An Adenine–Thiamin Auxotrophic Mutant of *Bacillus subtilis*

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**INTRODUCTION**

In *Salmonella typhimurium* and *Escherichia coli*, the five biosynthetic steps that lead from 5-phospho-α-D-ribose 1-pyrophosphate to 5'-phosphoribosyl-5-aminoimidazole are common to the pathways that lead to purine nucleotide synthesis and to the synthesis of the pyrimidine moiety of thiamin. Mutations that prevent the synthesis of 5' phosphoribosyl-5-aminoimidazole cause auxotrophy for both adenine and thiamin. Such mutations were first described by Yura (1956), and the explanation for their dual requirement was given by Newell & Tucker (1968). The identification of the genes for the five enzymes can be found in the bibliographies of the most recent editions of the genetic maps for *S. typhimurium* (Sanderson, 1972) and *E. coli* (Bachmann, Low & Taylor, 1976).

In 1974, as part of a classroom exercise, Mr Thomas Patterson isolated an adenine–thiamin (Ath−) auxotroph of *Bacillus subtilis* in my laboratory. After preliminary characterization we named the mutation *ath-I*. A strain carrying the mutation was given the accession number cu869 and placed in our stock collection.

Recently, Walter & Bacher (1977) described mutants defective in thiamin biosynthesis in *B. subtilis*. Although these authors isolated 224 adenine auxotrophs and 53 thiamin auxotrophs, they were unable to find any Ath− mutants. Their failure to isolate Ath− mutants might imply that *B. subtilis* has either a novel biosynthetic pathway, or duplicate pathways for the synthesis of purines and thiamin. The Ath− strain cu869 belies this conclusion. A brief description of strain cu869 seems appropriate.

**METHODS**

*Bacteria.* Bacterial strains were all derived from *Bacillus subtilis* strain 168, and are listed in Table 1. The trpC2, hisA1, purB6, leuA169 and ath-1 mutations caused auxotrophy for tryptophan, histidine, adenine, leucine, and adenine plus thiamin, respectively. The furB mutations permitted bacteria to grow in the presence of 40 μg uracil ml⁻¹ and 40 μg 5-fluorouracil ml⁻¹. The tre-12 mutation prevented growth when 400 μg trehalose ml⁻¹ replaced glucose in minimal agar.

*Culture media and growth conditions.* These have been described previously (Ward & Zahler, 1973). Minimal agar contained 10 μg tryptophan ml⁻¹. When required, 1 μg thiamin.HCl or 20 μg adenine ml⁻¹ was added.

*Materials.* The mutagen 6-chloro-9[(3-(2-chloroethyl)-amino)propyl]amino]-2-methoxyacridine (ICR-191) was a gift from Dr Hugh Creech. Other chemicals were purchased from Sigma.

*Mutagenesis.* A culture of *B. subtilis* strain cu614 (trpC2 leuA169) was grown to stationary phase in Antibiotic Medium No. 3 (Difco), and then diluted 10⁻⁴ into the same medium containing 10 μg ICR-191 ml⁻¹. (The presence of the leucine auxotrophy in this strain was not related to the subject of this communication.) The culture was incubated with aeration for 16 h at 37 °C. Then the cells were washed in buffer (0-1 M-NaCl, 0-05 M-sodium citrate) and appropriate dilutions were made. About 5000 bacteria were spread on each of several plates of minimal agar containing 40 μg leucine ml⁻¹. The plates were examined after 24 h incubation. Micro-colonies, being fed by the small colonies on the plate, were picked with the aid of a dissecting microscope and characterized with respect to new growth requirements. Among approximately 300 new auxotrophic mutants identified this way (and another 300 induced by N-methyl-N'-nitro-N-nitrosoguanidine and screened in the same way), only one was Ath−.

To test for reversions of cu869 to non-requirement for thiamin alone or for adenine alone, about 10⁸ bacteria were spread on minimal agar plates containing either adenine (for thiamin prototrophy) or 0-1 ml
Table 1. *Bacillus subtilis* strains

