An Adenine–Thiamin Auxotrophic Mutant of *Bacillus subtilis*

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(Received 22 February 1978)

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 μ g uracil ml^{−1} and 40 μ g 5-fluorouracil ml^{−1}. The *tre-12* mutation prevented growth when 400 μ g 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 μ g tryptophan ml^{−1}. When required, 1 μ g thiamin.HCl or 20 μ g adenine ml^{−1} was added.

Materials. The mutagen 6-chloro-9[(3-[(2-chlorethyl)-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 μ g 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 μ g 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

Strain no.	Strain genotype	Source or reference
BR19	<i>trpC2 hisA1</i>	J. Spizizen & B. E. Reilly
BR62	<i>trpC2 purB6</i>	J. Spizizen & B. E. Reilly
CU120	<i>trpC2</i>	J. Spizizen & B. E. Reilly (formerly strain 168; Spizizen, 1958)
CU614	<i>trpC2 leuA169</i>	Ward & Zahler (1973)
CU637	<i>trpC2 furB1</i>	Spontaneous fluorouracil-resistant mutant of CU120; this laboratory
CU660	<i>trpC2 purB6 furB4</i>	Spontaneous fluorouracil-resistant mutant of BR62; this laboratory
CU869	<i>trpC2 ath-1</i>	See text
CU1004	<i>trpC2 tre-12 furB4</i>	Constructed by PBS1 transduction of CU660 by phage grown in strain Q870 from J.-A. Lepesant

Antibiotic Medium No. 3 per plate and thiamin (for purine prototrophy). Then 5 μ l of a solution containing 500 μ g 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 μ g 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 frame-shift 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 10⁹ 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

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.

I thank Dr Hugh Creech for a gift of ICR-191. This research was supported in part by Grant 76-82221 from the National Science Foundation.

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