Identical amino acid sequence of the *aroA(G)* gene products of *Bacillus subtilis* 168 and *B. subtilis* Marburg strain

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A DNA fragment containing the *aroA(G)* gene of *Bacillus subtilis* 168, encoding 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) synthase-chorismate mutase, was cloned and sequenced. The N-terminus of the protein encoded by *aroA(G)* showed homology with chorismate mutase encoded by *aroH* of *B. subtilis* and with the chorismate mutase parts of proteins encoded by the *pheA* and *tyrA* genes of *Escherichia coli*. The C-terminus of the *aroA(G)* product has sequence similarity with 3-deoxy-D-manno-octulosonate 8-phosphate synthase of *E. coli*. It was shown that the proteins encoded by the *aroA(G)* gene of *B. subtilis* 168 and the *aroA* gene of *B. subtilis* ATCC 6051 Marburg strain are identical, so the observed differences in DAHP synthase activity from these two strains must result from other changes.

Keywords: *Bacillus subtilis*, aromatic amino acids, chorismate mutase, DAHP synthase

The aromatic amino acids phenylalanine, tyrosine and tryptophan are formed by a sequence of reactions starting with synthesis of 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP), which occurs by the enzymically catalysed condensation of D-erythrose 4-phosphate and phosphoenolpyruvate (Henner & Yanofsky, 1993). The synthesis is catalysed by DAHP synthase (EC 4.1.2.15), encoded in *Bacillus subtilis* by the *aroA* gene. This gene has been mapped at 265° on the circular map of the *B. subtilis* chromosome (Anagnostopoulos *et al.*, 1993). DAHP synthase of *B. subtilis* 168 is sensitive to feedback inhibition by chorismate and prephenate and is also responsible for the chorismate mutase activity in this strain (Huang *et al.*, 1974a, b). The chorismate mutase activity in *B. subtilis* 168 is assigned to the gene *aroG* (Hoch & Nester, 1973). The *aroA* and *aroG* genes have been placed in one locus on the chromosome map of *B. subtilis* 168 (Anagnostopoulos *et al.*, 1993). The enzyme encoded by *aroA(G)* has been partially purified and data on its amino acid composition obtained (Huang *et al.*, 1974a). It was shown (Llewellyn *et al.*, 1980) that DAHP synthase of *B. subtilis* 168 was distinct from the monofunctional DAHP synthase of *B.*

subtilis ATCC 6051 Marburg strain. It had an additional chorismate mutase activity, and it was postulated that the bifunctional enzyme arose from the monofunctional enzyme by conversion of an allosteric site for binding prephenate into an active site for chorismate mutase activity (Llewellyn *et al.*, 1980).

In this paper we present our data on the cloning and structural analysis of the *aroA(G)* gene of *B. subtilis* 168 and the *aroA* gene of *B. subtilis* ATCC 6051 Marburg strain.

The *aroA(G)* gene of *B. subtilis* 168 was cloned on a 2 kb DNA fragment by partial digestion of the chromosome with *Sau3A*, using the plasmid pIJ2 (Yomantas *et al.*, 1979) as the vector. The selection was carried out by complementation of the *aroG932* mutation of an *aroG932 leuA8 recE4* strain of *B. subtilis* (Yu. Yomantas, GNIgenetika, Moscow). The cloned fragment also complements the *aroA6* mutation of *B. subtilis*. The level of DAHP synthase and chorismate mutase activities in crude extracts of a *B. subtilis* strain carrying the *aroA(G)* fragment on a multicopy plasmid was about 10 times higher than in the recipient *B. subtilis* strain without the plasmid (data not shown).

The nucleotide sequence of the fragment is shown in Fig. 1. It contains two open reading frames, designated orf324 and orf1102. Both have putative ribosome-binding sites

Abbreviations: DAHP, 3-deoxy-D-arabino-heptulosonate 7-phosphate.

The EMBL accession number for the nucleotide sequence reported in this paper is X65945.

Fig. 1. Nucleotide sequence of the cloned DNA fragment. The sites for *Ava*II, *Eco*RI, *Eco*RV and *Pst*I are indicated. The amino acid sequences of the products of orf324 and orf1102 (*aroA*(G)) are indicated in single-letter code. Paired arrows under the nucleotide sequence denote inverted repeats. The transcriptional start point of *aroA*(G) mRNA (which was determined in primer extension experiments with oligonucleotide 5p3-2) is indicated by an asterisk. The -35 and -10 regions of the promoter and putative ATG start codons of *aroA*(G) are shown by capital letters. The oligonucleotides AG-N and AG-C2 were used for synthesis of the PCR product of the *aroA* gene of *B. subtilis* Marburg strain.

and ρ -independent transcription terminator structures. The start point of orf1102 mRNA was determined by primer extension analysis; -35 and -10 regions upstream of it resemble the consensus structure of σ^A promoters (Fig. 1). The protein encoded by orf1102 has a molecular mass and amino acid composition similar to purified DAHP synthase (Huang *et al.*, 1974a). Disruption of orf1102 by inserting a DNA fragment into the *Pst*I or *Eco*RI site resulted in the loss of the complementation of the *aroA* mutation in *B. subtilis*. These data support the identity of orf1102 as the *aroA*(G) gene.

