Journal of Biotechnology 216 (2015) 149–150

Contents lists available at ScienceDirect

Journal of Biotechnology

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

Genome announcement

Complete genome sequence of *Microbacterium* sp. CGR1, bacterium tolerant to wide abiotic conditions isolated from the Atacama Desert

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ARTICLE INFO

Article history:
Received 14 October 2015
Accepted 22 October 2015
Available online 29 October 2015

Keywords:
Microbacterium
Atacama Desert
Abiotic factors tolerance
Riboflavin
Arsenic biosensor

ABSTRACT

Microbacterium sp. CGR1 (RGM2230) is an isolate from the Atacama Desert that displays a wide pH, salinity and temperature tolerance. This strain exhibits riboflavin overproducer features and traits for developing an environmental arsenic biosensor. Here, we report the complete genome sequence of this strain, which represents the first genome of the genus *Microbacterium* sequenced and assembled in a single contig. The genome contains 3,634,864 bp, 3299 protein-coding genes, 45 tRNAs, six copies of 5S-16S-23S rRNA and a high genome average GC-content of 68.04%.

Bacteria isolated from extreme environments show remarkable tolerance to life-threatening conditions, making them important sources of prominent genes useful for biotechnological approaches. In this context, the Atacama Desert is especially attractive, since microorganism communities growing there are exposed to challenging environmental conditions, such as extremely low water availability, nutrient-poor soils, extreme solar radiation, large temperature oscillations, elevated salinity and high levels of arsenic (Crits-Christoph et al., 2013; Smedley and Kinniburgh, 2002). Recently, we recovered 30 isolates with different morphologies and abiotic tolerant features from a pH and salinity gradient transect located at 4480 m.a.s.l in the Atacama Desert, with ranging day/night temperatures from −3.9 °C to 24.4 °C. Among the isolates, a Gram positive, motile and yellow-intense pigmented bacterium, identified as a *Microbacterium* strain by 16S rDNA gene sequencing, had one of the widest pH (5–12) and salinity (0–7%) tolerance ranges, and so it was selected for whole genome sequencing. This isolate was named *Microbacterium* sp. CGR1 (RGM2230).

Genomic DNA from *Microbacterium* sp. CGR1 was purified from exponential growth cultures (1 mL, OD_{600}:0.5) using the DNeasy Blood & Tissue Kit for DNA (Qiagen). Genome sequencing was performed at GCB Genome Sequencing Shared Resource (Duke University) using four single-molecule-real-time (SMRT) cells of the PacBio RSII (Pacific Biosciences, Menlo Park, CA) platform with a 15 kb to 20 kb insert library and XL/C2 chemistry, producing a total of 299,922 reads post-filter with mean read length of 12,794 bp, N50 size of 17,569 bp and a total of 3,837,473,443 bp. *De novo* assembly was conducted using HGAP version 3 (SMRT Analysis version 2.3) with default parameters (Chin et al., 2013), resulting in a complete contig of 3,634,864 bp with coverage of 717*x*, representing the first genome of the genus *Microbacterium* assembled in a single contig.

Microbacterium sp. CGR1 was annotated using the NCBI Prokaryotic Genome Annotation Pipeline released 2013 (Tatusova et al., 2013) and approved on August 11th, 2015. We predicted 51 RNA genes (6 rRNA and 45 tRNA), a total of 3299 protein-coding genes and a high genome average GC-content of 68.04 mol% (see Table 1).

The genome of *Microbacterium* sp. CGR1 revealed the presence of the complete set of genes for riboflavin biosynthesis. Riboflavin (vitamin B2) is a yellow water-soluble vitamin produced by all plants, fungi and many microorganisms, but not by higher animals including humans. Vitamin B2 is known to be the central component of the cofactor's flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), which are necessary to all flavoproteins. For this reason, riboflavin is required for a large variety of cellular processes. Traditional chemical synthesis of riboflavin is now being replaced by low cost and less energy wasting commercially competitive biotechnological processes that use ascomycetes *Ashbya*

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http://dx.doi.org/10.1016/j.jbiotec.2015.10.020
0168-1656/

Table 1
Genome features of *Microbacterium* sp. CGR1 (RGM2230).

Features	Chromosome
Length (bp)	3,634,864
G + C content (%)	68.04
Plasmid	0
CDS	3299
rRNA genes	6
tRNA genes	45

gossypii, filamentous fungi *Candida famata*, or bacterium *Bacillus subtilis* (Stahmann et al., 2000). Compared to those organisms, *Microbacterium* sp. CGR1 is a natural overproducer of riboflavin and has a wider tolerance range to pH, which opens the possibility of producing riboflavin at a higher pH, where ferrous iron – a known repressor of almost all the riboflavin biosynthetic pathway enzymes (Tanner et al., 1945) – is rapidly converted into ferric iron that is almost completely insoluble at pH ≥ 7. Thus, the availability of *Microbacterium* sp. CGR1 genome sequence offers new opportunities for systems metabolic engineering of riboflavin-producing industrial strains.

The genome analysis to *Microbacterium* sp. CGR1 also revealed the existence of genes encoding arsenic (As) resistance proteins. Arsenic is broadly present in nature and in high concentration in the Atacama Desert (Smedley and Kinniburgh, 2002). Much attention has been paid to As dangerous effects on plants and animals including humans. Thus, it is important to monitor As presence in water, soil and foodstuffs used for human consumption. Whole cell (bacterial) biosensors that have been developed for this purpose employ the As-responsive transcriptional repressor ArsR to control expression of reporter genes in response to As(III) or As(V) (Kaur et al., 2015). To date, most of the As biosensors (80%) have been developed in laboratory strains of *Escherichia coli* (Kauer et al., 2015). However, *E. coli* may not be the most convenient host strain for testing environmental samples (Petanen and Romantschuk, 2003), since it lacks of many of the physiological features that are required for survival in soil, sediments or water environments. Therefore, the future development of improved biosensors for detection of As in environmental samples will depend on the ability to engineer indigenous bacteria to detect and provide a measurable response to a range of environmental As concentrations (Chang et al., 2007). In this scenario, seems relevant to further characterize the *ars*R gene found in the genome of *Microbacterium* sp. CGR1 that encodes a protein containing the *arsR*-type HTH domain, the common secondary structure α1-α2-α3-α4-β1-β2-α5 and three cysteine residues that form a 3S-coordinate As(III) binding domain within the C-terminal (Qin et al., 2007). The isolation of this bacterium from a highly concentrated As environment, the great tolerance adaptability of *Microbacterium* sp. CGR1 to different abiotic factors, and the described transformation capability of the *Microbacterium* genus (Zinniel et al., 2008), indicate that this isolate is an excellent candidate to become a sensitive and genetically stable environmental arsenic biosensor.

Nucleotide sequence accession number

The complete chromosome sequence of *Microbacterium* sp. CGR1 (RGM2230) has been deposited in GenBank under the accession number CP012299 (BioProject PRJNA291433). RGM2230 is available from Colección Chilena de Recursos Genéticos Microbianos—INIA (RGM, Chillán, Chile).

Acknowledgements

This work was supported by Fondap Grant 15090007Center for Genome Regulation and Fondecyt1151384 to MG.

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