A *Salmonella typhimurium* Strain Defective in Uracil Catabolism and \(\beta\)-Alanine Synthesis

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A selection procedure for uracil catabolism mutant strains involving indicator dye plates was developed. Using this method, a strain defective in uracil catabolism has been isolated in *Salmonella typhimurium* that was temperature-sensitive at 42°C where it required low concentrations of N-carbamoyl-\(\beta\)-alanine, \(\beta\)-alanine or pantothenic acid for growth. An extract of the mutant strain degraded uracil at 37°C at a significantly diminished rate compared to that observed for the wild-type strain under the same growth conditions. The conversion of dihydrouracil to N-carbamoyl-\(\beta\)-alanine was blocked at all temperatures examined in the mutant strain. By means of genetic analysis, the mutant strain was determined to be defective at two genetic loci. Transduction studies with bacteriophage P22 indicated that the panD gene is mutated in this strain, accounting for its \(\beta\)-alanine requirement. Episomal transfers between *Escherichia coli* and the mutant strain provided evidence that the defect in uracil catabolism was located in another region of the *S. typhimurium* chromosome.

**INTRODUCTION**

The biosynthesis of pantothenic acid in *Salmonella typhimurium* requires \(\beta\)-alanine as an intermediate (Fig. 1). Two possible sources of \(\beta\)-alanine exist in *S. typhimurium*; one from aspartic acid and the other from uracil catabolism (Ortega et al., 1975; Cronan, 1980). The pathway for uracil degradation (Fig. 1) consists of three enzymic steps which culminate in the release of \(\beta\)-alanine, carbon dioxide and ammonia (Vogels & van der Drift, 1976). This reductive catabolic pathway of uracil degradation appears to occur quite often in both prokaryotic (Campbell, 1957; Kramer & Kaltwasser, 1969; Ban et al., 1972; Hilton et al., 1975) and eukaryotic organisms (Woodward et al., 1957; Milstein & Bekker, 1976; Wasternack et al., 1979; Kaspari, 1981). The decarboxylation of aspartic acid by the enzyme aspartate-1-decarboxylase (EC 4.1.1.15) provides another source of \(\beta\)-alanine (Williamson & Brown, 1979; Cronan, 1980). It has been shown in *S. typhimurium* that aspartate-1-decarboxylase synthesizes \(\beta\)-alanine at a significant level (Cronan, 1980). The genetic locus that encodes the decarboxylase has been designated panD and has been shown recently to be genetically linked to the panC locus at 5 min on the *S. typhimurium* chromosome (Primerano & Burns, 1983). In an earlier study (Ortega et al., 1975), leaky temperature-sensitive *S. typhimurium* panD mutant strains were isolated that required \(\beta\)-alanine. The panD locus was located in these strains following transductional analysis at 89 min of the *S. typhimurium* linkage map. It has been suggested that this locus may encode a subunit of aspartate-1-decarboxylase (Primerano & Burns, 1983).

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In this study, we report the isolation of a temperature-sensitive *S. typhimurium* strain that is unable to synthesize enough β-alanine at 42 °C to meet its pantothenic acid requirement. The isolation of this strain was accomplished using indicator dye plates which screened for uracil catabolism mutant strains. This mutant has a growth requirement which is satisfied at 42 °C by only N-carbamoyl-β-alanine, β-alanine or pantothenate and has been noted to degrade uracil at a considerably reduced rate. By means of episomal transfers from *Escherichia coli* and bacteriophage P22 transductions, the mutant strain was determined to be defective at the panD locus as well as at a second genetic locus encoding an enzyme involved in uracil catabolism in *S. typhimurium*.

**METHODS**

**Media and organisms.** Minimal medium A (Davis & Mingioli, 1950), Luria broth and AG medium (Kelln et al., 1975) were prepared as previously described. For solid medium, 2% agar was added. Indicator dye plates consisted of 2% agar minimal medium A plates containing 0.02% bromthymol blue, 0.4% sodium acetate, 0.1% ammonium sulphate, 150 μg uracil ml⁻¹ and 2 μg calcium pantothenate ml⁻¹. The *S. typhimurium* and *E. coli* K12 strains utilized in this study are shown in Table 1. Phage P22 int-4 cly-2 (Ely et al., 1974) was used in the generalized transduction studies.

