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Research article

Revealing the biotechnological potential of *Delftia* sp. JD2 by a genomic approach

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Abstract: *Delftia* sp. JD2 is a chromium-resistant bacterium that reduces Cr(VI) to Cr(III), accumulates Pb(II), produces the phytohormone indole-3-acetic acid and siderophores, and increases the plant growth performance of rhizobia in co-inoculation experiments. We aimed to analyze the biotechnological potential of JD2 using a genomic approach. JD2 has a genome of 6.76Mb, with 6,051 predicted protein coding sequences and 93 RNA genes (tRNA and rRNA). The indole-acetamide pathway was identified as responsible for the synthesis of indole-3-acetic acid. The genetic information involved in chromium resistance (the gene cluster, *chrBACF*,) was found. At least 40 putative genes encoding for TonB-dependent receptors, probably involved in the utilization of siderophores and biopolymers, and genes for the synthesis, maturation, exportation and uptake of pyoverdine, and acquisition of Fe-pyochelin and Fe-enterobactin were also identified. The information also suggests that JD2 produce polyhydroxybutyrate, a carbon reserve polymer commonly used for manufacturing petrochemical free bioplastics. In addition, JD2 may degrade lignin-derived aromatic compounds to 2-pyrone-4,6-dicarboxylate, a molecule used in the bio-based

polymer industry. Finally, a comparative genomic analysis of JD2, *Delftia* sp. Cs1-4 and *Delftia acidovorans* SPH-1 is also discussed. The present work provides insights into the physiology and genetics of a microorganism with many potential uses in biotechnology.

Keywords: *Delftia*; plant-growth promotion; bioremediation; comparative genomic; plant-microbe interaction

1. Introduction

Delftia species are metabolically diverse β-proteobacteria commonly found in sea water, soil, plants and activated sludge. Most *Delftia* are able to degrade or transform a wide variety of organic and inorganic compounds [1–9]. In addition, some strains have been reported as plant growth promoting bacteria (PGPB) [4,10–12]. These reports give insights into the biotechnological potential of *Delftia* strains. The only concern is that a few isolates have also been found in clinical samples, suggesting that some *Delftia* strains could be opportunistic agents [13,14].

Delftia sp. strain JD2 is a heavy-metal (HM) resistant bacterium isolated from a HM-contaminated Uruguayan soil [4]. JD2 bio-accumulates Pb(II) and reduces Cr(VI) to Cr(III) in liquid medium [4,12]. Thus, this bacterium might be useful for cleaning up HM-contaminated environments (bioremediation).

In addition, JD2 is a PGPB that produces plant growth promoting molecules such as siderophores (that capture and solubilize iron) and indole-3-acetic acid (IAA; a phytohormone that increases the plant root surface area available for the absorption of nutrients). JD2 also functions as a "helper" bacterium that improves the performance of rhizobial inoculant strains during the co-inoculation of alfalfa and clover, in gnotobiotic and greenhouse conditions, enhancing legume nodulation and growth [4,11,12]. Interestingly, during the rhizobia-*Delftia*-alfalfa interaction (co-inoculation of plants), JD2 induces the plant production of luteolin (involved in the nodulation phenotype of rhizobia) and IAA, which might explains the significant increase of the root system during the co-inoculation [11]. The rhizobia-*Delftia* co-inoculation of legumes produces a huge increase in plant yield, compared to the rhizobial single inoculation, pointing the economically relevance of co-inoculation practices in countries with high production of legumes [11,15].

The aim of this work was to inform about the draft genome of *Delftia* sp. strain JD2 and to discuss some features of selected gene functions, with focus in the biotechnological potential of this microbe. We also describe aspects of the genome organization and gene composition, comparing the JD2 and two *Delftia* available genomes (comparative genomic), looking for JD2-specific genes that may give insigth into new biotechnological uses of this bacterium. This study contributes to our understanding of the physiology and evolution of *Delftia* strains. This information may be useful for the improvement of some agronomic, industrial and bioremediation traits of *Delftia* sp. strain JD2.

