY4124	This work
SP1051	As KL16 argG Mul⁺ but *mtr24*	This work
SP1095	As SP1051 but Δ*recA srl*::Tn*10*	This work
SP1000	As JM101 but Δ*recA srl*::Tn*10*	G. P. Zhao
SP1153	As JC7623 but *mtr*::*kan*	This work
SP1193	As SP867 but *mtr*::*kan*	This work
SP1281	As W3110 but *trpC*::Tn*10*	This work
SP1282	As KB3100 but *mtr*::*kan*	This work
SP1345	As KY4040 but *mtr*::*kan*	This work
Phages		
Mu d5005	Mu *cts62* A⁺B⁺ Kan^r Rep$_{pmB1}$	18
M13mp18		45
M13mp19		45
Plasmids		
pBR327$_{par}$		46
pEG5005	pBCO::Mu d5005	18
pSLW1	*mtr⁺ argG⁺*	This work (see Fig. 1)
pSLW6	*mtr⁺*	This work (see Fig. 1)
pSLW13	*mtr⁺*	This work (see Fig. 1)
pSLW14	*mtr*::*kan*	This work
pUC4KSAC	1.3-kb kanamycin cassette	2

SP1051 was mixed with 100 μl of mini-Mu lysate and incubated without shaking for 30 min at 30°C. Then 2 ml of L broth was added, and the mixture of infected cells was grown for 75 min at 30°C with shaking. The cells were spun down and washed in saline prior to plating on ArgG selection medium. Plates were incubated at 30°C.

DNA sequencing strategy. The 2.7-kb *Eco*RI-*Cla*I fragment from pSLW13 (Fig. 1) was purified electrophoretically and digested with either *Nsi*I, *Sau*3A, *Bgl*II, *Ssp*I, or *Pvu*II to create appropriate DNA fragments that could be ligated into M13mp19. For regions of the DNA sequence that could not be readily sequenced by using the available M13 subclones, specific oligodeoxynucleotide primers (19-mers) were synthesized to complete the sequencing of both strands.

DNA sequence analysis. DNA sequences were determined by the method of Sanger et al. (35), modified for use with [α-^{35}S]dATP as the labeling nucleotide. Recombinant M13 phage were used as templates. Both Sequenase and *Taq* polymerase were used in the sequencing reactions. Both the commercially available −20 and −40 universal primers and custom-made oligonucleotide primers were used as dictated by the available M13 clones and by sequence compression complications encountered during the course of the work.

Computer analysis. For promoter analysis, the homology program of Mulligan et al. (32) was used. The cross-correlation coefficient analysis of hydropathy plots was done by using the program of Shiver et al. (36). The remaining analyses were carried out by using the University of Wisconsin Genetics Computer Group programs.

Nucleotide sequence accession number. The nucleotide sequence reported in this paper has been submitted to GenBank (accession number M35417).

RESULTS AND DISCUSSION

Cloning *argG⁺*. The *mtr* gene, originally identified through the isolation of mutations that imparted resistance to 5-methyltryptophan, lies near the 69-min region of the *E. coli* chromosome, approximately 7 to 15 kb from *argG* (19, 25). Resistance to 5-methyltryptophan attributable to lesions in *mtr* can be observed only in the presence of phenylalanine (Table 2) (41). No method for the direct selection of *mtr⁺* DNA was available, so *mtr⁺* was cloned by exploiting its linkage to the selectable marker *argG*.

The *argG⁺* gene was cloned by an in vivo procedure based on the mini-Mu system of Groisman and Casadaban (18). A mini-Mu replicon, Mu d5005, having the capacity to package 23 kb of chromosomal DNA, was chosen. In this system, mini-Mu transposons with plasmid origins of replication become randomly distributed throughout the chromosome, where they serve as substrates for the Mu packaging reaction. The packaged DNA is then introduced into a Mu lysogen by infection. Intramolecular recombination leads to plasmids. Those carrying the desired gene are selected using an appropriate *rec⁺* tester strain.

A mini-Mu lysate generated within strain MC1040-2 was used to infect an *argG* strain, SP1051, that was lysogenic for Mul⁺. No *argG⁺* clones were obtained following selection

