Available online at www.sciencedirect.com
SCIENCE DIRECT®
Plasmid 55 (2006) 87–98
PLASMID
www.elsevier.com/locate/yplas

Replication regions of *Sinorhizobium meliloti* plasmids

Robert J. Watson *, Roselyn Heys

Research Branch, Agriculture and Agri-Food Canada, Ottawa, Ont., Canada KIA OC6

Received 1 March 2005, revised 21 July 2005
Available online 3 October 2005
Communicated by Donald Helinski

Abstract

The replication (*rep*) regions of small plasmids from three *Sinorhizobium meliloti* strains were cloned by marker rescue. Two unique replication regions were identified, one of which was common to two different strains. Plasmid pBB83 carried a 7.2 kbp *rep* region from a 42 kbp plasmid, and pBB84 carried a 4.5 kbp *rep* region from a 36 kbp plasmid. The cloned *rep* regions were of different compatibility types, and were capable of displacing their parent plasmids from *S. meliloti*. Neither could function in a PolA$^-$ strain of *Escherichia coli*. The cloned replication regions were less stable in *S. meliloti* than their parent plasmids. The *rep* genes for each plasmid were localized to less than 2.5 kbp segments. Sequencing data revealed that the pBB83 Rep protein is uncommon, with partial identity to a protein encoded by a plasmid from *S. meliloti* GR4 [Mercado-Blanco, J., Olivares, J., 1994. The large nonsymbiotic plasmid pRmeGR4a of *Rhizobium meliloti* GR4 encodes a protein involved in replication that has homology with the RepC protein of *Agrobacterium* plasmids. Plasmid 32, 75–79]. However, the cloned DNA fragment also contains a truncated segment of the common *repABC* genes, suggesting that the parent plasmid contained two sets of replication genes. Other genes and an IS-element within the insert are most closely related to sequences derived from the Rhizobiaceae family, suggesting that the plasmid has a limited host range. In contrast, the pBB84 *rep* region contained genes similar to those associated with several broad host-range plasmids, and its Rep protein is related to that of a *Pseudomonas aeruginosa* broad host-range plasmid, pVS1 [Heeb, S., Itoh, Y., Nishijyo, T., Schnider, U., Keel, C., Wade, J., Walsh, U., O'Gara, F., Haas, D., 2000. Small, stable shuttle vectors based on the minimal pVS1 replicon for use in gram-negative, plant-associated bacteria. Mol. Plant-Microbe Interact. 13, 232–237]. The pBB84 *rep* region also includes a probable origin of replication, consisting of DNA boxes flanking a series of direct repeats and an AT-rich sequence.

Keywords: *repABC*; IS element; Plasmid compatibility; Plasmid stability; Replication origin

1. Introduction

Sinorhizobium meliloti is an agriculturally important soil bacteria that fixes nitrogen within nodules on the roots of alfalfa. The process is a symbiotic relationship which is beneficial to the plant, since the bacterial endosymbiont converts atmospheric nitrogen to a form and in amounts which can satisfy its growth requirements. In return, the bacterium is able to proliferate within the nutritive environment provided by the nodules.

Genes required for nodulation and nitrogen fixation have been extensively studied in *S. meliloti*.

* Corresponding author. Fax: +1 613 759 1701.
E-mail address: watsonrj@agr.gc.ca (R.J. Watson).

0147-619X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.plasmid.2005.08.003

Many are located on two large plasmids, about 1.4 and 1.7 Mbp in size, known as the *nod* and *exo* megaplasmids, respectively (Finan et al., 1986; Rosenberg et al., 1981). The former, also known as the SYM plasmid, contains numerous nodulation and nitrogen fixation genes. The *exo* megaplasmid contains a number of genes necessary to support an effective symbiosis. These include genes for exopolysaccharide synthesis and dicarboxylate transport. These megaplasmids may more properly be referred to as chromosomes, not only because of their size, but since they include essential genes and are apparently common to all strains. In addition to the megaplasmids, *S. meliloti* typically contains conventional plasmids, varying in number and size among strains. A common characteristic of these plasmids is that they are relatively large, ranging from 100 to 600 kbp (Adachi et al., 1983; Casse et al., 1978). Few of these plasmids have been associated with a specific phenotype, and little is known about their replication functions.