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Strain genotype</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>br19</td>
<td>trpC2 hisA1</td>
<td>J. Spizizen &amp; B. E. Reilly</td>
</tr>
<tr>
<td>br62</td>
<td>trpC2 purB6</td>
<td>J. Spizizen &amp; B. E. Reilly</td>
</tr>
<tr>
<td>cu120</td>
<td>trpC2</td>
<td>J. Spizizen &amp; B. E. Reilly (formerly strain 168; Spizizen, 1958)</td>
</tr>
<tr>
<td>cu614</td>
<td>trpC2 leuA169</td>
<td>Ward &amp; Zahler (1973)</td>
</tr>
<tr>
<td>cu637</td>
<td>trpC2 furB1</td>
<td>Spontaneous fluorouracil-resistant mutant of cu120; this laboratory</td>
</tr>
<tr>
<td>cu660</td>
<td>trpC2 purB6 furB4</td>
<td>Spontaneous fluorouracil-resistant mutant of cu660 by phage grown in strain Q870 from J.-A. Lepesant</td>
</tr>
<tr>
<td>cu869</td>
<td>trpC2 ath-1</td>
<td>See text</td>
</tr>
<tr>
<td>cu1004</td>
<td>trpC2 tre-12 furB4</td>
<td>Constructed by PBS1 transduction of cu660 by phage grown in strain Q870 from J.-A. Lepesant</td>
</tr>
</tbody>
</table>

Antibiotic Medium No. 3 per plate and thiamin (for purine prototrophy). Then 5 μl of a solution containing 500 μg ICR-191 ml⁻¹ was placed on the surface of each spread plate. On incubation, a ring of revertant colonies appeared around the site where the mutagen had been applied.

**Transduction and transformation.** The genetic methods have been described previously (Ward & Zahler, 1973). A minor variation of the transformation technique of Anagnostopoulos & Spizizen (1961) was used. Recipients (1.0 ml) were grown for 4-5 h in the first-stage medium, and then diluted 10⁻¹ (without washing) into 0.45 ml of the second-stage medium lacking tryptophan. Selection for Ath⁺ was made on minimal agar containing, in some cases, 20 μg adenine ml⁻¹.

**Production of strain cu869.** DNA was extracted from the original ICR-191-induced auxotroph and used at high concentration to transform strain br19 to His⁺. Since excess DNA was used, some of the transformants were simultaneously transformed to His⁺ and to Ath⁻, although hisA1 and ath-1 are not linked genetically. One such congressant, strain cu869, was purified and used in the experiments described below.

**RESULTS AND DISCUSSION**

*Is ath-1 a single mutation?*

The ath-1 mutation present in strain cu869 was originally induced by ICR-191, a frameshift mutagen. As is usually the case with such mutations, reversions were also induced by ICR-191. In addition, rare spontaneous revertants were found at approximately one per 10⁶ cells plated. Of two spontaneous and 20 mutagen-induced mutations permitting growth without thiamin, all permitted growth without adenine as well. Of two spontaneous and 12 mutagen-induced mutations permitting growth without adenine, all permitted growth without thiamin as well. It is concluded that ath-1 is a single mutation rather than two closely linked mutations.

*Genetic mapping of the ath-1 mutation*

Preliminary tests showed that the transduction of strain cu869 with phage PBS1 grown in strain cu1004 resulted in cotransduction of tre-12 with ath⁺ in 20 of 44 Ath⁺ transductants. A PBS1-mediated transduction of strain cu869 by phage grown in strain cu637 gave cotransduction of furB1 with ath⁺ in 8 of 70 Ath⁺ transductants. The tre-12 marker is 29% cotransduced with purB6 (Lepesant-Kejzlarová et al., 1975). The furB1 marker is 10% cotransduced with purB6 (unpublished results). These data suggested that ath-1 is located near purB on the *B. subtilis* chromosome (Young & Wilson, 1975).

Strain cu869 was transduced to ath⁺ by phage PBS1 grown in strain cu660, which carries the purB6 marker. Selection for Ath⁺ was made on minimal agar containing adenine. Of 115 Ath⁺ transductants tested, 112 were Ade⁻ and carried the purB6 allele. Thus purB6 was 97% cotransduced with ath⁺.

To test for linkage between ath-1 and purB6 by transformation, cu869 was transformed with DNA extracted from strain br62, which carries purB6, and with DNA from the
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prototrophic strain cu120. The selective agar contained adenine. Of 122 Ath⁺ transformants tested from the transformations using br62 as donor, 103 were Pur⁻ (84% cotransformation of purB6 with ath⁺). Of 94 Ath⁺ transformants tested from the transformations using cu120 as donor, all were Pur⁺, as expected; the adenine and thiamin requirements were always transferred together.

It is concluded that ath⁻1 is a single mutation, tightly linked to purB6. No nearby markers are available for determining the orientation of ath⁻1 and purB6 on the chromosome.

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REFERENCES