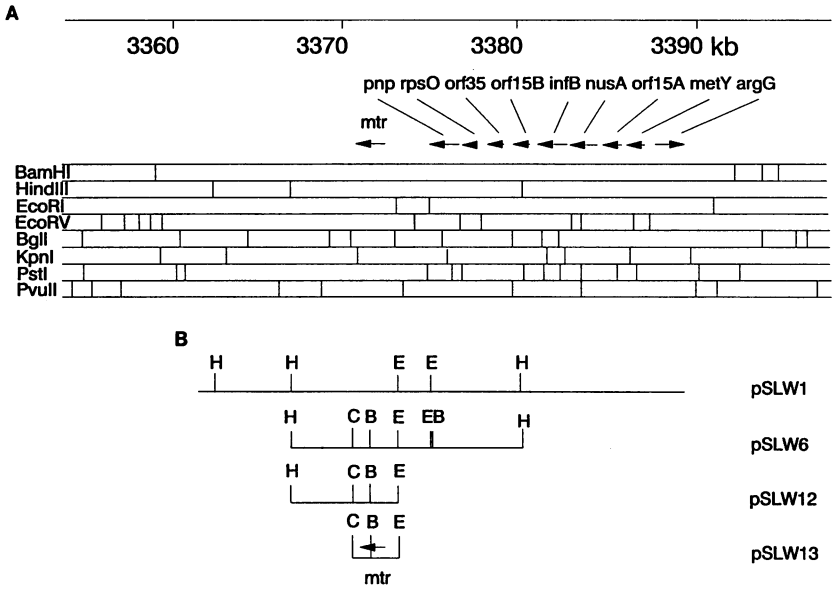


FIG. 1. Clones of *mtr*$^+$ in relation to the *E. coli* map. (A) Region of the map of Kohara et al. (26) of the *E. coli* chromosome that contains *mtr*. Known genes in this region of the chromosome are indicated, and the map coordinates for the region are shown. Arrows that designate genes are not drawn to scale. (B) Regions of chromosomal DNA that exist in plasmid constructions. pSLW1 represents the region of chromosomal DNA initially cloned using mini-Mu. The remaining plasmids were constructed by cloning DNA fragments from pSLW1 into an appropriate site in pBR327$_{par}$. The *Bgl*II site that disrupted the phenylalanine inhibition phenotype (see text) is found within the *mtr* open reading frame. Restriction enzymes: H, *Hin*dIII; E, *Eco*RI; C, *Cla*I; B, *Bgl*II.

on minimal medium, but 11 *argG*$^+$ clones were obtained when ArgG selection medium was used. The *arg*$^+$ mini-Mu clones were purified, and the corresponding plasmids were transformed into a *recA* background, SP1095, to prevent recombination between the cloned DNA and chromosomal DNA.

Identification of *mtr*$^+$ on an *argG*$^+$ plasmid. From linkage relationships determined by P1 transduction (19, 25) and previously published physical maps of the 69-min region of the *E. coli* chromosome (13, 26), it was judged that *mtr* should lie within a 12-kb *Hin*dIII fragment. Among 11 *argG*$^+$ mini-Mu clones that were analyzed, one isolate (pSLW1; Fig. 1) contained a 12-kb *Hin*dIII fragment. This *Hin*dIII fragment was isolated and inserted at the unique *Hin*dIII site of a high-copy-number plasmid, pBR327$_{par}$. The resulting construct, pSLW6, exhibited a new phenotype. The growth of strains bearing pSLW6 was inhibited on minimal medium supplemented with phenylalanine (50 mg/liter). The phenylalanine-mediated growth inhibition was reversed by tryptophan (10 mg/liter). Growth inhibition was also reversed on medium supplemented with acid-hydrolyzed casein. This medium contains a mixture of 16 amino acids but no L-tryptophan. No amino acid other than tryptophan, tested singly, was able to reverse the phenylalanine inhibition associated with pSLW6. The mechanism by which acid-hydrolyzed casein reverses phenylalanine inhibition is not understood.

TABLE 2. Scoring 5-methyltryptophan resistance attributable to mutations in *mtr*

Strain	Relevant genotype	Zone of inhibition (cm)	
		Minimal[a]	Minimal + F[b]
CSH26		3.6	3.6
SP1015	*mtr24*	3.6	No zone
JC7623		3.5	3.5
SP1153	*mtr*::*kan*	3.6	No zone

[a] Minimal medium supplemented with amino acids (40 mg/liter) required for growth of auxotrophic strains.
[b] Phenylalanine added to minimal medium at 50 mg/liter.

The role of aromatic amino acids in the phenotype conferred by pSLW6 suggested that the expression of *mtr* from a high-copy-number plasmid was the basis of the sensitivity to phenylalanine. This idea is plausible for two reasons. First, a similar phenotype was observed for a clone of the tyrosine-specific permease, *tyrP*$^+$ (24). Strains carrying *tyrP*$^+$ on a high-copy-number plasmid failed to grow in the presence of phenylalanine unless tyrosine was present. Phenylalanine has been shown to induce the expression of *tyrP*, and tyrosine has been shown to repress *tyrP* (23). Second, it is known that tryptophan-specific transport into cells is increased by exposure to phenylalanine in a manner that depends on TyrR (40). If phenylalanine induces the expression of *mtr*, it is reasonable to suppose that phenylalanine inhibition might be attributed to the overexpression of a protein, such as the product of *mtr*$^+$, that could not be tolerated in large amounts.

From the construction and analysis of a series of subclones (data not shown) of pSLW6, it was determined that the integrity of a *Bgl*II site was required for phenylalanine sensitivity. Using phenylalanine-mediated growth inhibition as a phenotypic marker for the integrity of the *mtr*$^+$ gene, a 2.7-kb *Eco*RI-*Cla*I fragment was subcloned. The resulting plasmid, pSLW13, contained the essential *Bgl*II site (Fig. 1).