**Isolation of mutant strain.** *S. typhimurium* LT2 cells were mutagenized with ethylmethane sulphonate for 2 h at 37 °C by the method of Miller (1972). The mutagenized cells were washed and resuspended in 5 ml AG medium. Following a 10-fold dilution of the cells into Luria broth, the cells were grown overnight at 37 °C. After this outgrowth period, the bacteria were washed with and resuspended in minimal medium A lacking any carbon source. Onto each indicator dye plate were spread approximately 240 bacterial cells. The dye plates were incubated at 37 °C and those colonies which appeared pale green (see Results) were picked with toothpicks (usually after 3–4 d growth). Such colonies were retested on the indicator dye plates at 37 °C and subsequently were tested for pantothenate auxotrophy. One pantothenate-requiring strain, W016, the focus of this study, was examined for its ability to grow on intermediates of uracil catabolism, β-alanine, aspartic acid or calcium pantothenate.

**Growth determinations.** In determining the growth rate of strain W016, AG medium was used. All bacterial cultures were rotated at 200 r.p.m. in a temperature-controlled shaker and growth was monitored at 540 nm with a Klett-Summerson colorimeter. The ability of strain LT2 and strain W016 to utilize minimal medium A
Table 1. Bacterial strains

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strain</th>
<th>Genotype*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. typhimurium</td>
<td>LT2</td>
<td>Wild-type</td>
<td>Gutnick et al. (1969)</td>
</tr>
<tr>
<td></td>
<td>PanC</td>
<td>panC2</td>
<td>Sanderson &amp; Demerec (1965)</td>
</tr>
<tr>
<td></td>
<td>SA2629</td>
<td>panB4</td>
<td>Sanderson &amp; Demerec (1965)</td>
</tr>
<tr>
<td></td>
<td>HD11</td>
<td>pyrH11</td>
<td>O'Donovan &amp; Gerhart (1972)</td>
</tr>
<tr>
<td></td>
<td>W016</td>
<td>panD urc</td>
<td>This study</td>
</tr>
<tr>
<td>E. coli K12</td>
<td>KL723</td>
<td>thr-1 leuB6 (gpt-proA)62 proA2 hisG4 recA13 argE3 thi-1 ara-14 lacY1 galK2 xyl-7 mitl-1 rpsL31 tss-33 λ- supE44/F’104</td>
<td>Low (1968)</td>
</tr>
<tr>
<td></td>
<td>JE5550</td>
<td>lacY1 galK2 manA4 araD6 gyrA12 recA1 rpsL700 mitl-1 argE3 tss-29 supE44/F’506</td>
<td>Movva et al. (1978)</td>
</tr>
</tbody>
</table>

* Genetic nomenclature is that of Sanderson & Roth (1983) for S. typhimurium and Bachmann (1983) for E. coli K12. The panD allele in strain W016 is temperature-sensitive.

Uracil catabolism in S. typhimurium

Cross-feeding studies. The ability of Pan⁺ auxotrophs and strain W016 to cross-feed each other were tested on AG minimal medium plates. Approximately 10⁸ cells ml⁻¹ of the particular strains being examined were cross-streaked on each plate.

Catabolism of uracil. Strains LT2 and W016 were grown at 42 °C in Luria broth containing 20 mM-β-alanine and harvested in exponential phase. Pellets were resuspended in 50 mM-Tris/HCl, pH 7.4 containing 10 mM-2-mercaptoethanol. 250 mM-sucrose, 0.1 mM-EDTA, 1 mM-phenylmethylsulphonyl fluoride and 1 mg aprotinin ml⁻¹ and sonicated. Conversion of [2-¹⁴C]uracil to [¹⁴C]thymidine was measured at 37°C or 42°C in a reaction mixture (0.4 ml) containing 50 mM-Tris/HCl, pH 7.4, 1 mM-dithiothreitol, 1 mM-NAPDH and 200 μM-[2-¹⁴C]uracil (22.5 Ci mol⁻¹, 832.5 GBq mol⁻¹). Reactions were initiated with sonicates containing 3–4 mg protein and quenched at varying times with 4 M-HClO₄. The [¹⁴C]thymidine generated was measured as previously described (Jones et al., 1978). Catabolism of [6-³H]uracil to its respective intermediates was performed in a total volume of 50 μl containing 50 mM-Tris/HCl, pH 7.4, 1 mM-NAPDH, 200 μM-[6-³H]uracil (2 Ci mol⁻¹, 74 GBq mol⁻¹), and 0.4 mg protein from the cell sonicate. The products of the reaction were separated by thin-layer chromatography on DEAE-cellulose plates as previously described (Traut & Loechel, 1984).