The best-known replication genes found in soil bacteria belong to the *repABC*-replication system (Nishiguchi et al., 1987; Tabata et al., 1989). Characteristically, such plasmids share homology throughout the three replication genes, yet may belong to different incompatibility groups. The *repAB* genes have regulatory roles associated with plasmid partitioning and stability. Analysis of the RepA and RepB protein sequences has indicated that they belong to a large family of pairs of associated proteins whose similarity extends to the SopAB and ParAB proteins from the well-known *Escherichia coli* F plasmid and P1 phage. This family also includes the KorB-IncC proteins encoded by the broad-host range incP-plasmid RK2, which is similar or identical to RP4 (for a review, see Williams and Thomas, 1992). Unlike the *repAB* genes, the *repC* gene is essential for replication, and probably encodes a protein necessary for replication initiation. In many instances, the *repC* gene is associated with upstream and downstream *cis*-acting sequences which may include determinants for incompatibility or an origin of replication.

In the Rhizobeaceae family, the *repABC*-type replicons predominate among plasmids in which *rep* regions have been studied. They have been identified in the octopine-type Ti plasmid pTiB6S3 (Tabata et al., 1989) and the nopaline-type Ti plasmid pTiC58 (Li and Farrand, 2000), both from *Agrobacterium tumefaciens*, and also in the *A. rhizogenes* hairy-root inducing plasmid pRiA4b (Nishiguchi et al., 1987). Symbiotic plasmids in Rhizobia identified as containing *repABC* replication systems include p42d from *Rhizobium etli* CE3 (Soberón et al., 2004), pNGR234a from *Rhizobium* sp. strain NGR234 (Freiberg et al., 1997), pRL1JI from *R.* leguminosarum bv. *viciae* (Danino et al., 2003) and pExo from *S. meliloti* 1021 (Chain et al., 2000). The ubiquity of *repABC* amongst cryptic plasmids in the Rhizobiaceae has been demonstrated by PCR techniques employing degenerate primers diagnostic for *repC* (Palmer et al., 2000). Also, cryptic plasmids belonging to this family of replicons have been cloned and analyzed from *R. etli* CFN42 (Cevallos et al., 2002) and *R. leguminosarum* 3841 (Turner and Young, 1995).

Few other types of plasmid replication systems have been identified in Rhizobia. Barran et al. (2001) identified a minimal replicon from an *S. meliloti* plasmid which replicates by a rolling-circle mechanism. Replication required a DNA segment encoding a putative replicator protein with several possible *cis*-acting sequences. The replication region from another *S. meliloti* plasmid, pRmeGR4a, has been localized by subcloning (Mercado-Blanco and Olivares, 1994). It includes a single ORF which must encode a replication protein. This protein had significant, but partial (<30%), sequence identity with known RepC proteins from Rhizobiaceae plasmids.

In this report, we describe the cloning and characterization of two *rep* regions from *S. meliloti* cryptic plasmids which do not belong to the *repABC* replicon family. Since *repABC* replicons are characteristically large and of low copy-number, we first screened a collection of *S. meliloti* isolates to identify a few strains containing relatively small plasmids (<50 kbp). Cloned *Eco*RI-fragments from these plasmids capable of replicating in *S. meliloti* were obtained by selecting those able to rescue cloning vehicle-associated antibiotic-resistance genes after transformation. A 7.2 kbp *rep* region, originating from a 42 kbp plasmid, encoded a replication protein with about 56% identity to that of *S. meliloti* pRmeGR4a. A second *rep* region, 4.5 kbp in size, originated from a 36 kbp plasmid and was most closely related to a plasmid previously characterized from *P. aeruginosa* (Itoh et al., 1984).

2. Materials and methods

2.1. Strains and plasmids

Bacterial strains and plasmids are listed in Table 1. *S. meliloti* strains screened for plasmid