Journal of Biotechnology 176 (2014) 40–41

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Genome Announcements

Genome sequence of the acid-tolerant strain *Rhizobium* sp. LPU83☆

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ARTICLE INFO

Article history:
Received 17 January 2014
Accepted 10 February 2014
Available online 17 February 2014

Keywords:
Rhizobia
Alfalfa
Broad-host-range
Acid-tolerance

ABSTRACT

Rhizobia are important members of the soil microbiome since they enter into nitrogen-fixing symbiosis with different legume host plants. *Rhizobium* sp. LPU83 is an acid-tolerant *Rhizobium* strain featuring a broad-host-range. However, it is ineffective in nitrogen fixation. Here, the improved draft genome sequence of this strain is reported. Genome sequence information provides the basis for analysis of its acid tolerance, symbiotic properties and taxonomic classification.

Rhizobia are able to fix atmospheric dinitrogen in symbiosis with host plants of the family *Fabaceae*. This symbiosis is of agricultural importance for the input of nitrogen into soil and legume crops. *Rhizobium* strains featuring high efficiency in nitrogen fixation and competitiveness in soil are applied as bioinoculants to enhance plant productivity. However, performance of bioinoculant strains may be limited due to adverse soil conditions such as acidity and/or salinity. Previously, several acid-tolerant alfalfa-nodulating rhizobia were collected and characterized (Del Papa et al., 1999). Strains that are very competitive for nodulation of alfalfa in acidic soils, but inefficient for biological nitrogen fixation were identified within this collection. These isolates feature an extended host range. They are able to nodulate *Phaseolus vulgaris* (Eardly et al., 1985), *Leucaena leucocephala* (Del Papa et al., 1999) and other legumes. These characteristics also apply to a new group of rhizobia, the so-called Oregon-like group, representing a potential risk factor in agricultural soils since they may outcompete efficient symbionts. Based on its 16S rRNA gene sequence, the strain *Rhizobium* sp. LPU83 belongs to this group and is very closely related to *Rhizobium* sp. Or191, originally isolated from acidic soils in Oregon (USA) (Eardly et al., 1985, 1995, 1992; Torres Tejerizo et al., 2011a). Phylogenetic studies supported that Oregon-like rhizobia are genetically connected to both, tropical legume-infecting rhizobia (i.e., the bean-nodulating *Rhizobium etli*) and temperate legume-infecting rhizobia (i.e., *Medicago*-nodulating sinorhizobia) (Laguerre et al., 2001; Torres Tejerizo et al., 2011a). Accordingly, their genomes represent a mosaic structure complicating taxonomic classification. Genetic flexibility of *Rhizobium* sp. LPU83 is facilitated by horizontally mobile plasmids (Torres Tejerizo et al., 2010). Here, the improved draft genome sequence of *Rhizobium* sp. LPU83 is presented to study its genetic features and genome structure.

Genomic DNA of *Rhizobium* sp. LPU83 was isolated (Schneiker-Bekel et al., 2011; Wibberg et al., 2013, 2011) and sequenced on the Genome Sequencer (GS) FLX platform (Roche) and on the MiSeq system (Illumina). A whole-genome-shotgun (WGS) and a paired-end (PE) sequencing run on the GS FLX in combination with an 8-k mate-pair sequencing run on the MiSeq yielded 4,784,417 sequence reads accounting for 875,461,560 bases total sequence information. Thus, a 116-fold coverage was achieved for the approximately 7.5 Mb *Rhizobium* sp. LPU83 genome. After hybrid assembly of all sequence reads by applying the GS *De Novo* Assembler Software (version 2.6), the draft genome consists of ten scaffolds representing five replicons. To facilitate gap closure and assembly validation, a PCR-based strategy was applied using the NEB LongAmp *Taq* DNA Polymerase (New England Biolabs, Ipswich, MA, USA) and primers designed on contig-ends.

☆ *Nucleotide sequence accession number*: The *Rhizobium* sp. LPU83 draft genome sequence was deposited in the EMBL database under the accession numbers HG916852 (chromosome), HG916853 (pLPU83a), CBYB010000001-58 (pLPU83b), HG916854 (pLPU83c) and HG916855 (pLPU83d). The strain is available from Prof. A. Lagares, IBBM (Instituto de Biotecnología y Biología Molecular), CCT-CONICET La Plata, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Calles 47 y 115, 1900 La Plata, Argentina.

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http://dx.doi.org/10.1016/j.jbiotec.2014.02.008
0168-1656/

Table 1
Rhizobium sp. LPU83 genome features.

Feature	Chromosome	pLPU83a	pLPU83b	pLPU83c	pLPU83d
Size (bp)	4,195,305	151,687	ca. 531,535	759,787	1,932,030
GC content (%)	60.19	59.65	57.86	59.18	59.16
Total number of genes	4210	161	582	805	1942
rRNA operons	3	0	0	0	0
tRNAs	51	0	0	0	1
Protein coding genes (cds)	4150	161	582	805	1941
Genes with a predicted function	2823	96	314	468	1187

In addition, primer-walking on gap-spanning fosmids was carried out. Sequences that closed gaps were integrated into the assembly applying the CONSED software package (Gordon et al., 1998). Four of five replicons were closed and finished. Due to a high number of repeated sequences, several gaps remained in the symbiotic plasmid pLPU83b. Sizes (and GC content) of the chromosome and the plasmids pLPU83a, pLPU83b, pLPU83c and pLPU83d are 4,195,305 bp (60.19%), 151,687 bp (59.65%), ca. 531,535 bp (57.86%), 759,787 bp (59.18%), and 1,932,030 bp (59.16%), respectively.

Annotation of the genome was done within the GenDB platform (Meyer et al., 2003). The chromosome of the strain harbors 4150 protein-coding sequences as well as 51 tRNA genes and three *rrn* operons. The coding capacities of the other replicons are summarized in Table 1. All known nodulation and nitrogen fixation genes were discovered on plasmid pLPU83b as described previously (Torres Tejerizo et al., 2010, 2011a,b). The high number of repeated sequences and transposable elements in the symbiotic plasmid (pSym) pLPU83b suggests that this plasmid represents a hot-spot for intra-specific differentiation as already described for other rhizobial pSym plasmids (Schneiker-Bekel et al., 2011; Wibberg et al., 2013). Genome features revealed that plasmid pLPU83d can be considered as a chromid sharing chromosomal and plasmid characteristics, whereas plasmids pLPU83a and pLPU83c represent accessory plasmids. Lipopolysaccharide and polysaccharide synthesis genes are encoded on plasmids pLPU83a and pLPU83d. Analysis and interpretation of the almost finished genome sequence by means of the comparative genomics tool EDGAR (Blom et al., 2009) revealed that the *Rhizobium* sp. LPU83 genome is not very closely related to other known rhizobia, e.g. *Rhizobium leguminosarum* or *R. etli*. Therefore the Oregon-like *Rhizobium* sp. group most probably comprises new species.

Availability of the first nearly complete genome sequence of an Oregon-like *Rhizobium* sp. strain provides the background for future studies addressing the ecological role of this important rhizobial group.

Acknowledgements

The bioinformatics support of the Bioinformatics Resource Facility (BRF) at the Center for Biotechnology (CeBiTec, Bielefeld University) is gratefully acknowledged. A.S. acknowledges the METAEXPLORE grant of the European Commission (KBBE-222625). This work was partially supported by the grant PICT-2012-0518 to M.P.

G.A.T.T., M.F.D.P., A.L. and M.P. are members of the Research Career of CONICET.

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