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Cloning and Genetic Analysis of the Tryptophan Genes of *Azospirillum lipoferum*

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Abstract

The *trp*-genes of *A. lipoferum* were subcloned from a cosmid gene bank of *A. lipoferum* by complementation of an *E. coli trp*$^-$ mutant which lacks the complete *trp* operon. On a fragment of about 20 kb in size the genes *trp*E, *trp*D, *trp*C and *trp*A were localized by complementation of different *E. coli trp*$^-$ mutants with subcloned DNA fragments. At least three different *trp* gene clusters could be identified. The *trp*E gene was subcloned in pUC18 and sequenced. After hybridization of the *trp*E gene against total genomic DNA two signals were observed. It therefore could be possible that *A. lipoferum* possess two copies of the *trp*E gene.

Keywords: *Azospirillum*, tryptophan genes, *trp*E sequence

Abbreviations: B: *BamH*I, E: *EcoR*I, H: *Hind*III, K: *Kpn*I, P: *Pst*I, S: *Sal*I, Sm: *Sma*I, X: *Xba*I

1. Introduction

The soil bacterium *Azospirillum* is of interest because of its ability to produce phytohormonal substances (Zimmer and Bothe, 1988, Ruckdäschel, 1987). Inoculation experiments indicated that the production of indole-acetic-acid (IAA) stimulates plant growth.

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As a precursor of IAA the amino acid tryptophan is of interest. For *Azospirillum lipoferum* there was a lack of information about the genes involved in the *trp*-biosynthesis and about the organization of these genes. The results of our work provide some information about these aspects.

2. Materials and Methods

All DNA manipulations were done as described previously (Abdel-Salam and Klingmüller, 1987). For DNA sequencing a pUC18 sequencing kit was used (Boehringer Mannheim, Germany). Hybridization was done at high stringency conditions (20×SSC, 66°C) using a non-radioactive Digoxigenin detection kit (Boehringer Mannheim, Germany).

3. Results and Discussion

Cloning of the trp*-genes*

Total genomic DNA of *A. lipoferum* was partially digested with Sau3A and fragments with a size of 20–22 kb were cloned into the *BamH*I-site of the cosmid vector pRK312 (Ruckdäschel, 1987). This gene bank was conjugated into an *E. coli* strain which lacks the complete *trp*-operon (Yanofsky et al., 1981). By using complementation analysis two cosmid-clones, named pK1 and pT, were isolated that allowed the mutant to grow on minimal medium without tryptophan. Both cosmids had a DNA-region of about 20 kb in common and must carry all genes necessary for *trp*-biosynthesis. Till now we subcloned the genes *trp*E, *trp*D, *trp*C and *trp*A of *A. lipoferum* (Fig. 1). *Trp*B and *trp*F could not yet be identified.

Sequencing of trp*E gene*

The *trp*E coding for anthranilate – synthase -α- subunit was sequenced (Fig. 2).

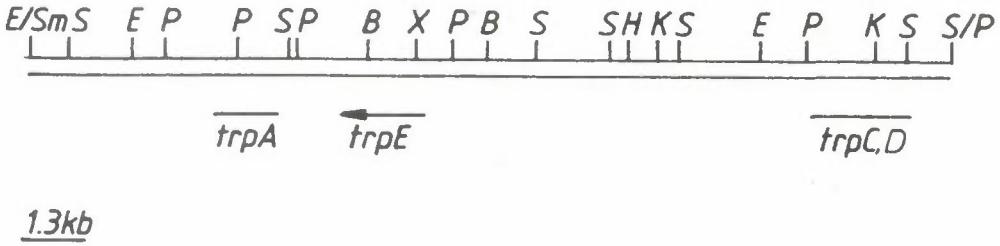

Figure 1. Restriction map of the *trp*-region of *A. lipoferum* and location of different *trp*-genes.