Genetic techniques. Complementation of the mutations in strain W016 was accomplished using episomes (Dunn & Snell, 1979). A drop of various episomal donors of E. coli and a drop of strain W016 were mixed on AG minimal medium plates at 42 °C. The appearance of colonies on the minimal medium indicated complementation of the transductants. Complementation of the uracil catabolism defect using episomes was accomplished using episomes (Dunn & Snell, 1979). Complementation of the mutations in strain W016 was accomplished using episomes (Dunn & Snell, 1979). The resultant transductants were individually scored on AG minimal medium plates and AG plates at 30 °C and 42 °C. The panC⁺ panB transductants were isolated at 37 °C on AG medium plates containing 50 μg pantoate ml⁻¹ while the panD⁺ panB transductants were isolated on the same medium at 42 °C. These transductants were scored on AG minimal medium plates and AG minimal medium plates containing 50 μg pantoate ml⁻¹. The panB⁺ pyrH transductants and panC⁺ pyrH transductants were isolated on AG medium plates at 37 °C while the panD⁺ pyrH transductants were isolated on AG medium plates at 42 °C. The resultant transductants were individually scored on AG medium plates and AG medium plates containing 10 μg 5-fluorouracil ml⁻¹.

Chemicals. Calcium pantothenate, β-alanine, 5-fluorouracil, N-carbamoyl-β-alanine, bromothymol blue, uracil, ethylmethane sulphonate, aspartic acid, dihydrouracil and pantoyl lactone were purchased from Sigma. K¹⁴CNO, [2-¹⁴C]uracil, [6-³H]uracil and [¹H]β-alanine were obtained from ICN, Irvine, Calif. USA. DEAE-cellulose plates and silica gel plates were from Brinkman Instruments (Westbury, NY, USA). Potassium pantoate was prepared by a procedure described elsewhere (Primarano & Burns, 1983).
Table 2. Ability of mutant strain W016 to utilize intermediates of uracil catabolism and pantothenic acid biosynthesis for growth

The medium utilized was AG medium containing uracil, dihydrouracil, pantoate and aspartic acid at 50 μg ml⁻¹; N-carbamoyl-β-alanine at 10 μg ml⁻¹; and β-alanine and calcium pantothenate at 2 μg ml⁻¹. Growth was observed at the temperatures indicated at 24 h: −, no visible growth; +, growth.

<table>
<thead>
<tr>
<th>Additive</th>
<th>42 °C</th>
<th>30 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Uracil</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Dihydrouracil</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>N-Carbamoyl-β-alanine</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>β-Alanine</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pantoate</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Pantothenate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

RESULTS

The basis for mutant isolation on the green indicator dye plates was that if a bacterial colony is unable to catabolize uracil at 37 °C, it will not degrade uracil to β-alanine and CO₂. The resultant HCO₃⁻ produced by wild-type cells will acidify the medium and its colour will change from a green to a yellow-red colour. The colonies of S. typhimurium LT2 (wild-type) appeared red after several days on the dye plates. A mutant strain unable to catabolize uracil will not produce CO₂ and thus does not produce acid, leaving the indicator dye plates unchanged. The acid formed by the bacterial colonies during growth on the indicator dye medium with glucose as a carbon source causes a non-specific yellow colour in the medium surrounding all colonies. By substituting acetate for glucose as the carbon source in the medium, this yellow colour was avoided. Using ethylmethane sulphonate mutagenesis (Miller, 1972) and the indicator dye plates, several colonies unable to catabolize uracil and also requiring pantothenate were isolated from S. typhimurium LT2.

