Genomics 106 (2015) 384–392

Contents lists available at ScienceDirect

Genomics

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

Comparative genome analysis of *Lysinibacillus* B1-CDA, a bacterium that accumulates arsenics

Aminur Rahman [a,c], Noor Nahar [a], Neelu N. Nawani [b], Jana Jass [c], Sibdas Ghosh [d], Björn Olsson [a,1], Abul Mandal [a,*,1]

[a] Systems Biology Research Center, School of Bioscience, University of Skövde, P.O. Box 408, SE-541 28 Skövde, Sweden
[b] Dr. D. Y. Patil Biotechnology and Bioinformatics Institute, Dr. D. Y. Patil Vidyapeeth, Tathawade, Pune, 411033, India
[c] The Life Science Center, School of Science and Technology, Örebro University, SE-701 82 Örebro, Sweden
[d] School of Arts and Science, Iona College, New Rochelle, NY 10801, USA

ARTICLE INFO

Article history:
Received 11 May 2015
Received in revised form 29 July 2015
Accepted 15 September 2015
Available online 24 September 2015

Keywords:
Toxic metals
Bioremediation
Lysinibacillus sphaericus B1-CDA
Genome sequencing
de novo assembly
Gene prediction

ABSTRACT

Previously, we reported an arsenic resistant bacterium *Lysinibacillus sphaericus* B1-CDA, isolated from an arsenic contaminated lands. Here, we have investigated its genetic composition and evolutionary history by using massively parallel sequencing and comparative analysis with other known Lysinibacillus genomes. Assembly of the sequencing reads revealed a genome of ~4.5 Mb in size encompassing ~80% of the chromosomal DNA. We found that the set of ordered contigs contains abundant regions of similarity with other Lysinibacillus genomes and clearly identifiable genome rearrangements. Furthermore, all genes of B1-CDA that were predicted be involved in its resistance to arsenic and/or other heavy metals were annotated. The presence of arsenic responsive genes was verified by PCR *in vitro* conditions. The findings of this study highlight the significance of this bacterium in removing arsenics and other toxic metals from the contaminated sources. The genetic mechanisms of the isolate could be used to cope with arsenic toxicity.

1. Introduction

Worldwide various anthropogenic activities such as mining, chemical industries, use of arsenic-based pesticides, and natural occurrences continue to cause major environmental and health problems by releasing heavy metals into the soil and water alike [1], and exposing millions of people directly or indirectly to toxic metals including arsenic (As). Long-term exposure to As leads to skin diseases, such as hyper- and hypo-pigmentation, hyperkeratosis and melanosis, as well as gangrene, skin cancer, lung cancer and bladder cancer [2]. Poisoning occurs from drinking of contaminated water and/or consuming crops cultivated by irrigation with As-contaminated groundwater [3–5]. Studies by the Food and Agricultural Organization of the United Nations (FAO) indicate that arsenic is accumulated in different parts of the cultivated crops, such as the grain and straw of rice, a major staple food [6]. It is therefore important that we develop efficient, yet affordable, technologies to clean arsenic from soil and water.

Remediation of toxic metal using microbes has been shown to be more proficient than physical and chemical methods [7]. In fact, bacteria have developed several metabolic processes and strategies to transform As, including respiratory arsenate reduction, cytoplasmic arsenate reduction and arsenite methylation [8]. Furthermore, certain bacteria have evolved the necessary genetic components that confer resistance mechanisms, allowing them to survive and grow in environments containing levels of As that would be toxic to most other organisms. The high-level resistance to As in bacteria is conferred by the arsenical resistance (ars) operon comprising either three (*arsRBC*) [9] or five (*arsRDABC*) genes arranged in a single transcriptional unit located on plasmids [10] or chromosomes [11]. ArsB, an integral membrane protein that pumps arsenite out of the cell, is often associated with an ATPase subunit, arsA [12]. The *arsC* gene encodes the enzyme for arsenate reductase, which is responsible for the biotransformation of arsenate [As(V)] to arsenite [As(III)] prior to efflux. ArsR is a trans-acting repressor involved in the basal regulation of the ars operon, while arsD is a second repressor controlling the upper levels of expression of ars genes [13]. Several researchers have studied As transformation mechanisms using genetic markers such as *arsB* and *arsC* genes in the ars operon for arsenic resistance [12,14], the arrA gene for dissimilatory As(V) respiration (DAsR) [15–17], and the aoxB gene for As(III) oxidation [18,19]. Moreover, some studies detected that in spite of clear evidence of the As-transforming activity by microorganisms, no amplicon for arsenite oxidase (aoxB) or As(V) respiratory reductase (arrA) was attained using the reported polymerase chain reaction (PCR) primers and protocols [17,20,21]. Here we report a bacterial strain, *Lysinibacillus*

* Corresponding author.
E-mail address: abul.mandal@his.se (A. Mandal).
[1] Authors have the equal contribution.

http://dx.doi.org/10.1016/j.ygeno.2015.09.006
0888-7543/

Table 1
Summary of the genome with nucleotide content and gene count levels.