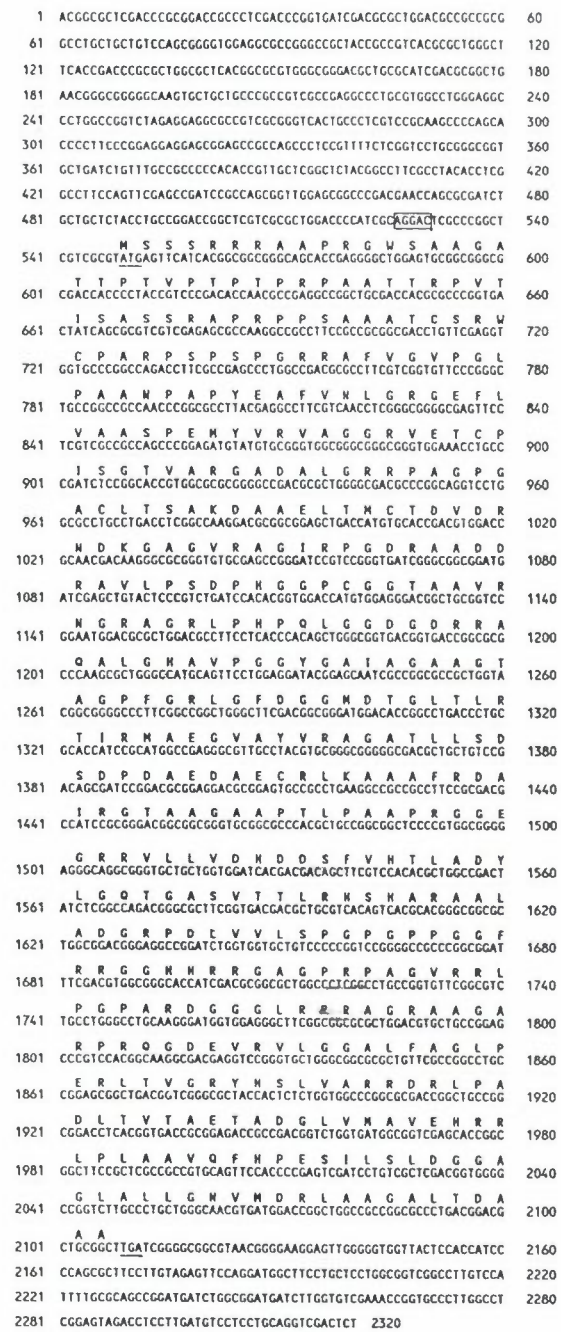

Figure 2. Nucleotide and amino acid sequence of the *trp*E-gene of *A. lipoferum*. Start and stop condons are underlined. Upstream of the start codon, at position 525, a possible ribosomal binding site is boxed (from Ramschütz, 1991).

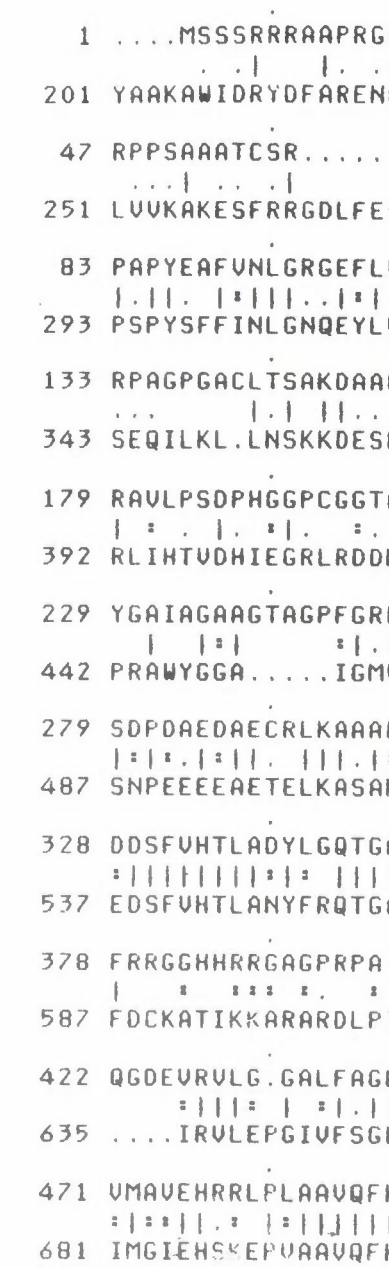

Figure 3. Amino acid sequence (as deduced from the nucleotide sequence) of the *trp*E-protein for *A. lipoferum* (upper line) and *R. meliloti* (lower line). Identities are indicated by vertical bars.

