A new insertion sequence from Sinorhizobium meliloti with homology to IS1357 from Methylobacterium sp. and IS1452 from Acetobacter pasteurianus

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Abstract

The insertion sequence ISRm8 was identified by sequence analysis of the cryptic plasmid pRmeGR4b of Sinorhizobium meliloti GR4. ISRm8 is 1451 bp in length and carries 22/24-bp terminal imperfect inverted repeats with seven mismatches and a direct target site duplication of 3 bp. ISRm8 carries a unique open reading frame whose putative protein showed significant similarity to the insertion sequences IS1357 and IS1452, isolated from Methylobacterium sp. and Acetobacter pasteurianus, respectively. Two copies of this IS element were found in strain GR4; one of them is linked to plasmid pRmeGR4b, whereas the other is localized out of the non-pSym plasmids. In S. meliloti field populations ISRm8 shows a limited distribution (50% of the strains tested carry the IS element), with a copy number ranging from 1 to 6.

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1. Introduction

The genome of nitrogen-fixation bacteria of the genus Rhizobium is characterized by the presence of repetitive DNA sequences including inserion sequence (IS) elements. These small genetic entities, unlike drug resistance transposons, do not contain selectable genes [1]. In rhizobia, some of the identified IS elements have been isolated indirectly by transposition to symbiotic genes [2,3] but they were also found by sequence analysis [4–6]. In addition, different techniques have been recently developed to trap mobile elements from different rhizobial genomes [7].

Sinorhizobium meliloti is a Gram-negative saprophytic soil bacterium which is capable of forming a symbiotic relationship with alfalfa (Medicago sativa). This symbiotic interaction results in the formation of specialized nitrogen-fixing root nodules. S. meliloti strain GR4 harbors, in addition to the symbiotic megaplasmids or pSymS, two non-pSym plasmids named pRmeGR4a (114 MDa) and pRmeGR4b (140 MDa) [8]. The nfe (nodule formation efficiency) region located on plasmid pRmeGR4b [8–10] is surrounded by IS elements. ISRm3 [9] and ISRm4 [5]
are located to the right of \textit{nfeD} [10], at 128 bp and 2.5 kb, respectively. IS\textit{Rm6} is localized 4.3 kb to the left of the former loci [6]. In the course of sequencing the adjacent DNA region to the \textit{nfe} genes and 11 kb to the right of \textit{nfeD}, we identified a new \textit{S. meliloti} IS element designated IS\textit{Rm8}. This IS element exhibits a high homology to the insertion sequences IS1357 and IS1452, isolated from \textit{Methyllobacterium} sp. and \textit{Acetobacter pasteurianus}, respectively. In this report, we present the entire nucleotide sequence of IS\textit{Rm8}, and analyze the presence and distribution of IS\textit{Rm8} in \textit{S. meliloti} native populations.

2. Materials and methods

2.1. Bacterial strains and plasmids

\textit{S. meliloti} strains and plasmids used in this work are shown in Table 1.

2.2. DNA sequencing and analysis

The \textit{EcoRI} 17 restriction fragment of pRmNT40 [8] cloned in pUC18 was used as original source of DNA template for sequencing reactions and DNA probes. A nested set of deletions from cloned DNA were created by controlled exonuclease III-S1 nuclease digestion (Erase-a-base kit, Promega Biotech.), overlapping deletion clones were selected. Plasmids were propagated in \textit{Escherichia coli} DH5\textalpha{} (Bethesda Research Laboratory). Plasmid DNA for sequencing was isolated and purified with the Magic Minipreps kit (Promega Biotech.). Sequencing was performed in both strands by the chain termination method [13] in an Automatic Laser Fluorescent DNA Sequencer (Applied Biosystems) using universal primers (M13 forward and reverse primers) and synthetic oligonucleotides when needed. DNA sequence edition, translation and analysis were performed with the GeneWorks software package (IntelliGenetics, Inc.) and programs FASTA and BLAST Network Service at the National Center for Biotechnology Information (NCBI) were utilized for homology searching.

2.3. DNA hybridization and IS fingerprinting

Total DNA was digested with \textit{EcoRI} and electrophoretically separated in an 0.8% Tris-borate agarose gel and vacuum blotted onto nylon filters (Hybond-N, Amersham) according to the manufacturer’s instructions. DNA probe was obtained by labelling an internal fragment of IS\textit{Rm8} (403 bp) resulting from the double digestion with \textit{EcoRI}-\textit{SalI} enzymes. Labelling with digoxigenin-11-dUTP, hybridization, washing and immunological detection were performed as specified by the manufacturer’s instructions (Boehringer Mannheim). The filters were washed 2\times{}5 min at room temperature with 50 ml of 2\times{}SSC (NaCl 0.3 M; Na-citrate, 0.03 M; pH 7.0); sodium dodecyl sulfate (SDS), 0.1% (w/v)
and 2×15 min at 50°C with 0.1×SSC; SDS, 0.1% (w/v).

3. Results and discussion

In the course of sequencing the adjacent region of \( nfe \) genes on plasmid pRmeGR4b [8] 7.3 kb to the right of insertion sequence ISRm4 [5], a new IS element was identified (Fig. 1), named hereafter ISRm8. ISRm8 is 1451 bp in length and contains 24/22-bp imperfect inverted repeats (IR) with seven mismatches and a 3-bp target site duplication. Sequence analysis revealed a unique open reading frame...
(ORF) spanning most of the insertion sequence, with a translational ATG start codon (nt 128–130) and terminating with a TGA translational stop codon (nt 1286–1288). This ORF encodes a protein of 389 residues with a calculated molecular mass of 42.8 kDa. The nucleotide sequence described in this work has been deposited in the EMBL, GenBank and DDBJ databases under accession number Y13433.

A computerized search in protein databases showed significant homology between the predicted protein encoded by ISRm8 and the putative transposase of the insertion sequences IS1357 (39% identity) and IS1452 (36% identity) from *Methylobacterium* sp. and *A. pasteurianus* [14], respectively. Moreover, this similarity reached higher levels in some regions of the transposases, mainly in the NH2-terminal regions (Fig. 2). The homology found among ISRm8, IS1357 and IS1452 elements suggests that they belong to a particular group of insertion sequences.

DNA hybridization experiments indicate that *S. meliloti* strain GR4 carries two copies of ISRm8; one of them is the one we sequenced on plasmid pRmeGR4b whereas the other is located somewhere else in the genome but out of the non-pSym plasmids (Fig. 3). Furthermore, ISRm8 was not present in strain Rm2011 (Fig. 3, lane 5). Analysis of the distribution of ISRm8 within *S. meliloti* field isolates (Table 1) revealed a limited distribution (50% of the strains tested carry the IS element) with a copy number ranging from 1 to 6 (Fig. 4).

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