A 1.3-kb *Bam*HI-ended cassette from pUC4KSAC containing the Tn*903* kanamycin resistance (*kan*) gene (2) was inserted at the *Bgl*II site of pSLW13. The resulting plasmid, pSLW14, no longer imparted hypersensitivity to phenylalanine. To show that the kanamycin cassette had inactivated *mtr*, a chromosomal homolog of this construction was iso-

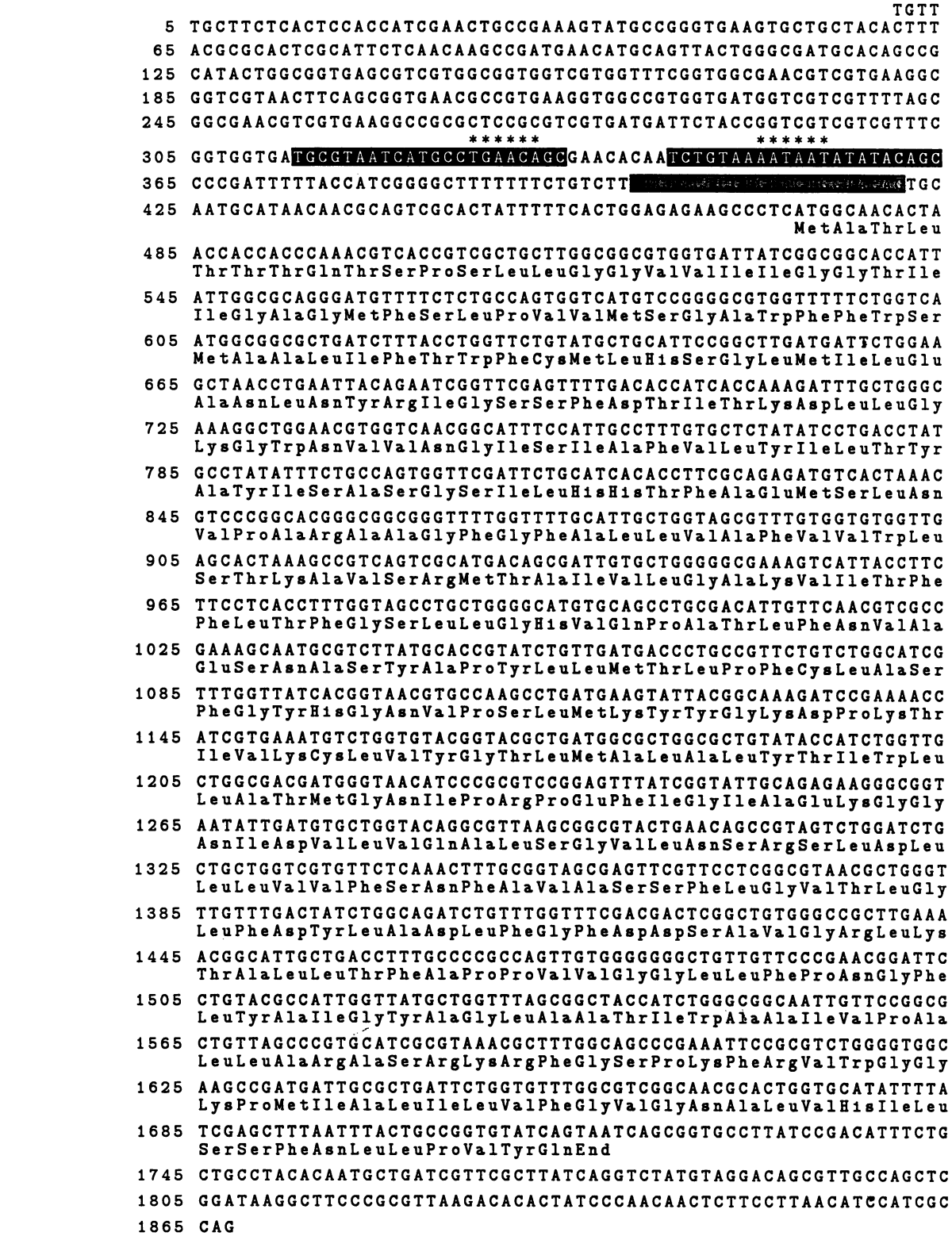


FIG. 2. DNA sequence and deduced amino acid sequence of *mtr*. The DNA sequence and flanking sequences of *mtr* are shown above, with translation of the open reading frame shown below. Two possible TyrR operators are indicated by black boxes. A possible TrpR operator is indicated by a gray box. The putative −10 and −35 hexamers identified by the program of Mulligan et al. (32) are indicated by asterisks. A purine-rich region is located −14 to −6 relative to the start site of translation.