One S. typhimurium mutant strain (designated W016) was examined for its ability to utilize compounds involved in uracil catabolism as well as pantothenic acid biosynthesis to solve its auxotrophy (Table 2). At 42 °C on AG medium plates containing uracil, dihydrouracil, pantoate or aspartic acid, no significant growth was evident after 48 h. Only the supplementing of N-carbamoyl-β-alanine, β-alanine or pantothenate to the plates allowed rapid growth of strain W016 after 48 h at 42°C. This strain required a higher concentration of N-carbamoyl-β-alanine (10 μg ml⁻¹) than β-alanine or pantothenate for growth (Table 2). This may be indicative of cell permeability differences. The effect of shifting the temperature from 30 °C to 42 °C was examined for strain W016 (Fig. 2). It is evident that the growth of strain W016 rapidly ceased unless pantothenate was supplemented at 42 °C. The production of a thermolabile protein at 42 °C was indicated. The growth rates of strain W016 and strain LT2 were compared under a variety of conditions (T. P. West & M. S. Shanley, unpublished results). The mutation in strain W016 did not significantly affect its growth relative to the parent strain (generation time 48 min).

Cross-feeding studies were performed with strain W016 and other pan mutant strains on minimal medium agar plates at 42 °C. It was determined that strain W016 and strain SA2629 were able to cross-feed each other. On the other hand, strain PanC could not be cross-fed by strain W016 or by strain SA2629. Strains W016 and SA2629 were cross-fed by strain PanC. Thus, from cross-feeding studies in E. coli W strains, strain W016 appeared very similar to the panD mutant strains isolated in that micro-organism (Cronan et al., 1982).

To examine whether strain W016 contained a panD mutation, complementation of the β-alanine requirement was attempted using 19 E. coli K12 strains that harbour episomes which contain different sections of the E. coli K12 chromosome. Of those episome-containing strains
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![Graph showing growth rate over time](image)

**Fig. 2.** Effect of a shift in temperature at 185 min from 30 °C to 42 °C on the growth of strain W016 in AG medium with no addition (●) or with 2 μg calcium pantothenate ml⁻¹ (■).

**Table 3. Transductional analysis of the β-alanine biosynthesis mutation in strain W016**

Phage P22 *int*-4 *cly*-2 lysates of donor strains were prepared at 25 °C as stated in Methods. Selected donor marker transductants were obtained by mixing donor strain lysate and recipient cells at a multiplicity of infection of 5–7.

<table>
<thead>
<tr>
<th>Transductional donor</th>
<th>Recipient</th>
<th>Selected marker</th>
<th>Unselected marker</th>
<th>Unselected/selected</th>
<th>Percentage cotransduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>W016</td>
<td>PanC</td>
<td><em>panC</em></td>
<td><em>panD</em></td>
<td>348/351</td>
<td>99</td>
</tr>
<tr>
<td>SA2629</td>
<td>W016</td>
<td><em>panB</em></td>
<td><em>panB</em></td>
<td>344/352</td>
<td>97</td>
</tr>
<tr>
<td>SA2629</td>
<td>PanC</td>
<td><em>panC</em></td>
<td><em>panB</em></td>
<td>325/341</td>
<td>95</td>
</tr>
<tr>
<td>HD11</td>
<td>SA2629</td>
<td><em>panB</em></td>
<td><em>pyrH</em></td>
<td>2/200</td>
<td>1</td>
</tr>
<tr>
<td>HD11</td>
<td>W016</td>
<td><em>panD</em></td>
<td><em>pyrH</em></td>
<td>0/200</td>
<td>0</td>
</tr>
<tr>
<td>HD11</td>
<td>PanC</td>
<td><em>panC</em></td>
<td><em>pyrH</em></td>
<td>0/200</td>
<td>0</td>
</tr>
</tbody>
</table>

Examined, only one episomal transfer was able to complement the β-alanine requirement at 42 °C of strain W016. The F¹04 episome, which is maintained within strain KL723 (Table 1), was able to relieve the requirement after the mating transfer on minimal medium. This episome contains that section of the *E. coli* K12 chromosome between 98 and 6 min on the linkage map which also includes the *pan* genes (Bachmann & Low, 1980).