An ORF of 1560 bp encoding a 520 aa-polypeptide as identified. The G/C content was 75%. The *A. lipoferum trp*E gene showed a low amino acid-homology to the *E. coli trp*E gene (43%), and to 13 other *trp*E-genes (*Leptospira biflexa, Pseudomonas syringae, Pseudomonas putida, Corynebacterium glutamicum, Methanobacterium thermoautotrophicum, Spirochaeta aurantia, Thermus thermophilus, Vibrio parahaemolyticus, Bacillus lactofermentum, Lactobacillus biflexa, Acinetobacter calcoaceticus, Salmonella typhimurium* and *Clostridium thermocellum*). However, stronger homologies at the amino acid level to the *trp*E sequence of *Rhizobium meliloti* were found (Fig. 3; *Rhizobium* data from Bae et al., 1989). Neither leader/attenuator regions nor typical promoter regions could be identified in a sequenced region of *Azospirillum* of more than 500 bp upstream of the *trp*E startcodon (data not shown).

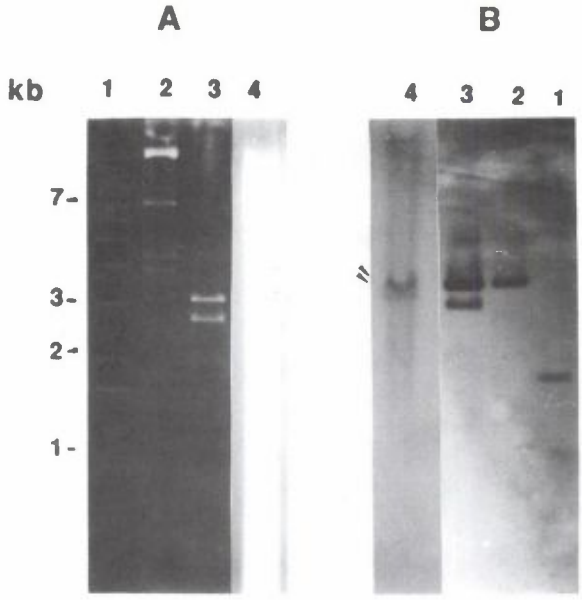

Figure 4. Hybridization with the *BamHI/ XbaI* fragment of pT, carrying an internal part of the *trp*E gene of *A. lipoferum*. Hybridization was done in 20×SSC at 66°C using a non-radioactive Digoxigenin detection kit (Boehringer Mannheim, Germany). Target DNA were 25 μg of the genomic DNA of *A. lipoferum*, cut with *Pst*I. 1: KBL; 2: pT, cut with *Pst*I; 3: pUC18, carrying *trp*E gene of *A. lipoferum* on a *Pst*I fragment; 4: total genomic DNA of *A. lipoferum*, cut with *Pst*I.

Organization of the trp*-genes*

In contrast to *E. coli* (Crawford, 1989) the *trp*-genes of *A. lipoferum* are not organized in a single operon but presumably there are at least three different gene clusters. While the genes *trp*D and *trp*C are localized on one restriction fragment of about 3 kb in size, *trp*E is about 10 kb distant from *trp*D/ *trp*C and *trp*A again is about 2 kb distant from *trp*E (Fig. 1).

Tn5-mutagenesis and hybridization experiments with the trp*E gene*

By using the plasmids pGS9 and pSUP2021 for Tn5-mutagenesis in *A. lipoferum*, it had not been possible to isolate a *trp*$^-$ mutant (Abdel-Salam and Klingmüller, 1987). We therefore addressed the question whether there could exist several copies of the *trp* genes. A *BamHI*/ *Xba*I – internal fragment of the *trp*E of *A. lipoferum* was labeled and used for hybridization against total DNA of *A. lipoferum* digested with *Pst*I.

Two signals were obtained – one with the expected size of 3 kb and one with a size of 2.8 kb (Fig. 4). Further experiments will be done to confirm the existence of two copies of the *trp*E gene in *A. lipoferum*.

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