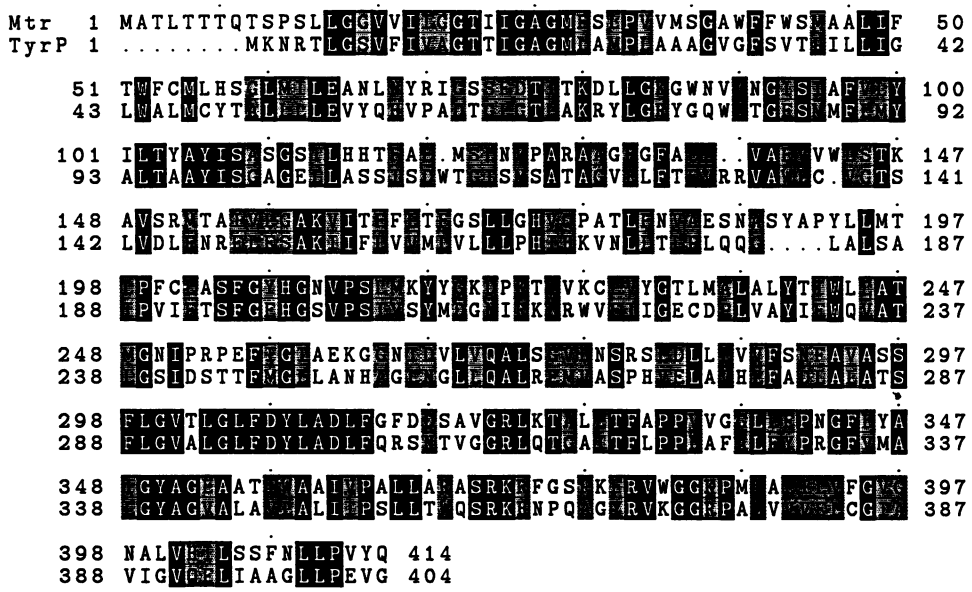


FIG. 3. Amino acid sequence comparison of Mtr and TyrP. The amino acid sequence of Mtr was compared with that of TyrP by the algorithm of Needleman and Wunsch (33). The proteins are 33% identical. Amino acid identities are indicated by black highlighting. Amino acid similarities (16) are indicated by gray highlighting. Only four gaps are introduced to align the sequences, with the largest gap covering only four residues.

lated according to the procedure of Winans et al. (42). This was done by linearizing pSLW14 with *Bam*HI, transforming an exonuclease V-deficient strain, JC7623, and selecting for kanamycin-resistant, ampicillin-sensitive derivatives. The resulting chromosomal construct, SP1153, exhibited the characteristic Mtr$^-$ phenotype (41): the strain was resistant to 5-methyltryptophan only in the presence of phenylalanine (Table 2). Table 2 compares the phenotype of a prototypical *mtr* mutation, *mtr24*, with that of the *mtr*::*kan* mutation present in SP1153. When mtr::*kan* was transferred by P1 transduction into a variety of other *E. coli* strains, the Mtr mutant phenotype always accompanied the Kan^r phenotype (data not shown). The chromosomal *mtr*::*kan* marker was 74% linked by P1 transduction to *argG*. This linkage is the same as the reported linkage of previously isolated *mtr* mutations (19, 25), providing further evidence that the manipulations described above had insertionally inactivated the chromosomal *mtr*$^+$ gene.

Identification of the *mtr* coding region. Nucleotide sequence data were obtained from a series of overlapping M13 clones generated from the *Eco*RI-*Cla*I fragment. This structural analysis revealed an open reading frame of 414 codons within the 2.7-kb *Eco*RI-*Cla*I fragment. The *Bgl*II site used to insertionally inactivate *mtr* lies within the open reading frame. The DNA sequence and the inferred amino acid sequence of the *mtr* gene are shown in Fig. 2.

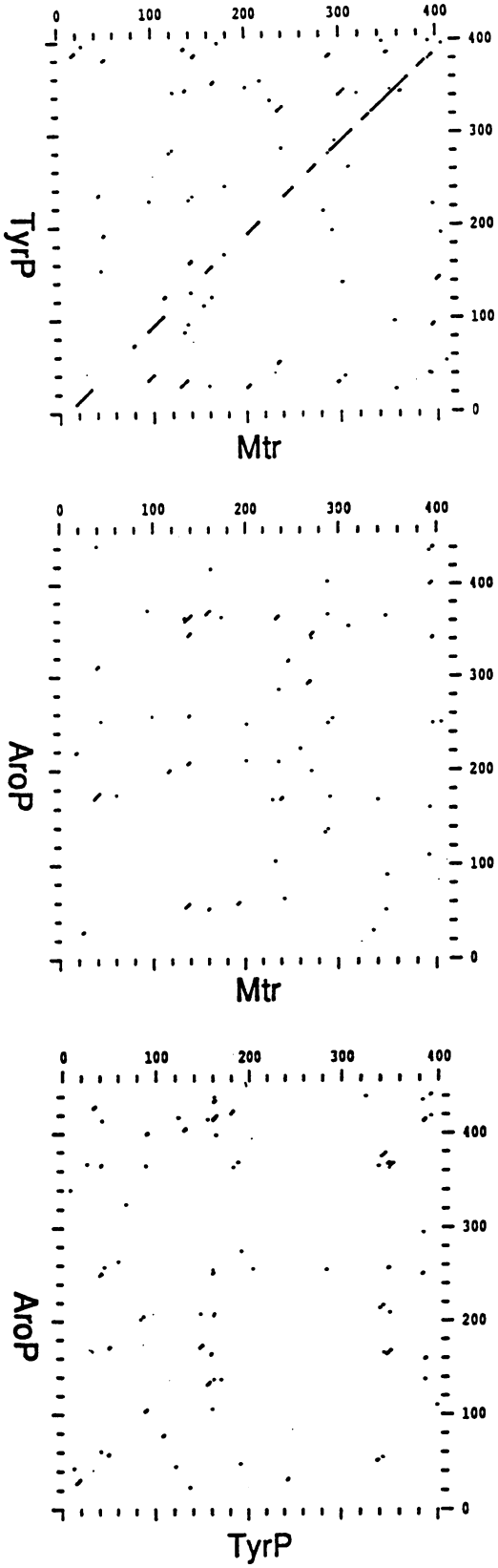
Codon preference and GC bias analysis (4, 17) facilitated the identification of protein-coding regions in the sequenced DNA. These algorithms identify regions of a DNA sequence that show a strong codon preference or an unusual GC composition bias in the third position of the codon. Both analyses pointed to the 414 codon open reading frame as a probable protein-coding region of the DNA (data not shown).