The amino acid sequence of the *aroA*(G) gene product (DAHP synthase-chorismate mutase) was compared with the PIR/NBRF protein data bank. The N-terminal part of the protein (amino acids 1-60) shows homology with the chorismate mutase parts of bifunctional enzymes encoded by the *pheA* and *tyrA* genes of *E. coli* (Hudson & Davidson, 1984) and monofunctional chorismate mutase encoded by the *aroH* gene of *B. subtilis* ATCC 6051 Marburg strain (Gray *et al.*, 1990). Multiple alignments of these proteins are presented in Fig. 2(a). The C-terminal part of the amino acid sequence of DAHP synthase-

(b)

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aroA (G)    164 P-RTSPYDFQGLGVE-GLQILKRVADFEFLAVISEITVPAHIEEALDYIDVIQIGARNMQ
               . * * . . . * * * * : : : : : : : : : : * : * : * : * : *
kdsA        61 ANRSSIHSYRGPGLGEEMKPIQLKLTPGVKIITDVHEPSQAQPVADVVDVIQLPAFLAR
               . . . . . . . . . . . . . . . . . . . . . . . . . . . . .

aroA (G)    222 NFELLKAAGAVKKPVLLKRGLAATISSEFINAAEYIMSQNDQIILCERGIR-TYETATRN
               . : * : * . . . : * : . . . : * . . . . * : : * : * : * : *
kdsA        121 QTDLVEAMAKTGAVINVKKPQFVSPQGMINVDKFKEGGNEKVILCDRGANFGYDNLVVD
               . . . . . . . . . . . . . . . . . . . . . . . . . . . . .

aroA (G)    281 TLDISVPILKQETHLPVFVDVTH-----STGRNLLLLPTAKAALAIGADGVMA
               . : * : * : : : : : : : : : : : : : : : : : : : : : : : :
kdsA        181 MLGFSIMKKVSGNSPFV-IF-DVTHALQCRDPFGAASGGRRQAVALERAGMVLGLAGLFI
               . . . . . . . . . . . . . . . . . . . . . . . . . . . . .

aroA (G)    340 EVHPDPSPVALSDSAQQMAIPEFEKWLNELKPMVKV
               * : * : * . * . . . . . . . . . . . . . . . . . . . . .
kdsA        239 EAHPDPEHAKDGPSSALPLAKLEPFLKOMKAIIDL
               . . . . . . . . . . . . . . . . . . . . . . . . . . . . .

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Fig. 2. Protein sequence alignments of DAHP synthase-chorismate mutase (*aroA(G)*) encoded by the *aroA(G)* gene of *B. subtilis* with: (a) monofunctional chorismate mutase (*aroH*) encoded by the *aroH* gene of *B. subtilis* ATCC 6051 Marburg strain (Gray et al., 1990), chorismate mutase-prephenate dehydratase (*pheA*), chorismate mutase-prephenate dehydrogenase (*tyrA*) encoded by the *pheA* and *tyrA* genes of *E. coli* (Hudson & Davidson, 1984); and (b) 3-deoxy-D-manno-octulosonate 8-phosphate synthase (*kdsA*) encoded by the *kdsA* gene of *E. coli* (Woisetschlager & Hogenauer, 1987). Similarities in amino acid sequences are marked by asterisks (same amino acid), colons (strong similarity) and dots (family similarity). Alignments were done with the DNA-SUN software (A. Mironov, GNIIoGenetika).

The 1116 base PCR product of the *aroA* gene (amplified with oligonucleotides AG-N and AG-C2; Fig. 1), which encodes monofunctional DAHP synthase in *B. subtilis* Marburg strain, was used for DNA sequencing and was cloned in the *Bam*HI site of plasmid pCB22 (Sorokin & Khazak, 1990) downstream of expression unit EU19035. The recombinant plasmid complemented *aroA* and *aroG* mutations in *B. subtilis*. The complete nucleotide sequence of PCR product was determined; it has only one T $\rightarrow$ C substitution at position 912, which does not change the encoded amino acid. Thus the product encoded by the *aroA* gene of the Marburg strain is the same as that of the *aroA*(G) gene of strain 168. We propose that the different properties described for DAHP synthases isolated from *B. subtilis* 168 and *B. subtilis* Marburg (Llewellyn *et al.*, 1980; Huang *et al.*, 1974a) could be explained by particularities of the multienzyme complex formed in these two strains and mimic effects of high active chorismate mutase encoded by *aroH* in the Marburg strain.

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