Having localized the mutation leading to the β-alanine requirement in strain W016 to a particular region of the *E. coli* K12 chromosome containing the *pan* genes, transductional analysis of the mutation to various gene loci in *S. typhimurium* was next investigated. Utilizing bacteriophage P22 *int*-4 *cly*-2 lysates of strain W016, strain SA2629 and strain HD11 as transductional donors, recipient strains were transduced as shown in Table 3. The mutant locus in strain W016 was 99% cotransducible with the *panC* locus at 42 °C. Further, the *panB* locus was cotransduced with the mutant locus in strain W016 at a frequency of 97% and with the *panC* locus at a frequency of 95%. The *pyrH* mutation in strain HD11 was cotransduced 1% with the *panB* locus in strain SA2629 while it was not cotransduced with the *panC* mutation or the mutation in strain W016. From the cotransduction frequencies, it was apparent that the mutation within strain W016 was *panD*, which is located at 5 min on the *S. typhimurium* linkage map, due to its genetic linkage to the *panC* and *panB* loci. Considering the β-alanine requirement and the transductional analysis, the above results agree with the findings of Primerano & Burns (1983). They have recently shown in *S. typhimurium* *panD* strains that the cotransduction frequency between *panD* and *panC* is 99%.

**Transductional Selected Unselected/ Percentage**

**marker**

**selected**

**cotransduction**
Fig. 3. Uracil catabolic products in strain W016 (a) and strain LT2 (b) at 42 °C. Catabolic products of [6-3H]uracil were separated as described in Methods. Dihydrouracil (■) and N-carbamoyl-β-alanine plus β-alanine (●) were quantified following thin-layer chromatographic separation.

Table 4. Growth of strains LT2 and W016 on uracil and products of uracil catabolism as sole nitrogen source at 30 °C

Strains were grown in minimal medium A containing 0.2% nitrogen source and 0.2% glucose as the carbon source from an inoculum of 3000 cells ml⁻¹ on a rotary shaker for 48 h. Viable cells were counted on AG medium agar plates. Results are expressed as 10⁻⁴ × cells ml⁻¹ where each value represents the mean of at least two separate determinations.

<table>
<thead>
<tr>
<th>Nitrogen source</th>
<th>Strain</th>
<th>LT2</th>
<th>W016</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uracil</td>
<td></td>
<td>312</td>
<td>1.45</td>
</tr>
<tr>
<td>Dihydrouracil</td>
<td></td>
<td>190</td>
<td>1.38</td>
</tr>
<tr>
<td>N-Carbamoyl-β-alanine</td>
<td></td>
<td>750</td>
<td>1800</td>
</tr>
<tr>
<td>β-Alanine</td>
<td></td>
<td>2270</td>
<td>2850</td>
</tr>
</tbody>
</table>

The presence of a second mutation in strain W016 was established by biochemical and genetic studies. The ability of strain W016 to degrade [2-14C]uracil was investigated at 37 °C and 42 °C. The subsequent release of 14CO₂ resulting from the catabolism of uracil was monitored in cell extracts. Uracil catabolism is substantially reduced in the mutant strain. At 37 °C, strain W016 [3.2 pmol CO₂ released min⁻¹ (mg protein)⁻¹] degraded only 10% of the amount of [2-14C]uracil determined for strain LT2 [32 pmol CO₂ released min⁻¹ (mg protein)⁻¹] under the same conditions. At 42 °C, strain W016 [0.13 pmol CO₂ released min⁻¹ (mg protein)⁻¹] was noted to catabolize 2% as much [2-14C]uracil as strain LT2 [6.4 pmol CO₂ released min⁻¹ (mg protein)⁻¹]. This suggested a defect in one of the three enzymic steps in the uracil catabolic pathway (Fig. 1). To determine which step was defective, cellular extracts of strains LT2 and W016 were incubated with [6-3H]uracil at 42 °C. Following various incubation periods, dihydouracil as well as N-carbamoyl-β-alanine plus β-alanine were separated using thin-layer chromatography (Traut & Loechel, 1984) and quantified (Fig. 3). In the mutant strain, dihydouracil continued to accumulate while the N-carbamoyl-β-alanine plus β-alanine were formed slowly (Fig. 3a). In the wild-type strain, dihydouracil reached a steady-state concentration because it was efficiently converted to N-carbamoyl-β-alanine plus β-alanine (Fig. 3b). This indicates that the dihydropyrimidinase activity in strain W016 was significantly diminished since its product had become rate-limiting.