Analysis of sequences upstream of *mtr*. A promoter homology program (32) identified a possible site for transcription initiation upstream of the open reading frame. A purine-rich region, characteristic of a ribosome-binding site (27), is found between coordinates −14 to −6 relative to the predicted start point of translation. If the prediction of the −10 and −35 hexamers is correct, a potential stem-loop structure can form near the beginning of the transcript.

The DNA sequence was examined for possible operator sites. Using the consensus sequence for TyrR binding proposed by Baseggio et al. (3), two possible TyrR boxes were found. The presumptive −10 and −35 hexamers required by RNA polymerase for promoter recognition are situated within the presumptive TyrR boxes (Fig. 2). A possible Trp repressor target site was also identified downstream of the possible TyrR boxes (Fig. 2). The regulation of *mtr* expression involves multiple factors and will be described in a future publication.

Comparison of *mtr* with other aromatic amino acid permeases. The nucleotide sequence of the gene coding for the tyrosine-specific permease, *tyrP*, predicts a protein of 404 amino acids. By using the algorithm of Needleman and Wunsch (33), the deduced amino acid sequence of Mtr was compared with that of TyrP. The two amino acid sequences proved to be 33% identical. The regions of greatest identity between the two proteins are toward the C termini. Between residues 297 to 313 of Mtr there are 17 amino acids that are identical to TyrP except for one nonidentity, where threonine is found instead of alanine (Fig. 3). By using the same algorithm, no significant sequence identities were observed between either of the specific permeases, Mtr and TyrP, and the general aromatic amino acid permease, AroP. Dot plot analysis confirmed the amino acid identities between Mtr and TyrP but registered no significant identities between either of these permeases and AroP (Fig. 4). Dot plot analysis did not reveal any internally repeated sequences within Mtr.

A hydropathy plot (28) of Mtr suggests that this protein is very hydrophobic (Fig. 5). This is a characteristic of many membrane proteins. A comparison of the hydropathy plots



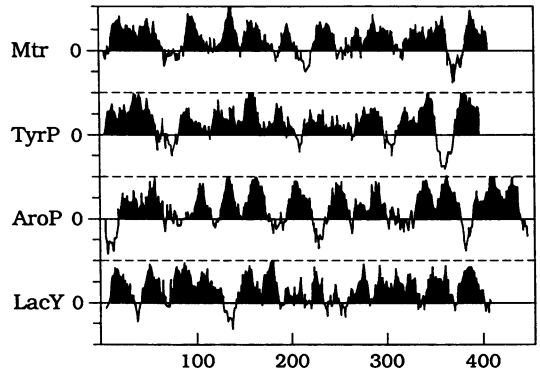


FIG. 5. Comparison of hydropathy plots. The hydropathy plots for Mtr, TyrP, AroP, and another bacterial single-component membrane permease, LacY (7), are shown. The hydrophobic regions are positive and are shown above the line in black. The hydrophilic regions are negative and are shown below the line in white. The hydrophobicity scale ranges from +3 to −3 for each plot. The hydrophobic regions of Mtr and TyrP are similar but are different from those of AroP and LacY. (The Kyte-Doolittle plots were generated by using a window of 11 residues.)

of Mtr and TyrP (Fig. 5) reveals that these two proteins have similarly distributed hydrophobic regions. The hydropathy plot of Mtr does not resemble that of two other membrane permeases, AroP and the lactose permease, LacY (Fig. 5).