Attribute	Value	% of total
Genome size (bp)	4,509,276	100.00
DNA GC content (bp)	1,690,719	37.49
DNA coding region (bp)	3,911,574	86.75
Number of replicons	1	
Extrachromosomal	0	
Total genes	4601	100.00
rRNA genes	11	0.24
tRNA genes	77	1.67
Protein coding genes	4513	98.09
Genes assigned to RAST functional categories	2671	58.05
Genes assigned Gene Ontology terms by Blast2GO	3050	66.29

sphaericus B1-CDA as potential candidate for heavy metal bioremediation. This bacterial strain was isolated from cultivated land in the Chuadanga district of Bangladesh, where soil, sediment, and ground water have been contaminated with arsenics for many years.

In this study, we provide *in vitro* findings of potential arsenic responsive genes in B1-CDA and summarize a set of phenotype features for *L. sphaericus* B1-CDA, together with a description and annotation of its genome sequence. To improve our understanding of genes involved in metal binding activity and reduction of metal by the B1-CDA strain, we performed massively parallel genome sequencing to investigate the metal responsive genes, predicted by RAST and/or Blast2GO. Employing comparative analyses with other available *Lysinibacillus* genome sequences, we investigated genetic composition and evolutionary history of strain B1-CDA and characterized the genetic differences among the various lineages to understand the evolutionary processes involved in shaping the genomes of these bacteria.

2. Methods

2.1. Strain isolation

The soil samples were collected from cultivated land in the Chuadanga district of Bangladesh, a highly arsenic-contaminated region located in the south-west region of this country. The soil was collected from the surface at 0–15 cm in depth, retained in plastic bags and kept at 4 °C until further analysis. Isolation of bacteria from the collected soil, the characterization of the soil samples and the content of metal ions has been described previously [22]. Previously, we have reported that the strain *L. sphaericus* B1-CDA is highly resistant to arsenic and it accumulates arsenic inside the cells [22].

2.2. Genomic DNA extraction and electrophoresis

Genomic DNA was extracted from the isolate, B1-CDA using Master pure™ Gram positive DNA purification kit (Epicenter, USA) with a minor modification. Bacteria were cultured in Luria Bertani (LB) medium and pellets were collected from 1.0 ml of bacterial cultures by

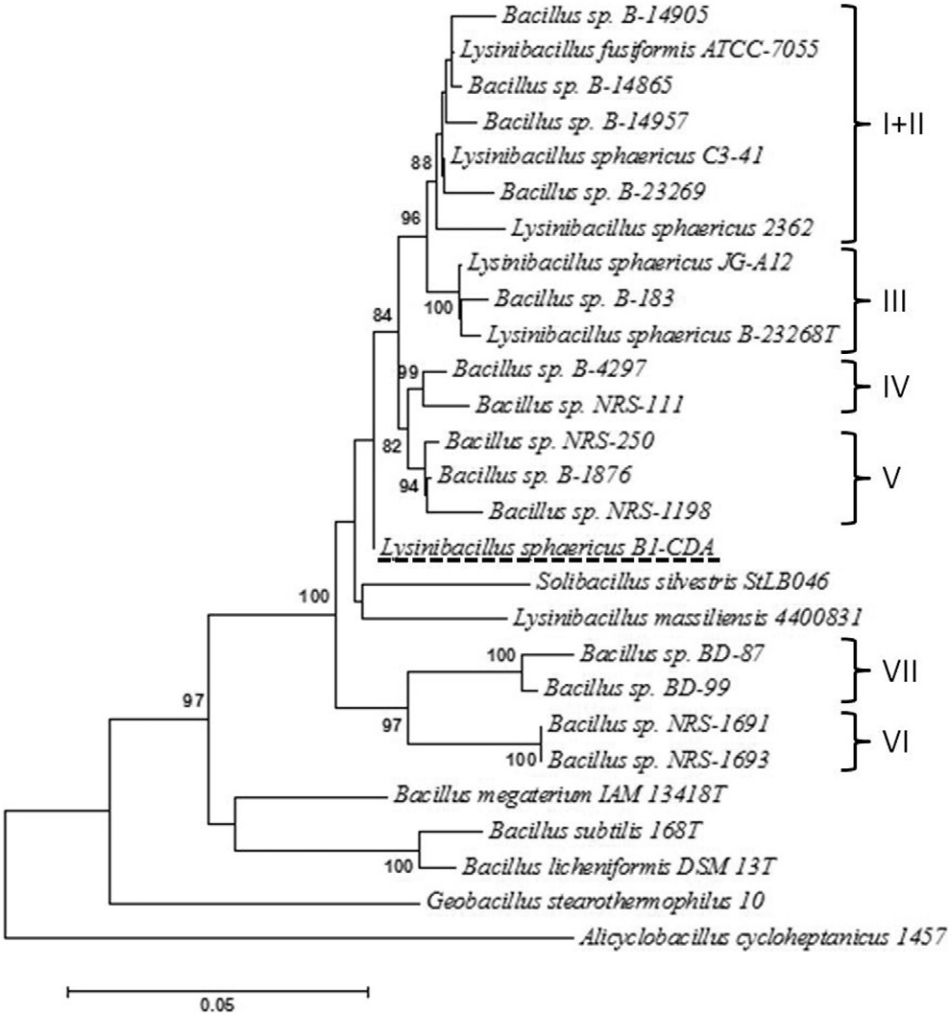

Fig. 1. Phylogenetic tree depicting the position of *Lysinibacillus sphaericus* B1-CDA relative to the available type strains and other non-assigned species within the family *Bacillae*. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches.