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Orotic Acid Excretion in Some Wild-Type Strains of *Escherichia coli* K-12

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During rapid growth, the excretion of pyrimidines, predominantly uracil, is a common phenomenon in procaryotes and eucaryotes. In *Escherichia coli*, some K-12 strains excrete orotic acid and not uracil. This is caused by a mutation in the *pyrF* gene.

The de novo synthesis of UMP appears to be the same for all prototrophic organisms (15). The series of reactions converts carbon dioxide, NH_3 (from ammonia or glutamine), and aspartic acid into orotic acid by a series of four reactions. Orotic acid, the first pyrimidine, is then converted to UMP by two reactions (Fig. 1). The six enzymes are coded for by the unlinked genes *pyrA-F* in *Escherichia coli* and *Salmonella typhimurium* (15).

The first enzyme in the pathway, carbamyl phosphate synthetase, condenses HCO_3^-, NH_3, and a phosphate from ATP to yield carbamyl phosphate. Carbamyl phosphate is then used for both arginine and pyrimidine biosynthesis (Fig. 1). Carbamyl phosphate synthetase is repressed by a cytidine phosphate and by arginine (1). It is allosterically inhibited by UMP (2).

The second enzyme in the pathway, the first enzyme unique to pyrimidine biosynthesis, is aspartate transcarbamylase. This enzyme catalyzes the condensation of carbamyl phosphate and L-aspartate to carbamyl aspartate plus inorganic phosphate (8). Aspartate transcarbamylase is also highly regulated. It is allosterically inhibited by CTP (8) and repressed by a uridine compound (10, 18).

The remaining four enzymes are not subject to allosteric regulation; however, each is repressible by one or more uridine and/or cytidine compounds (10, 18). Thus, this pathway is regulated at two points by allosteric inhibition and at all steps by end product repression (for a review see reference 15).

For these reasons, a report by Machida and Kuninaka (11) is somewhat surprising. They reported that *E. coli* K-12 strains, but not *E. coli* B strains, excrete between 94 and 441 mg of orotic acid per liter of growth medium during exponential phase. They further reported that maximal orotic acid excretion occurred at about 32°C when the initial medium pH was 6.0. We decided to further investigate these observations.

Figure 2B shows the absorption spectra of the spent cell-free medium from two *E. coli* K-12 strains (W3110 *trpA33* and JC3312), one *E. coli* B, and one *S. typhimurium* (*pyrF146*) (19). Figure 2A shows the absorption spectra of uracil and orotic acid under the same conditions. The *pyrF146* strain was grown in uracil minimal medium to mid-exponential phase and then starved for uracil, which causes it to excrete large quantities of orotic acid; thus, this is also the control.

The data show that the *E. coli* W3110 *trpA33*, a strain shown to overproduce orotic acid (11), excretes a compound with a UV absorption very similar to the *S. typhimurium* strain and nearly identical to orotic acid. Paper chromatography by published procedures (11) confirms that the majority of the UV-absorbing material is orotic acid (data not shown). However, the other K-12 strains and the *E. coli* B strain do not excrete this compound. Paper chromatography of the spent medium used to grow strain JC3312 and the *E. coli* B showed that the UV-absorbing compound excreted by these strains was uracil (Womack and O'Donovan, manuscript in preparation).

A closer inspection of the list of strains tested by Machida and Kuninaka (11) shows that they tested eight *E. coli* K-12 strains that excrete orotic acid and five K-12 strains that do not excrete it; thus, orotic acid excretion is not a property of all *E. coli* K-12 strains. To clarify this point, we tested all of the *E. coli* K-12 strains from our laboratory. The results of our tests plus those of the previous study show that all of the strains found to excrete orotic acid are listed by Bachmann in the same chart (reference 3, chart 8).