The degree of similarity between the hydropathy plots of the known aromatic amino acid permeases was evaluated computationally by calculating cross-correlation coefficients (37), using the program of Shiver et al. (36). The cross-correlation coefficient is a numerical statement of the degree of similarity between hydrophobic functions. The coefficient of two dissimilar hydropathy functions is near 0, whereas the coefficient of two similar hydropathy functions is near 1.0. The cross-correlation coefficient for the Mtr and TyrP hydropathy plots was determined to be 0.72, using hydropathy values determined by Kyte and Doolittle (28) and an averaging interval of 11 residues. The optimum alignment of the two plots was achieved by omitting the N-terminal 10 residues of Mtr. A correlation coefficient of this magnitude is consistent with coefficients obtained for another family of similar proteins, the cytochrome *b* polypeptides, using Kyte-Doolittle values and the same averaging interval (36). The hydropathy plots of the cytochrome *b* polypeptides had cross-correlation coefficients of between 0.54 and 0.80. The maximum coefficient determined by comparing the best alignment of the hydropathy plot of Mtr with that of AroP was 0.51. In a comparison of the plot of TyrP with that of AroP, the maximum coefficient was 0.45.

Other phenotypes associated with the *mtr*::*kan* mutation. It was reported that *aroP* strains bearing an additional mutation that inactivated either the tyrosine- or the phenylalanine-specific transport system excreted tyrosine or phenylalanine, respectively (39). A double mutant strain that carried *aroP* and *mtr*::*kan* mutations, SP1282, was tested for

FIG. 4. Dot plot comparison of aromatic amino acid permeases. Dot plot analysis indicates identities between Mtr and TyrP. The region of greatest identity between the two proteins is toward the C termini. By using the same stringency, no identity to AroP was observed with either Mtr or TyrP. (A window of 11 residues and a stringency of 9 were used.)

its ability to secrete tryptophan by a standard auxanography test, using as a tester strain the tryptophan auxotroph SP1281. The test for feeding was done on minimal medium with glucose to prevent expression of the tryptophan permease in the *tna* operon. The insertionally inactivated *mtr* mutation, in combination with *aroP*, did not lead to the excretion of tryptophan.

A tryptophanyl-tRNA synthetase mutation, *trpS5*, shown to encode an unstable protein, imposes a tryptophan requirement. The tryptophan auxotrophic phenotype of the *trpS5* mutation can be suppressed by certain mutations in *mtr* (22). A *trpS5 mtr*::*kan* double mutant, SP1345, was constructed. The *mtr*::*kan* mutation did not reverse *trpS5*-mediated tryptophan auxotrophy.

Transport studies (40) have shown that a 20-fold excess of one aromatic amino acid inhibits uptake of the other two aromatic amino acids via the general aromatic amino acid permease. An *mtr*::*kan trpC* double mutant was constructed. The resultant strain, SP1193, failed to grow on minimal tryptophan (15 mg/liter) medium when phenylalanine (150 mg/liter) was present. The isogenic *mtr*$^+$ strain, SP867, was unaffected by phenylalanine. In all likelihood, this result reflects the inability of tryptophan to enter cells when the tryptophan-specific uptake system is mutationally disabled and the transport of tryptophan through the AroP system is blocked by phenylalanine.

Concluding comments. Our results from cloning and sequencing the *mtr* gene predict that the Mtr polypeptide is a tryptophan-specific permease that contains 414 amino acids. Attempts to visualize the protein product by whole-cell protein gels or by in vitro transcription and translation assays have so far proven unsuccessful. Insertional inactivation of the cloned *mtr* gene, followed by insertion of the *mtr* null mutation into the chromosome, led to strains having the previously described *mtr* phenotype. The null mutation created by in vitro manipulation lay in the same region of the chromosome as do previously identified *mtr* mutations. The direction of transcription of *mtr* (Fig. 1) is codirectional with replication (5).

The genes for the tyrosine-specific permease, *tyrP*, and the general aromatic amino acid permease, *aroP*, are regulated by TyrR. The *mtr* gene is probably part of the TyrR regulon as well, given the fact that tryptophan-specific transport is induced by phenylalanine in a manner that depends on TyrR (40). We have detected two possible TyrR operators and a TrpR operator upstream of *mtr* (Fig. 2). Studies to better define the promoter and the presumptive operators of *mtr* are in progress.

The predicted amino acid sequences of Mtr and TyrP are 33% identical (Fig. 3), suggesting that the two proteins are evolutionarily related. There is no significant amino acid sequence identity between Mtr and AroP or between TyrP and AroP. AroP does have some sequence identity to two yeast amino acid permeases specific for arginine and histidine (20).

Certain *mtr* mutations were able to reverse the auxotrophic phenotype of a *trpS5* mutation. However, insertional inactivation of the *mtr* gene in a *trpS5* mutant background does not lead to prototrophy. The same *mtr* mutation was unable to stimulate tryptophan secretion in an *aroP* background, although the *tyrP474* and *pheP367* mutations in a similar genetic arrangement led to tyrosine and phenylalanine secretion, respectively. Possibly Mtr protein requires specific types of amino acid substitutions to reverse the *trpS5* phenotype. By the same token, specifically altered TyrP and PheP proteins may be required in an *aroP* background to facilitate the secretion of their respective substrates. Thus, the insertional inactivation of *mtr* described here would be unable to either suppress the *trpS5* mutation or affect tryptophan secretion.