2. Materials and Methods

2.1. Sequencing, annotation and functional categorization

Delftia sp. strain JD2 was aerobically growth (200 rpm) in TY medium at 30 ℃ as described by [16], and the total DNA was prepared according to the method of [17]. A highly pure genomic DNA sample was sequenced at Macrogen, Inc., South Korea, using a Hiseq2000 Illumina system, by shotgun library sequencing. Graphical assessment of the quality of reads data was conducted using the software program FASTQC (www.bioinformatics.bbsrc.ac.uk/projects/fastqc/). Assembly of raw data was done using the SPAdes assembly software [18]. Contigs were merged and oriented using ABACAS (http://abacas.sourceforge.net; [19], as guided by two openly available *Delftia* genome sequences: *D. acidovorans* SPH-1 ([20], NCBI reference genomic sequence NC_010002.1) and *Delftia* sp. Cs1-4 ([6], NCBI reference genomic sequence NC_015563.1), as scaffolds. The JD2 draft genome was annotated using both, the RAST server [21], and the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP; http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html). The predicted genes were functionally categorized using SEED subsystems [22] at the RAST server (http://rast.nmpdr.org). Proteins conserved functional domains were identified using the NCBI Conserved Domain Search Service (CD Search; [23]).

2.2. Organism and sequence data

In addition to JD2, 31 genomes of members of the family *Comamonadaceae* (distributed in 11 genera; Table S1, supplementary resource) were downloaded from the ftp site of NCBI (ftp.ncbi.nih.gov) and used when indicated.

2.3. Identification of homologous clusters and putative orthologs

Homologous clusters, as well as putative conserved orthologous sequences among the 32 genomes were identified using the OrthoMCL method [24], by means of the get_homologous software [25]. Blastp searches were done with a minimal identity value cutoff of 30% and minimal query coverage of 75%.

2.4. Phylogenetic analyses

Phylogenetic trees of the family *Comamonadaceae* were constructed using the 513 orthologous gene groups identified using OrthoMCL. Protein sequences were aligned using Clustal Omega v1.2.0[26], and phylogenetic trees were inferred using the maximum-likelihood method with an amino acid LG+G model by means of Phyml version 3.1 with three random starting trees [27,28]. The default SH-like test was used to evaluate branch supports in each analysis [28]. Finally, a consensus tree was inferred from the phylograms, using the sumtrees.py program [29]. The node support shown in the consensus tree represents the relative number of phylogenies in which certain node appears.

The 16S ribosomal rRNA genes was also used as input for phylogenetic analysis. The full gene sequence was compared automatically using the BLAST against the sequences of bacteria available in NCBI database (http://www.ncbi.nlm.nih.gov/, last accessed December, 2014). The phylogenetic analysis was constructed using the neighbor-joining algorithm (NJ) with bootstrap analysis for 2000 replicates, in the MEGA 6.0 software [30].

2.5. Comparative genomics

Similarities among *Delftia* genomes were analyzed by means of the average nucleotide identity (ANI). Assembled contigs longer than 200 bp were used to estimate ANI values between *Delftia* sp. JD2 and *Delftia* sp. Cs1-4 (6.68 Mb) or *D. acidovorans* SPH-1 (6.76 Mb). This index is used to delineate species using genome sequence data [31]. Genomes displaying an ANI value of 95% or higher are considered to belong to the same species. Two-way ANI (reciprocal best hits based comparison) was estimated by means of the ani.rb script developed by Luis M. Rodriguez-R and available at enveomics.blogspot.com.

The comparative genomes analysis was performed for JD2, SPH-1 and Cs1-4 using the homologous gene clusters identified using the get_homologs software [25]. Some unique genes, only found in JD2 (JD2-specific genes), were further characterized using Blast against the NCBI database (http://www.ncbi.nlm.nih.gov/, last accessed December, 2014). Blastp searches were done with a minimal identity value cutoff of 30% and minimal query coverage of 75%. The phylogenetic analyses between *Delftia* strains and other β-proteobacteria were constructed using the NJ algorithm and the maximum-likelihood (ML) method, with bootstrap analysis for 2000 replicates, in the MEGA 6.0 software [30].

The draft genome sequence has been deposited in the GenBank database under the accession number LFJT00000000 (the first version, described in this work).

3. Results

3.1. General features of Delftia sp. JD2 draft genome

A total of 15,287,096 reads, covering 1,543,996,696 bases (101 bp average read lengths), were generated. After *de novo* assembly, 219 contigs with an average length of 30,894 bp (274,616 bp the longest and 77 bp the shortest) were obtained. The results suggest that JD2 might have a single circular chromosome (66.4% GC content) with a total sequence length of 6.76 Mb.

For genome annotation and gene prediction, results from the RAST and the NCBI PGAAP servers were compared, showing similar results. The genome was predicted to have 6,051 protein coding sequences (CDS) and 87 tRNA. Two copies of the 16S, 5S and 23S rRNA genes were found. Among the predicted protein coding genes, 25.7% were considered as hypothetical and 2,647 CDS were classified into 500 subsystems (Figure 1).