Mutants blocked in one of the last two steps of UMP biosynthesis (Fig. 1) excrete orotic acid upon starvation for uracil. Mutants with this

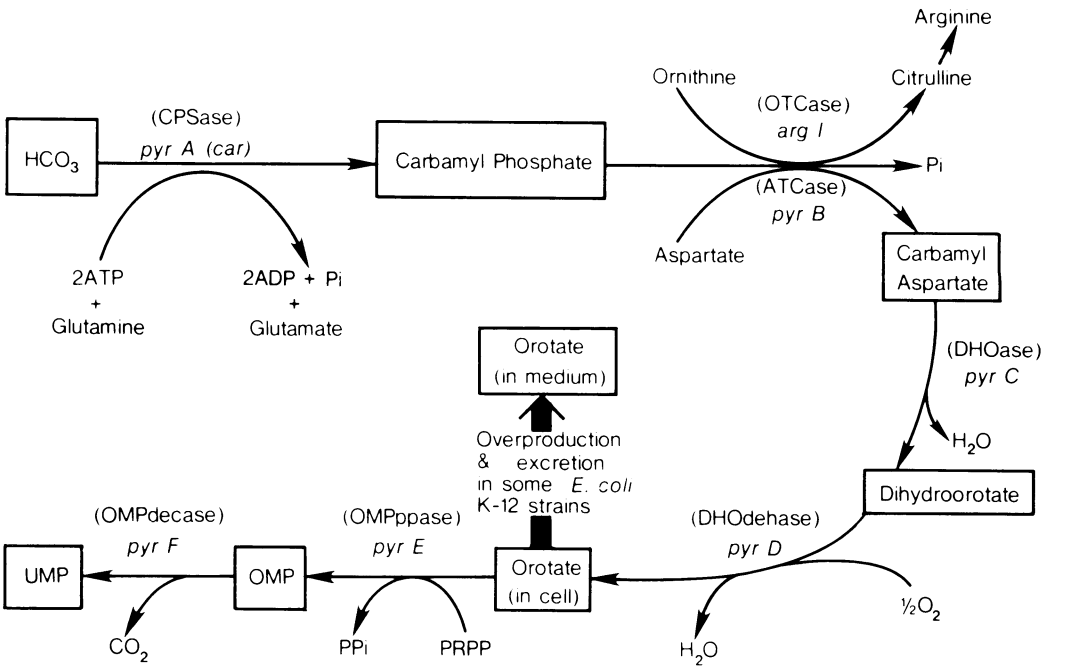

FIG. 1. *Pyrimidine biosynthetic pathway and partial arginine pathway in Escherichia coli K-12. Genetic symbols for the enzymes are shown in italics. The enzymes shown in parentheses are abbreviated as follows: carbamyl phosphate synthetase (CPSase); aspartate transcarbamylase (ATCase); dihydroorotase (DHOase); dihydroorotate dehydrogenase (DHOdehase); orotidine-5'-phosphate pyrophosphorylase (OMPppase); orotidine-5'-phosphate decarboxylase (OMPdecase); ornithine transcarbamylase (OTCase).*

phenotype have been found in *E. coli* (21), *Aerobacter aerogenes* (20), *Serratia marinorubra* (5), *S. marcescens* (9), *Neurospora crassa* (12), and even in *Homo sapiens* (17). Thus, this phenotype seems to be universal.

Other studies have shown that most organisms excrete orotic acid when grown in the presence of 5-fluorouracil or 6-azauracil. These uracil analogs are converted to the substituted 5'-monophosphates, which then inhibit the conversion of orotic acid to UMP, causing orotic acid to be excreted (7, 15). Thus, orotic acid excretion is most commonly associated with loss of activity of orotidine monophosphate (OMP) pyrophosphorylase, the *pyrE* product, or OMP decarboxylase, the *pyrF* product, either by a genetic defect or by analog inhibition.