Strains LT2 and W016 were grown on minimal medium containing either uracil, dihydouracil, N-carbamoyl-β-alanine or β-alanine as the sole nitrogen source at 30 °C (Table 4).
The results indicate that both strains can metabolize N-carbamoyl-β-alanine and β-alanine as their sole nitrogen source at 30°C. In contrast, strain WO16 did not utilize uracil or dihydrouracil as its sole source of nitrogen, unlike strain LT2 where uracil or dihydrouracil supported significant growth. This provides additional evidence that the conversion of dihydrouracil to N-carbamoyl-β-alanine is blocked in strain WO16. Thus, uracil catabolism in strain WO16 was significantly decreased at all temperatures examined and a defect at the second enzymic step of the uracil catabolic pathway was demonstrated.

To support the enzymic investigation of strain WO16, a genetic analysis involving episomal matings with strain WO16 was done at 37°C on the bromthymol blue indicator plates. As mentioned earlier, strain WO16 appears green on these plates while strain LT2 appears yellow-red. After the episomal matings with strain WO16, the colour of the resultant growth was examined. The episome F'506, which resides in E. coli K12 strain JE5550 (Table 1), after transfer to strain WO16 produced growth with a yellow-red appearance, indicating that the uracil catabolic defect was complemented. This particular episome complements those mutations between 30 and 37 min on the E. coli K12 linkage map (Bachmann & Low, 1980). The complementation by episome F'506, as well as the biochemical data pertaining to the mutant strain, indicate that this mutation is distinct from the β-alanine requirement in strain WO16. The second mutation has been designated genotypically as urc representing a uracil catabolism defect. Strain WO16 containing episome F'506 exhibited no growth at 42°C in AG medium supplemented with either uracil or dihydrouracil at 50 µg ml⁻¹ after 48 h in liquid culture. Therefore, the β-alanine requirement of a panD urc+ mutant cannot be satisfied by the β-alanine produced by uracil catabolism in S. typhimurium.

**DISCUSSION**

While screening for uracil catabolism mutant strains in *S. typhimurium* by a novel indicator dye plate selection procedure, a strain has been isolated that is temperature-sensitive at 42°C for N-carbamoyl-β-alanine, β-alanine or pantothenate. Transduction with bacteriophage P22 of selected strains provided evidence that the β-alanine auxotrophy results from a temperature-sensitive panD mutation in the strain since it is linked to the panC and panB genes at 5 min on the *S. typhimurium* linkage map (Sanderson & Roth, 1983). The cross-feeding experiments also provide evidence that a panD mutation is present. The presence of pantothenate in the dye plate procedure may have inadvertently allowed the isolation of the panD mutation in strain WO16 since panD urc+ transductants appear yellow-red on the indicator dye plates. A second mutation has been detected in uracil catabolism, corresponding to the type of mutation originally selected for, in the mutant strain WO16. This strain has a diminished capacity to degrade uracil or dihydrouracil at those temperatures examined. It appears that the activity of dihydropyrimidinase is greatly reduced due to the inability of strain WO16 to process dihydrouracil. The genetic locus has been designated urc, representing the uracil catabolism defect. With respect to the urc locus, episomal matings with strain WO16 indicate that the uracil catabolism mutation exists independently of the panD mutation responsible for β-alanine biosynthesis. The episome which complements the urc gene carries a section of the *E. coli* K12 chromosome containing the 30 to 37 min region, unlike the episome which complements the β-alanine requirement (Bachmann & Low, 1980). The latter episome contains chromosomal DNA that is found between 98 and 6 min on the linkage map (Bachmann & Low, 1980). The findings of this study indicate that the uracil catabolic pathway is capable of producing some β-alanine. It has been shown that aspartate-1-decarboxylase is necessary for β-alanine synthesis in *S. typhimurium* (Cronan, 1980). In contrast, uracil catabolism is not integral to β-alanine biosynthesis in *S. typhimurium* since the growth of a panD urc+ mutant strain failed to resume in the presence of uracil or dihydrouracil at 42°C.

In summary, WO16 can best be characterized as a double mutant strain of genotype panD urc where the temperature-sensitive β-alanine auxotrophy is separate from the uracil catabolism mutation. In addition, the mutant strain characterized here is not leaky at 42°C, as were previous strains of *S. typhimurium*, which should prove useful in labelling acyl-carrier protein by labelled β-alanine.
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