It has been suggested that several bacterial sugar/cation symporters consist of 12 membrane-spanning segments divided symmetrically by a central hydrophilic region (29). This same organizational pattern appears to be shared by an organophosphate permease, GlpT, which has been shown to have 12 transmembrane regions (15). The hydropathy plots of Mtr and TyrP (Fig. 5) are consistent with the notion that these polypeptides likewise possess 12 transmembrane regions and thus may have the same general pattern of organization as other bacterial transport proteins. Although these analogies provide quite compelling hints about a common mode of organization, further structural studies of Mtr and TyrP are needed before a 12-transmembrane-helix model may be considered proven.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grant GM22131 and by David Ross grant XR G901561 from the Purdue Research Foundation. Computer analyses involving the University of Wisconsin Genetics Computer Group programs was supported by grant NIH AI27713.

For their generous contributions of bacterial strains, viruses, and plasmids, we thank B. Wanner, B. Bachmann, K. D. Brown, N. Glansdorff, M. Casadaban, T. Yura, G.-P. Zhao, and F. Bolivar. We thank W. McClure and W. Cramer for providing computer programs. We also thank B. D. Aronson and R. J. Rolfes for invaluable advice and R. P. Westerman for computer assistance.

REFERENCES

1. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
2. **Barany, F.** 1985. Single-stranded hexameric linkers: a system for in-phase insertion mutagenesis and protein engineering. Gene **37**:111–123.
3. **Baseggio, N., W. D. Davies, and B. E. Davidson.** 1990. Identification of the promoter, operator, and 5′ and 3′ ends of the mRNA of *Escherichia coli* K-12 gene *aroG*. J. Bacteriol. **172**: 2547–2557.
4. **Bibb, M. J., P. R. Findlay, and M. W. Johnson.** 1984. The relationship between the base composition and codon usage in bacterial genes and its use for the simple and reliable identification of protein-coding sequences. Gene **30**:157–166.
5. **Brewer, B. J.** 1988. When polymerases collide: replication and the transcriptional organization of the *E. coli* chromosome. Cell **53**:679–686.
6. **Brown, K. D.** 1970. Formation of aromatic amino acid pools in *Escherichia coli* K-12. J. Bacteriol. **104**:177–188.
7. **Büchel, D. E., B. Gronenborn, and B. Müller-Hill.** 1980. Sequence of the lactose permease gene. Nature (London) **283**:541–545.
8. **Castilho, B. A., P. Olfson, and M. J. Casadaban.** 1984. Plasmid insertion mutagenesis and *lac* gene fusion with mini-Mu bacteriophage transposons. J. Bacteriol. **158**:488–495.
9. **Charlier, M., R. Crabeel, R. Cunin, and N. Glansdorff.** 1979. Tandem and inverted repeats of arginine genes in *Escherichia coli*. Structural and evolutionary considerations. Mol. Gen. Genet. **174**:75–88.
10. **Chye, M. L., J. R. Guest, and J. Pittard.** 1986. Cloning of the *aroP* gene and identification of its product in *Escherichia coli* K-12. J. Bacteriol. **167**:749–753.
11. **Chye, M. L., and J. Pittard.** 1987. Transcriptional control of the *aroP* gene in *Escherichia coli* K-12: analysis of operator mutants. J. Bacteriol. **169**:386–393.
12. **Cohen, S. N., A. C. Y. Chang, and L. Hsu.** 1972. Nonchromosomal antibiotic resistance in bacteria: transformation of *Escherichia coli* by R-factor DNA. Proc. Natl. Acad. Sci. USA

69:2110–2114.
13. **Crofton, S., and P. O. Dennis.** 1983. Cloning and orientation of the gene encoding polynucleotide phosphorylase in *Escherichia coli*. J. Bacteriol. **154**:58–64.
14. **Edwards, R. M., and M. D. Yudkin.** 1982. Location for the gene for the low-affinity tryptophan-specific permease of *Escherichia coli*. Biochem. J. **204**:617–619.
15. **Gött, P., and W. Boos.** 1988. The transmembrane topology of the *sn*-glycerol-3-phosphate permease of *Escherichia coli* analysed by *phoA* and *lacZ* protein fusions. Mol. Microbiol. **2**:655–663.
16. **Gribskov, M., and R. R. Burgess.** 1986. Sigma factors from *E. coli*, *B. subtilis*, phage SP01 and T4 are homologous proteins. Nucleic Acids Res. **14**:6745–6763.
17. **Gribskov, M., J. Devereux, and R. R. Burgess.** 1984. The codon preference plot: graphic analysis of protein coding sequences and prediction of gene expression. Nucleic Acids Res. **12**:539–549.
18. **Groisman, E. A., and M. J. Casadaban.** 1986. Mini-Mu bacteriophage with plasmid replicons for in vivo cloning and *lac* gene fusing. J. Bacteriol. **168**:357–364.
19. **Hiraga, S., K. Ito, T. Matsuyama, H. Ozaki, and T. Yura.** 1968. 5-Methyltryptophan-resistant mutations linked with the arginine G marker in *Escherichia coli*. J. Bacteriol. **96**:1880–1881.
20. **Honoré, N., and S. T. Cole.** 1990. Nucleotide sequence of the *aroP* gene encoding the general aromatic amino acid transport protein of *Escherichia coli* K-12: homology with yeast transport proteins. Nucleic Acids Res. **18**:653.
21. **Ish-Horowicz, D., and J. Burke.** 1981. Rapid and efficient cosmid cloning. Nucleic Acids Res. **9**:2989–2998.
22. **Ito, K., S. Hiraga, and T. Yura.** 1969. Tryptophanyl transfer RNA synthetase and expression of the tryptophan operon in the *trpS* mutants of *Escherichia coli*. Genetics **61**:521–538.
23. **Kasian, P., B. E. Davidson, and J. Pittard.** 1986. Molecular analysis of the promoter operator region of the *Escherichia coli* K-12 *tyrP* gene. J. Bacteriol. **167**:556–561.
24. **Kasian, P., and J. Pittard.** 1984. Construction of a *tyrP-lac* operon fusion strain and its use in the isolation and analysis of mutants derepressed for *tyrP* expression. J. Bacteriol. **160**:175–183.
25. **Kitakawa, M., E. R. Dabbs, and K. Isono.** 1979. Genes coding for ribosomal proteins S15, L21, and L27 map near *argG* in *Escherichia coli*. J. Bacteriol. **138**:832–838.
26. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50**:495–508.
27. **Kozak, M.** 1983. Comparison of initiation of protein synthesis in procaryotes, eucaryotes and organelles. Microbiol. Rev. **47**:1–45.
28. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157**:105–132.
29. **Maiden, M. C. J., E. O. Davis, S. A. Baldwin, D. C. M. Moore, and P. J. F. Henderson.** 1987. Mammalian and bacterial sugar transport proteins are homologous. Nature (London) **325**:641–643.
30. **Messing, J.** 1979. A multipurpose cloning system based on the single-stranded DNA bacteriophage M13. Recombinant DNA Technical Bulletin, NIH Publication no. 79–99, vol. 2, no. 2, p. 43–48. National Institutes of Health, Bethesda, Md.
31. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
32. **Mulligan, M. E., D. K. Hawley, R. Entriken, and W. R. McClure.** 1984. *Escherichia coli* promoter sequences predict *in vitro* RNA polymerase selectivity. Nucleic Acids Res. **12**:789–800.
33. **Needleman, S. B., and C. D. Wunsch.** 1970. A general method applicable to the search for similarities in the amino acid sequence of two proteins. J. Mol. Biol. **48**:443–453.
34. **Oxender, D. L.** 1975. Genetic approaches to the study of transport systems, p. 214–231. *In* H. N. Christensen (ed.), Biological transport, 2nd ed. W. A. Benjamin, New York.
35. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
36. **Shiver, J. W., A. A. Peterson, W. R. Widger, P. N. Furbacher, and W. A. Cramer.** 1989. Prediction of bilayer spanning domains of hydrophobic and amphipathic membrane proteins: application to the cytochrome b and colicin families. Methods Enzymol. **172**:439–461.
37. **Sweet, R. M., and D. Eisenberg.** 1983. Correlation of sequence hydrophobicities measures similarity in three-dimensional protein structure. J. Mol. Biol. **171**:479–488.
38. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.
39. **Whipp, M. J., D. M. Halsall, and J. Pittard.** 1980. Isolation and characterization of an *Escherichia coli* K-12 mutant defective in tyrosine- and phenylalanine-specific transport systems. J. Bacteriol. **143**:1–7.
40. **Whipp, M. J., and J. Pittard.** 1977. Regulation of aromatic amino acid transport systems in *Escherichia coli* K-12. J. Bacteriol. **132**:453–461.
41. **Williams, M. V., T. J. Kerr, R. D. Lemmon, and G. J. Tritz.** 1980. Azaserine resistance in *Escherichia coli*: chromosomal location of multiple genes. J. Bacteriol. **143**:383–388.
42. **Winans, S. C., S. J. Elledge, J. H. Krueger, and G. C. Walker.** 1985. Site-directed insertion and deletion mutagenesis with cloned fragments in *Escherichia coli*. J. Bacteriol. **161**:1219–1221.
43. **Wookey, P. J., and A. J. Pittard.** 1988. DNA sequence of the gene (*tyrP*) encoding the tyrosine-specific transport system of *Escherichia coli*. J. Bacteriol. **170**:4946–4949.
44. **Wookey, P. J., J. Pittard, S. M. Forrest, and B. E. Davidson.** 1984. Cloning of the *tyrP* gene and further characterization of the tyrosine-specific transport system in *Escherichia coli* K-12. J. Bacteriol. **160**:169–174.
45. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.
46. **Zurita, M., F. Bolivar, and X. Soberon.** 1984. Construction and characterization of new cloning vehicles. VII. Construction of plasmid pBR327par, a completely sequenced, stable derivative of pBR327 containing the *par* locus of pSC101. Gene **28**:119–122.