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Short Sequence-Paper

Cloning and sequence of the *Rhizobium leguminosarum* biovar *phaseoli* *fixA* gene

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We report the identification and cloning of *Rhizobium leguminosarum* biovar *phaseoli* *fixABCX* homologous genes and the complete nucleotide sequence of the *fixA* gene. The corresponding gene product is highly homologous to the *Rhizobium meliloti* and *Azorhizobium caulinodans* FixA proteins. Putative NtrA- and NifA-binding sites are identified in the *fixA* promoter region.

The process of nitrogen fixation requires the activation of a number of *nif* and *fix* genes. *FixA*, *fixB*, *fixC* and *fixX* genes have been identified in several nitrogen-fixing genera including *Rhizobium* [1–4], *Bradyrhizobium* [5], *Azorhizobium* [6], *Azospirillum* [7] and *Azotobacter* [8,9]. *FixABCX* genes are indispensable for symbiotic as well as free-living nitrogen fixation. *FixX* encodes a ferredoxin-like protein [8] while *FixB* is homologous to eukaryotic flavoproteins [10].

Rhizobium leguminosarum biovar *phaseoli* is able to fix nitrogen in symbiosis with common beans. In spite of its agricultural importance as inoculant, the regulation of its nitrogen fixation genes is still poorly understood. This is the first report on the isolation of *fix* genes from the *R. leguminosarum* bv. *phaseoli* genome.

To isolate the *fixABC* homologous genes from *R. leguminosarum* bv. *phaseoli*, a *Sau3A* lambda EMBL3 gene bank of CNPAF512 [11] was hybridized with a 6 kb *Bam*HI-*Sal*I fragment containing the *Azospirillum brasilense* *fixABC* genes [12]. From the positive clones, two *Sal*I fragments of 4.2 and 1.5 kb, respectively, showing homology to the probe, were subcloned into pUC19 [13]. The physical map of the *fixABCX* region was determined (data not shown).

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Abbreviations: aa, amino acid(s); bp, base pair(s); Da, dalton; *fix*, gene(s) encoding Fix; Fix, nitrogen fixation protein(s); kb, kilobase(s) or 1000 bp; *nif*; gene(s) encoding Nif; Nif, nitrogen fixation protein(s); ORF, open reading frame.

To confirm the identity of the isolated fragments, we determined the nucleotide sequence of a fragment of 1045 bp (Fig. 1). Double-stranded DNA was sequenced by the AutoRead Sequencing kit (Pharmacia-LKB). Sequences were run on an automated sequencer (A.L.F., Pharmacia-LKB). Computer assisted sequence analyses were performed by using the software package of PC GENE (IntelliGenetics Inc.). Sequence analysis revealed the presence of a 837-bp ORF encoding a protein of 279 aa with a calculated molecular mass of 30083 Da. This polypeptide displays 72.8% identity with each of the FixA proteins of *Rhizobium meliloti*

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1  CAAATGTCGGGAAGCTTACAAATTCAGTCATATGCTAGCAACGTCGGAAATCGAAGCCATTTT
61  AATGTCATAGAGACGCCAAACGCTTGGCAGAAATGTCAGCAACGTCATGCTAGTAC
121  AAGCCCGCGCCATAGAGAGGAGGAGTAAAGAACCCGCTGACATAGTATCTCTGTAT
      M H I V I C I
181  CAAACAGGTACCGGACTCCGACAAATACGAGTCGATCCGCTGACGAACACAAATCATGCG
      K Q V P D S A Q I R V D P V T M T I M R
241  TCAGGGGGTCCCAACCATCATCAACCCCTATGATCTGTTCCGCTTGAAGAGCGCTCCAG
      Q G V P T I I H P Y D L F A L E S R S S
301  TTGCGCGAGCCATGGCGGAGGTGACCGTCTCACCATGGGGCCGCCATGGCAGAGGA
      C A S E G G E V T V L T M G P P M A E D
361  CGCTTGGCAGGCGCTCACCCTAGCGGACCGCGCTCTTGAACGAGCGCTCATTT
      A L R K A L T V A D R A V L L T S R E F
421  TGGCGCTCCGACACTCTGGGACCTCGTTCTCTTTGCGAGCCATCGCAAGATCGG
      A G S D T L A T S F A L S R A I A K I G
481  AGAGCGCTTCCGTACGCGGATATGCTCTTACGGCCAAACGAGCATCGACGCGATAC
      E A P G T P D I V F T A K Q T I D G D T
541  CGCCAGGTTGGGCTGCTGCGCAAGGCGCTCGACTCTGCGAGCTCAGCTACCTTGC
      A Q V G F G I A K R L D L L Q L T V Y A
601  GAAATCGCTCTGTGACATCAATGGCGCGAGATTACGCTGAGCGCGCTCGGAGG
      K I V S V D I N G R E I T V E R R S E G
661  GGGCAGCAGACGCTGATGAGCAAGCTCCCTTCCTAATACAACTCTCGAAGGCACAAA
      G T Q T L M S K L P C L I T M L E G T N
721  CGAAATCGCGCGGCTCGCTCGATGACCGCTACGGGCGCGCGCAGCAGATCGTGAA
      E I R R G S L D D A L R A A R S Q I V K
781  GTGGATGCGGCGGACGCTGGCATAGAGGACCTCACCAGTGGCGCTCGCTGCTCGCC
      W N A A D A G I E D L T K C G L R G S P
841  AACCGTCTTAAGAGGCTTTTGGCCCTCTGAGCGGCGCGGAAAGCGGAGCAGATCGA
      T V V E R V F A P P E R A E K A E Q I D
901  CACCGCGGAAACACCGCGCATCTGCGGAGGAGTTGATCGCTGGATCTTTTTCG
      T A E K T P R D L A E E L I A G I F L R
961  CAGCGCGCTCGAAGCGAACTCGCTTTGACGCGAATTAAGCGAGGAGCGAGCATGTT
      S G V E S E L A P D A N -
1021  GAGCAGCAATCGAGAGGCTCTCTC

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Fig. 1. Nucleotide sequence of the *R. leguminosarum* bv. *phaseoli* *fixA* gene and 160 bp of the *fixA* promoter region. The predicted amino acid sequence is shown below the DNA sequence. The putative NifA-binding site and the $-24/-12$ promoter in front of *fixA* are underlined.