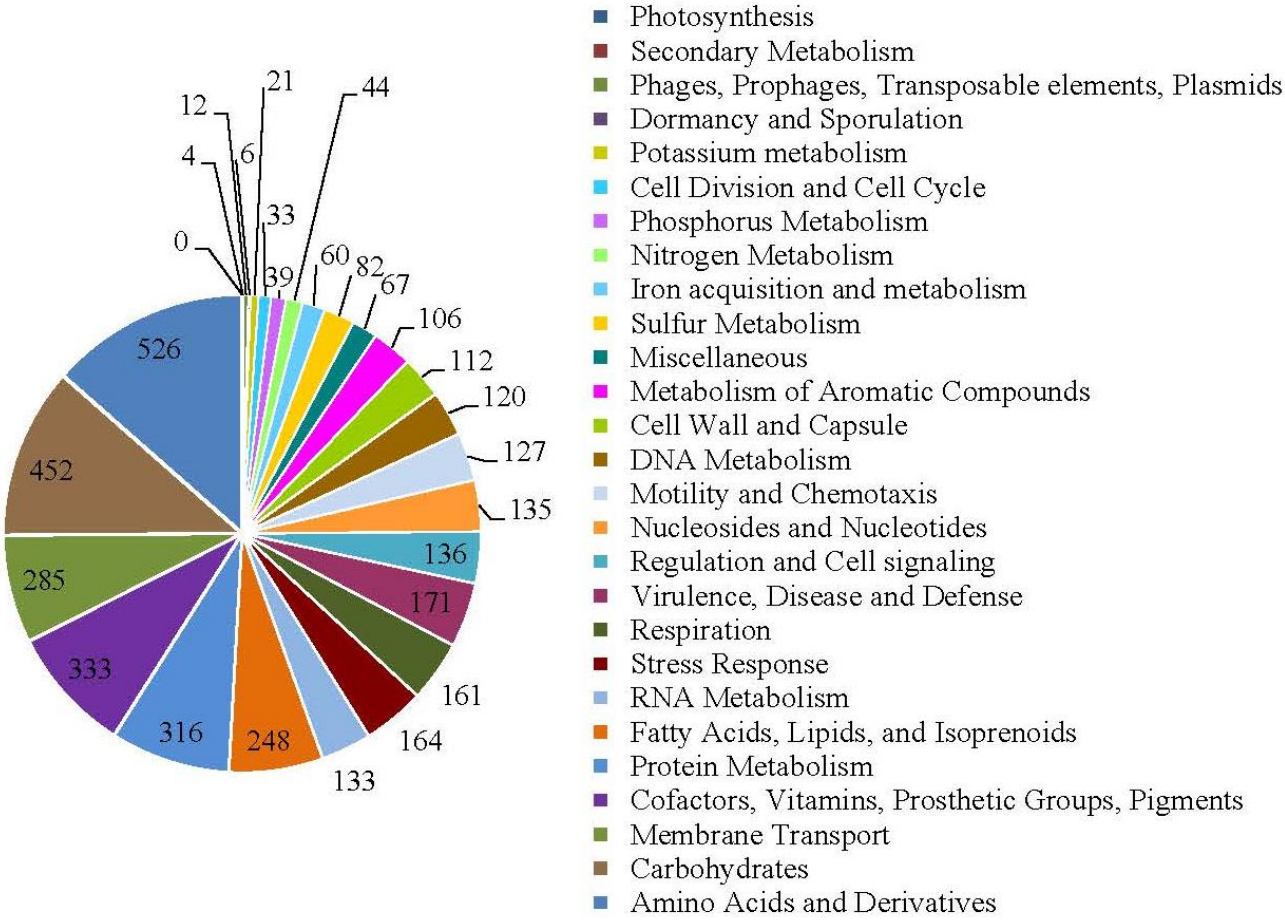

Figure 1. Subsystem categorization for the genome of *Delftia* sp. JD2 as annotated by RAST. The pie chart represents the relative abundance of each subsystem category, and numbers depict subsystem feature counts.

3.2. Taxonomic features of Delftia sp. JD2

513 putative orthologous genes were identified among the 32 analyzed genomes of the family *Comamonadaceae*, and used as the input for maximum-likelihood phylogenetic analysis. Phylogenetic relationships were also inferred using the 16S rRNA sequence. All the constructed trees presented similar topologies (Figure 2).

