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The tight-adhesion proteins TadGEF of *Bradyrhizobium diazoefficiens* USDA 110 are involved in cell adhesion and infectivity on soybean roots

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ABSTRACT

Adhesion of symbiotic bacteria to host plants is an essential early step of the infection process that leads to the beneficial interaction. In the *Bradyrhizobium diazoefficiens*-soybean symbiosis few molecular determinants of adhesion are known. Here we identified the tight-adhesion gene products TadGEF in the open-reading frames blr3941–blr3943 of the *B. diazoefficiens* USDA 110 complete genomic sequence. Predicted structure of TadG indicates a transmembrane domain and two extracytosolic domains, from which the C-terminal has an integrin fold. TadE and TadF are also predicted as bearing transmembrane segments. Mutants in *tadG* or the small cluster *tadGEF* were impaired in adhesion to soybean roots, and the root infection was delayed. However, nodule histology was not compromised by the mutations, indicating that these effects were restricted to the earliest contact of the *B. diazoefficiens* and root surfaces. Knowledge of preinfection determinants is important for development of inoculants that are applied to soybean crops worldwide.

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1. Introduction

Bradyrhizobium diazoefficiens (previously, *Bradyrhizobium japonicum*, Delamuta et al., 2013) belongs to the important bacterial class of rhizobia, which fixes atmospheric N_2 in symbiosis with legume plants. This symbiosis is the main contributor to N-nutrient input into the biosphere, constitutes a key step in the N biogeochemical cycle, and is applied in sustainable agriculture worldwide. The symbiosis is achieved after a complex process of infection of legume roots, in which bacterial adhesion to host root surfaces is an essential early step (Oldroyd and Downie, 2008). Adhesion was carefully studied in the *B. diazoefficiens*-soybean symbiosis, where exopolysaccharide, lectins, and pili formed by polymerization of a 21-kDa MW subunit were recognized as determinants (Dardanelli et al., 2003; Ho et al., 1988, 1990a,b, 1994; Lodeiro and Favelukes, 1999; Lodeiro et al., 2000a; Loh et al., 1993; Oehrle et al., 2000; Pérez-Giménez et al., 2009; Pueppke, 1984; Smith and Wollum, 1991; Vesper and Bauer, 1985; Vesper et al., 1987). However, molecular details of this process are unknown, since none of the above-mentioned studies made use of site-directed mutants in adhesins that might be directly involved in the contact of bacteria and root surfaces.

Among bacterial pili, fimbrial low-molecular weight protein (Flp) pili constitute a special class. The structural Flp subunit is around 6–8 kDa in size (Inoue et al., 1998; Kachlany et al., 2000) and is generally encoded among tight adherence (*tad*) loci, which contain other structural and regulatory genes for Flp pilus biosynthesis. The tad genes are widely distributed in bacteria (Imam et al., 2011; Tomich et al., 2007), but evidence on their functions was reported only in *Aggregatibacter actinomycetemcomitans* (Kachlany et al., 2001; Rosan et al., 1988), *Caulobacter crescentus* (Skerker and Shapiro, 2000), *Micrococcus luteus* (Angelov et al., 2015), *Pectobacterium* sp. (Nykyri et al., 2013), and *Pseudomonas aeruginosa* (de Bentzmann et al., 2006). These works confirmed the function of *flp/tad* genes in cell adhesion, biofilm development, host colonization and virulence, and extended their roles to

