An Adenine–Thiamin Auxotrophic Mutant of Bacillus subtilis

By STANLEY A. ZAHLER
Division of Biological Sciences, Cornell University, Ithaca, New York 14853, U.S.A.

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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-1. 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 10−6 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>
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<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 cu614; this laboratory</td>
</tr>
<tr>
<td>cu869</td>
<td>trpC2 ath-1</td>
<td>Constructed by PBS1 transduction of cu660 by phage grown in strain qs870 from J.-A. Lepesant</td>
</tr>
<tr>
<td>cu1004</td>
<td>trpC2 tre-12 furB4</td>
<td>See text</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 10⁸ 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