The information showed that JD2, Cs1-4 and SPH-1 clustered as a sister group to the genus *Comamonas*, showing a node value of 85%. Interestingly, strains of the genus *Acidovorax* clustered with *Verminephrobacter*, *Delftia*, *Comamonas* or *Alicycliphilus* strains, as a polyphyletic group.

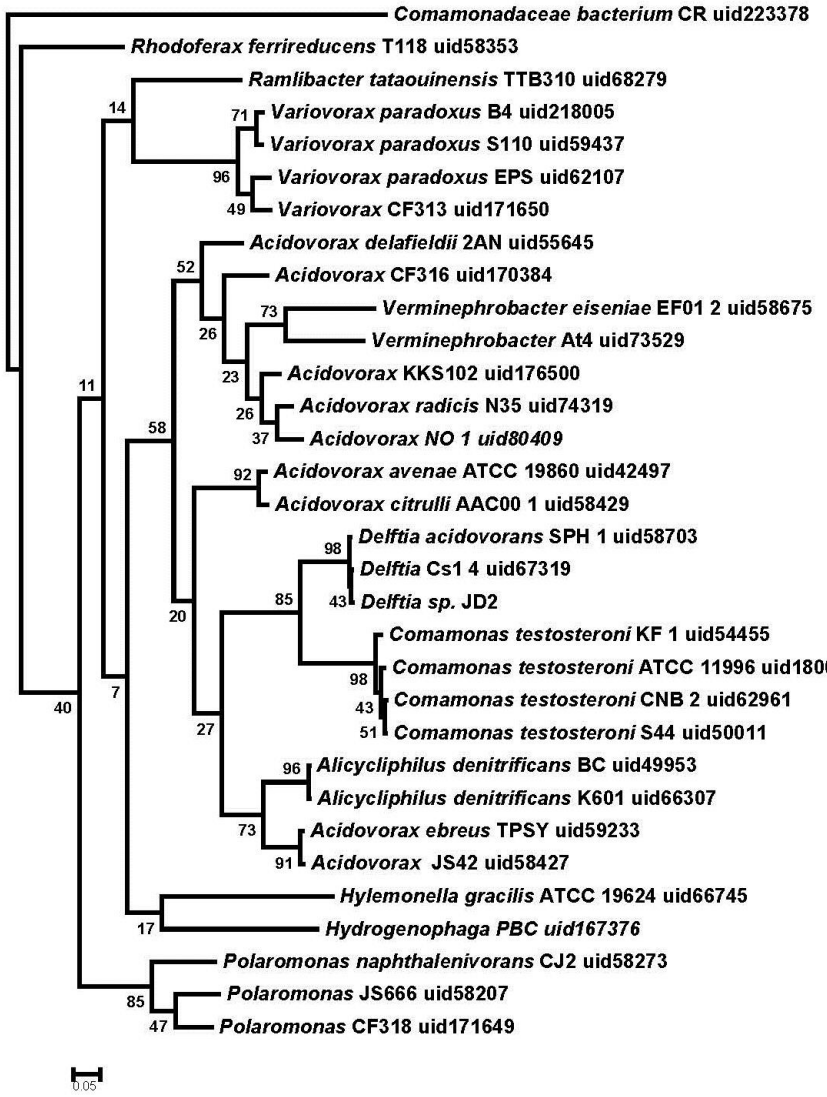

Figure 2. Phylogenetic relationships among members of the family *Comamonadaceae*. The figure represents the consensus tree inferred from 513 phylograms constructed with the orthologs genes identified. In this way, note that the node-values represent the percentage of independent phylogenetic analysis, that support each node.

3.3. Comparative genomics between JD2, SPH-1 and Cs1-4 genomes

In order to get information about the evolutionary processes acting on JD2 genome, a comparative genomic study was performed among JD2, SPH-1 and Cs1-4 genomes.The estimated ANI values among *Delftia* genomes were above 98% (98.65% and 98.07% identity between JD2 and Cs1-4 and SPH-1, respectively). The pan-genome analysis showed that the full complement of genes could be classified into 7,625 families (Figure 3). The 6,051 predicted CDS for JD2 clustered into 5,987 homologous families (5,836 and 5,764 families for SPH-1 and Cs1-4, respectively). As shown in the Venn diagram, the three *Defltia* strains share a core genome of 4,638 families (approximately 76–79% of the pan-genome; Figure 3). It was also shown that approximately 14.5% of JD2 CDS are

singletons or JD2-specific genes (875 CDSs with non-homologous genes in neither SPH-1 nor Cs1-4 genome; Figure 3).

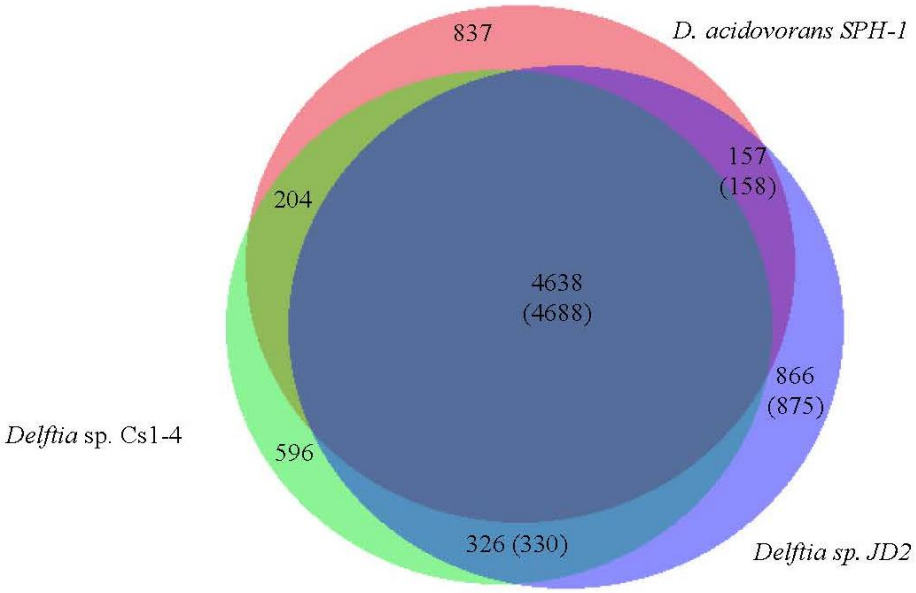

Figure 3. Venn diagram of homologous genes in three *Delftia* spp. strains. The Venn diagram shows the pan-genome of JD2, SPH-1 and JD2 generated using BioVenn [32]. The numbers in circles indicate the numbers of homologous protein families. Overlapped regions represent common homologous families shared between genomes. The number outside the overlapped regions indicates the homologous families exclusively found in each genome. In addition, for JD2, the number in parenthesis indicates the number of CDS in each region.

3.4. Exploring the strain-specific CDS in the JD2 genome

3.4.1. Resistance to heavy metals

Many predicted JD2-specific genes were associated with the HM-resistance phenotype reported for JD2 [4,12]. The RAST and PGAAP annotations also detected 171 genes involved in virulence, disease and defense, including 137 genes coding for resistance to antibiotics and toxic compounds. In terms of HM resistance, 6, 27 and 56 genes involved in chromium resistance, copper homeostasis, and cobalt, cadmium and zinc resistances, respectively, were predicted.

A cluster of chromium resistance genes (*chr*), with a syntenic organization (*chrBACF*), was detected in JD2. We also found two extra *chrA* genes dispersed throughout the genome. This chromium efflux system was also found in the *Delftia* sp. strain 670 genome (GenBank loci accessions: KEH10411.1, KEH10412.1, KEH10410.1 and KEH10409.1) and in *D. acidovorans* strain CM13 (NCBI Ref. Seq.: WP_046238144.1, WP_046238145.1 and WP_046238146.1). Interestigly, the *chr* cluster or the dispersed genes were not detected in the SPH-1 and Cs1-4 genomes (or in other partially sequenced *Delftia* strains). The overall results showed that the *chrBACF* cluster is conserved in a few members of the *Comamonadaceae* and *Burkholderiaceae*, in

particular in *Alicycliphilus denitrificans* K601 and *Burkholderia xenovorans* LB400, respectively, within the Burkholderiales Order (Figure 4 and 5).

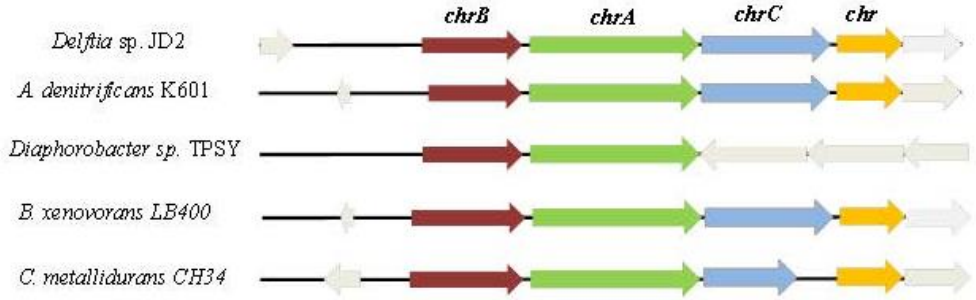

Figure 4. Gene arrangement and synteny of the *chrBACF* cluster in JD2 and other related bacteria. *A. denitrificans* K601 and *Diaphorobacter* sp. TPSY; and *B. xenovorans* LB400 and *C. metallidurans* CH34 belong to *Comamonadaceae* and *Burkholderiaceae* families, respectively.

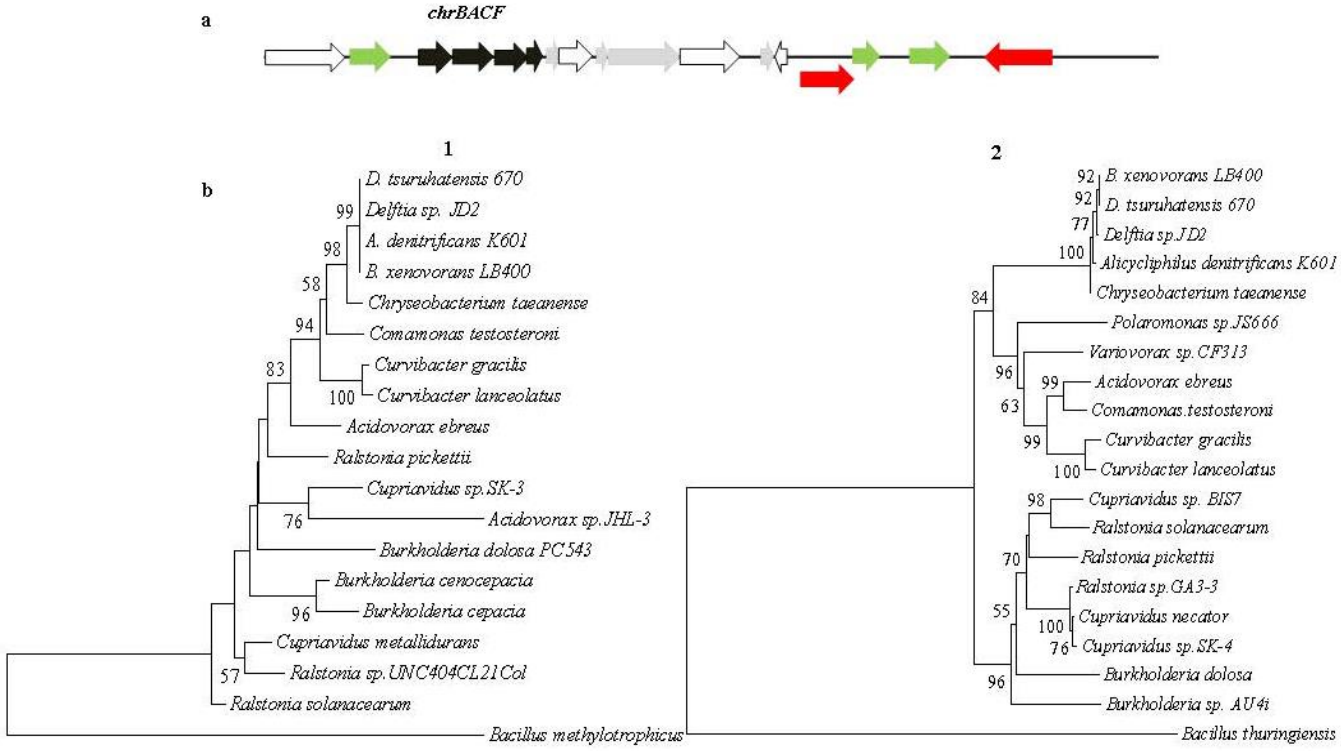

Figure 5. a) Gene arrangement of CDS in the vicinity of *chrBACF* cluster (in black arrows). Mobile genetic elements are shown in red (Int) and green arrows (Tnp). Grey and white arrows show the hypothetical and putative proteins, respectively. b) The evolutionary relationships of ChrB (1) and ChrA (2) was performed using NJ and ML, showing similar topologies; thus, only the results obtained with NJ are shown. ChrC and ChrF trees showed similar topology to ChrB and ChrA trees, as shown in the Figure S1 (supplementary resource). Values between clades (1000 replicates) represent the bootstrap.

A total of 28 mobile genetic elements, transposases (Tnp) and integrases (Int), were predicted throughout the JD2 genome. Immediately down- and up-stream to the *chrBACF* cluster, and separated by 735 and 7,523bp, respectively, a Tnp gene and a group of four syntenic mobile genetic (Tnp+Int) elements were found (Figure 5a). In order to analyze if the *chr* genes could be adquired by horizontal gene transfer, phylogenetic trees were reconstructed [Figure 5b and Figure S1 (supplementary resource)]. Although *chr* genes are absent in SPH1 and CS1-4, *chr* genes of JD2 and other members of the family *Comamonadaceae* clustered together, suggesting that these genes are usually present in the family.

3.4.2. Production of indole 3-acetic acid and tryptophan

The occurrence of genes involved in tryptophan (Trp; precursor of IAA synthesis) biosynthesis and degradation, and IAA biosynthesis was also searched.

The bacterial IAA-producing routes were analyzed in JD2, and the information suggests that only the IAM-pathway might be present in this bacterium. This is a two enzyme path (Trp-2-mono-oxygenase, IaaM; IAM-hydrolase, IaaH). A coding sequence for IaaH was found in JD2, but the coding sequence for IaaM was not detected. Twenty mono-oxygenases (Alkanesulfonate-, ammonia-, or 4-hydroxyphenylacetate 3-monooxygenase, among others) were detected in the JD2 genome. One or some of them are likely candidates for IaaM, probably due to the annotation of this gene (*iaaM*) as another class of mono-oxygenase. For instance, Blastx analysis showed that JD2 and Cs1-4 present *iaaH* orthologous genes with 99% and 96% identity and coverage, respectively. These results suggest that JD2 produces IAA through the IAM-pathway, using Trp as precursor.

The Trp biosynthetic and degradative pathways were searched into the JD2 draft genome. The shikimate pathway is the route for the biosynthesis of aromatic amino acids (Trp, tyrosine and phenylalanine), and uses erythrose-4-phosphate and phosphoenolpyruvate as precursors. This is a complex pathway that works through many enzymes (DAHP synthase, 3-dehydrokinase synthetase, dehydrokinate dehydrase, shikimate dehydrogenase, shikimate kinase, enolpyruvylshikimate phosphate synthase and chorismate synthase) for the synthesis of chorismate. The chorismic acid is the branch-point in aromatic acid synthesis. If chorismate is transform into anthranilate, the pathway continues until the end product, Trp. The enzymes anthranilate synthase (*trpE*), anthranilate phosphoribosyl transferase (*trpD*), N'-(5'-phosphoribosyl) anthranilate isomerase (*trpF*), indole-3-glycerol phosphate synthase (*trpC*) and Trp synthase (*trpAB*) are responsible of this biochemical transformation. All the enzymes responsible for the biosynthesis of Trp, using erythrose-4-phosphate and phosphoenolpyruvate as precursors, were detected in the JD2 genome. The Trp biosynthetic genes of JD2 were found organized in minor clusters. These clusters were located in different contigs, following the structure *trpBA*, *trpE* and *trpGDC*, as described for *P. aeruginosa*, *P. putida* and *P. syringae*. In these species of *Pseudomonas*, *trpBA* is under the control of TrpI. TrpI is a transcriptional regulator that belongs to the LysR family, and *trpI* is frequently located close to *trpBA*. The search for TrpI showed that JD2 has many homologous LysR transcriptional regulators (56% identity with TrpI of *P. aeruginosa* PAO1), distantly located from *trpBA*.

3.4.3. Siderophores

The search of genes involved in Fe acquisition, uptake and utilization of heme and hemin containing compounds, and Ton-like transporters, showed that the JD2 genome possesses several subsystems related to these functions. The entire pathways for pyoverdine synthesis, maturation and exportation, and the membrane receptor for its uptake, were found through the JD2 genome.

In addition, JD2 presents the genetic machinery for the acquisition of pyochelin and enterobactin, but not for their biosynthesis, suggesting that this strain may recognize and uptake different kinds of siderophores, beyond the siderhophores produced by itself. The uptake of the complex siderophore-Fe in bacteria is conducted through their specific recognition by a membrane receptor, followed by the reception by a periplasmic protein and finally their passage to the cytoplasm by an ABC-transporter. The energy used during this process is reliant on TonB [33]. During the annotation of the draft genome of JD2, more than 40 putative coding sequences related to Ton and Tol subsystems were found, which may be involved in the uptake of Fe and polymers, respectively.

3.4.4. Others

Interestingly, a bifunctional chitinase/lysozyme JD2-specific gene was detected. These enzymes have antagonistic abilities that might be used during the biological control of plants and microbial pathogens.