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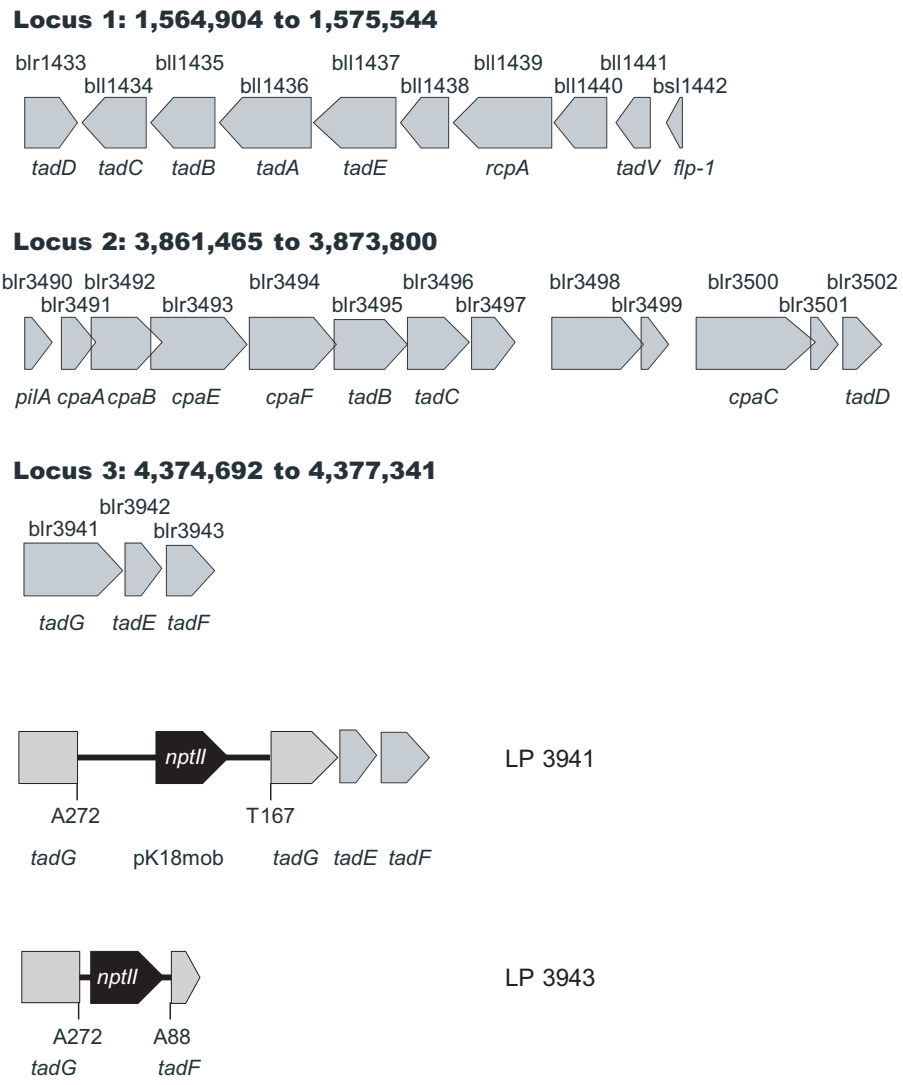

Fig. 1. Organization of *tad* and related genes in *B. diazoefficiens* USDA 110. The genes were identified with BLAST alignments and subsequently found clustered in three separate loci. This organization is in contrast to other species, where these genes are in a single operon. At the bottom the mutants obtained in this work are sketched. In LP 3941 the pK18mob suicide plasmid was inserted into *tadG* coding region between the aminoacid positions indicated (note that the recombination fragment was duplicated after the single crossing-over). In LP 3943 the fragment comprised between the aminoacid positions indicated was replaced by a Km^r cassette.

bacterial transformation. However, although in *Ensifer* (*Sinorhizobium*) *meliloti* the Flp pili are important for competitive nodulation (Zatakia et al., 2014), the tad genes were not studied yet in rhizobia–legume symbiosis. To advance in our knowledge on adhesion of *B. diazoefficiens*, here we studied the role of tadGEF, which are thought to encode components of the apparatus that anchors the Flp pilus to the cell surface (Tomich et al., 2007).

2. Materials and methods

2.1. Bioinformatics

Sequence similarity searches were done using BLAST and PSI-BLAST at NCBI site. Retrieved sequences were aligned using CLUSTALX and a phylogenetic estimation was performed using maximum likelihood calculations with the program PhyML (Guindon et al., 2009) and using the model JTT with gamma rate variation among sites. To validate the topologies obtained, 100 replicants in a non-parametric bootstrapping were used. To further characterize the ORF blr3941, predictions of putative transmembrane domains were performed with TMHMM (http://www.cbs.dtu.dk/services/TMHMM/last access date: 8-26-15), DAS (Cserzo et al., 1997) and TMpred (http://www.ch.embnet.org/software/TMPRED_form.html last access date: 8-25-15). Fold assignment methods as FFAS03 (Jaroszewski et al., 2005), HHPred (Söding, 2005) and Phyre (Kelley et al., 2015) were also applied. Using the putative templates detected, structural models were built using the program MODELLER (Rosan et al., 1988). Structural models were also obtained using *ab initio* methods as I-TASSER (Zhang, 2008).

2.2. Strains and culture conditions

Insertion and deletion mutants of *B. diazoefficiens* USDA 110 were obtained as already described (Quelas et al., 2010) with the strains, plasmids and primers indicated in Table 1. Briefly, to construct the LP 3941 gene disruption mutant, a 317 bp fragment (fragment A) was amplified using primers A-fw and A-rv. This PCR fragment was cloned in pGEM T-Easy (pEJM01) and then the fragment A was transferred to the EcoRI site of pK18mob (pEJM02). Finally, pEJM02 was conjugated from *Escherichia coli* DH5α to *B. japonicum* USDA 110 by triparental mating using the helper plasmid pRK2013, and the candidate mutants were selected in YM agar

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