The observations that the strains which excrete orotic acid all share a common ancestor (3) and that mutants in *pyrE* and *pyrF* (encoding OMP pyrophosphorylase and OMP decarboxylase; Fig. 1) excrete orotic acid suggested that the most likely explanation of orotic acid excretion in these strains was a defective *pyrE* or *pyrF*. This was tested genetically as follows: HfrWD1012, which donates *pyrF* and *trp* early (Fig. 3) in mating experiments, does not excrete orotic acid, and is *thyA*, was chosen as the male. The mating of HfrWD1012 with strain W3110 *trpA33*, an F^- strain, was by previously published procedures (16). Recombinants were selected for their ability to grow in the absence of thymine and tryptophan. None of the Trp^+ recombinants excreted orotic acid. Figure 4 gives an example of one such recombinant, WD1010. Recently, by cotransducing the wild-type trp^+ $pyrF^+$ region from a non-orotate-excreting donor into an orotate-excreting *trp* recipient, and selecting for Trp^+ recombinants, Slaughter (Ph.D. thesis, Texas A&M University, College Station, Tex., 1976) has shown that orotic acid excretion cotransduces to a high degree with *trp*. In addition we have shown that the Trp^- phenotype is not associated per se with orotic acid excretion. Thus, we conclude that orotic acid excretion in some *E. coli* K-12 strains is caused by a leaky OMP decarboxylase, the product of *pyrF*. Enzyme assays for OMP decarboxylase from crude cell extracts do not show a significant difference among orotic acid-excreting and orotic acid-nonexcreting strains. The detailed enzymology is now in progress.

The fact that some *E. coli* K-12 strains excrete orotic acid into the medium, whereas other *E.*

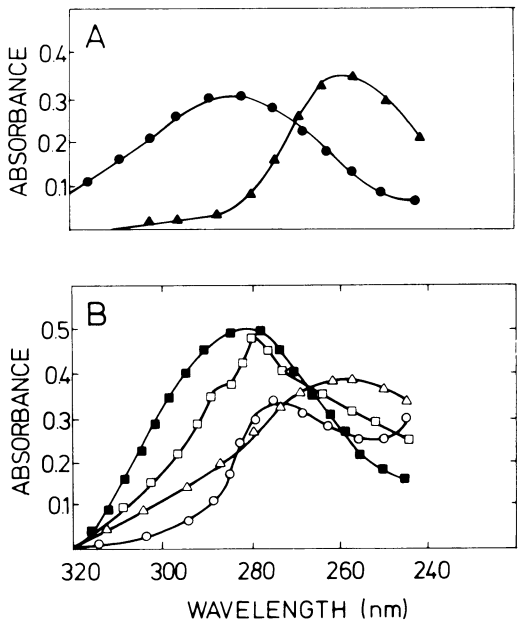

FIG. 2. *Absorption spectra of the spent medium from four bacterial strains and two standards. (A) UV absorption of orotic acid (●) and uracil (▲). Orotic acid or uracil was added to fresh tris(hydroxymethyl)aminomethane medium (18) containing 0.1% acid-hydrolyzed casein and 0.2% glucose. After mixing, 0.1 ml was removed and added to 0.9 ml of 10% perchloric acid. This mixture was then scanned from 340 to 220 nm in a Beckman DB-GT dual-beam spectrophotometer. Tris(hydroxymethyl)-aminomethane medium, without uracil or orotic acid, was in the reference cell. (B) UV absorption of the spent medium from four bacterial strains: S. typhimurium LT2 strain pyrF146 (□); E. coli K-12 strain W3110trpA33 (■); E. coli B (△); and E. coli K-12 strain JC3312 (○). All strains were grown in tris(hydroxymethyl)aminomethane medium containing 0.2% glucose, 0.1% hydrolyzed casein and tryptophan (20 μg/ml). The pyrF146 strain was supplied with 5 μg of uracil per ml which allows about 50% total growth. Growth was at 32°C in a reciprocal shaker. When the cultures were in late-exponential phase, 1 ml of each culture was removed and centrifuged to remove the cells. Then 0.1 ml of supernatant was added to 0.9 ml of 10% perchloric acid before being scanned. The reference cell contained tris(hydroxymethyl)aminomethane medium from before the addition of cells.*

coli K-12 strains and all *E. coli* B and *S. typhimurium* strains excrete uracil, affords a ready explanation for the following previously reported anomalous observations. (i) Certain *E. coli* K-12 strains (those that excrete orotic acid) show derepressed levels of de novo pyrimidine enzymes (O'Donovan and Kelln, unpublished data). (ii) RNA from *E. coli* K-12 strains (orotic acid and not uracil excreted into the medium) can be quite effectively labeled with radioactive uracil (6) in a hydrolyzed casein medium, whereas *S. typhimurium* (J. Neuhard, personal communication) and *E. coli* B (J. Womack, unpublished data) are not easily labeled in this medium because they excrete uracil. (iii) *upp* mutants are isolated by selecting for resistance to 5-fluorouracil (15). However, 10 μg of 5-fluorouracil per ml is required for *E. coli* B and *S. typhimurium*, whereas only 1 to 2 μg/ml is needed for *E. coli* K-12 strains (13). (iv) *E. coli* K-12 strain JC411 is reported to excrete uracil (14), not orotic acid, as would be predicted if all *E. coli* K-12 strains excreted orotic acid.

These observations are explained by our finding that certain closely related strains (3) contain a defective *pyrF* gene, causing them to have lower-than-normal UMP pool levels. The im-

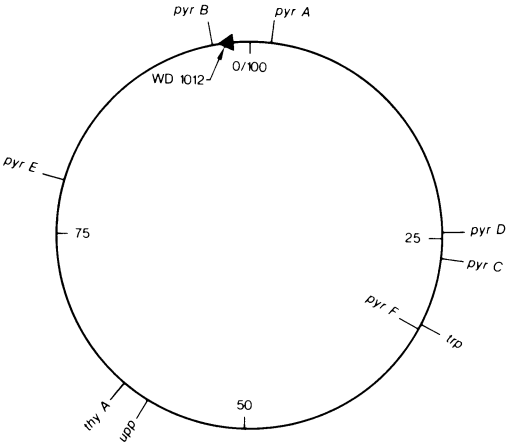

FIG. 3. *Linkage map of pyrimidine genes in E. coli. The position and direction of transfer of the Hfr donor strain, WD1012, are given by the symbol ▲.*

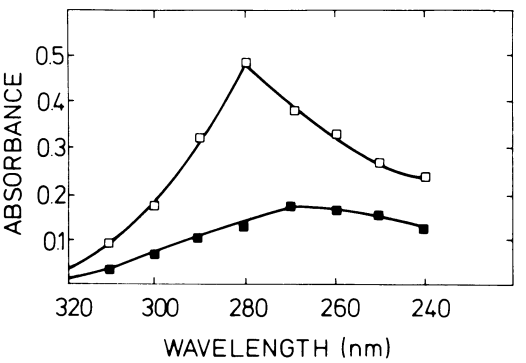

FIG. 4. *Absorption spectra of the medium from WD1010, the exconjugant (■), and the F⁻ strain, W3110 trpA33 (□). WD1010 was constructed from the mating of HfrWD1012 × F⁻ W3110 trpA33. The growth medium, temperature, and method of analysis were identical to those given in the legend to Fig. 2B.*

portance of this finding is that many of the strains used in the analysis of the physiology of *E. coli* K-12 in nucleic acid studies come from this family of strains (e.g., *E. coli* CR63; W3110). Thus, many of the observations long considered to be a property of *E. coli* K-12 wild-type strains are in fact the property of *E. coli* K-12 strains with a defective *pyrF* gene encoding OMP decarboxylase which catalyzes the sixth step of UMP synthesis. Purification of OMP decarboxylase from an *E. coli* B strain will be required to pinpoint this defect. This work is now in progress in our laboratory.

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