Also, an hydrolytic enzyme responsible for the hydrolysis of short chain amides to organic compounds and ammonium was found as JD2-specific gene. This gene was located upstream to a cluster of urea ABC transporter (urea binding and permease proteins, including *urtBC*).

3.5. Genomic exploration and identification of potential biotechnological uses

3.5.1. Lignin-derived aromatic compounds degradation

Genomic analysis of *Delftia* sp. JD2 revealed the presence of genes encoding a central intermediate pathway in bacterial degradation of diverse aromatic compounds. The identification of the protocatechuate (PCA) ring cleavage pathway (*lig* genes) [34], suggests that JD2, SPH-1 and Cs1-4 might mineralize aromatic compounds, including some lignin-derived ones, such as vanillate and ferulate. At least 13 catabolic genes for aromatic compounds were detected in the JD2 genome, as was reported for *C. testoteroni* BR6020 (*pmdKEFDABC* cluster; [35]). Among the predicted genes, the following CDS were found: phydroxybenzoate transporter, *pmdk*; 4-oxalomesaconate hydratase (EC 4.2.1.83), *pmdE (ligJ)*; 4-carboxy-4-hydroxy-2-oxoadipate aldolase (EC 4.1.3.17), *pmdF (ligK);* 2-pyrone-4,6-dicarboxylic acid hydrolase, *pmdD* (*ligI)*; protocatechuate 4,5-dioxygenase alpha chain (EC 1.13.11.8), *pmdA* (*ligA)*; protocatechuate 4,5-dioxygenase beta chain (EC 1.13.11.8), *pmdB* (*ligB)* and 4-carboxy-2-hydroxymuconate-6-semialdehyde dehydrogenase, *pmdC (ligC)*. A transcriptional regulator gene *ligR*, that positively regulates the expression of PCA 4,5-cleavage pathway genes, was also detected. LigR has similarity to members of the LysR transcriptional regulator family [36]. The search in JD2, Cs1-4 and SPH-1 genomes showed that they have also a CDS for a multi-copper polyphenol oxidoreductase, a putative laccase.

3.5.2. Lactic acid fermentation

A JD2-specific cluster of genes involved in the uptake of monosaccharides (ABC transporter system, RbsBAC), and a cluster of genes involved in L-fucose metabolism, immediately downstream to *rbsBAC*, were found. L-fucose is a widely distributed monosaccharide that can be used as sole carbon source by two separate metabolic pathways. L-fucose might be metabolized to L-lactaldehyde and dihydroxyacetone phosphate [37], or transformed to pyruvate and L-lactate [38]. The inspection of the JD2 genome showed that this bacterium present all the genetic machinery required for the uptake and transformation of L-fucose to pyruvate and L-lactate.

3.5.3. Polyhydroxybutyrate metabolism

Polyhydroxybutyrate (PHB) is a water insoluble polymer synthesized by some bacteria when growing in high carbon-to-nitrogen ratio media, and is commonly accumulated as intracellular granules [39]. We found the information for the biosynthesis and depolymerization of PHB dispersed in the JD2 genome. The entire pathway for the generation of the PHB-precursor (R)-3-hydroxybutanoyl-CoA from acetoacetate, crotonyl-CoA and (S)-3-hydroxybutanoyl-CoA was detected. The complete pathway for PHB polymerization include: a 3-hydroxybutyryl-CoA epimerase (EC 5.1.2.3), 3-hydroxybutyryl-CoA dehydratase (EC 4.2.1.55), D(-)-3-hydroxybutyrate oligomer hydrolase (EC 3.1.1.22), polyhydroxyalkanoic acid synthase, intracellular PHB depolymerase (EC 3.1.1.-), acetyl-CoA acetyltransferase (EC 2.3.1.9), D-beta-hydroxybutyrate dehydrogenase (EC 1.1.1.30), cetoacetyl-CoA reductase (EC 1.1.1.36), D-beta-hydroxybutyrate permease, enoyl-CoA hydratase (EC 4.2.1.17), acetoacetyl-CoA synthetase (EC 6.2.1.16), 3-ketoacyl-CoA thiolase (EC 2.3.1.16), 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) and 3-hydroxybutyryl-CoA dehydrogenase (EC 1.1.1.157). Interestingly, some of these genes were found in several copies among the genome. In addition, a CDS for a phasin family protein was also found. Phasins are polyhydroxyalkanoate surface coating proteins that form a proteinaceous sheet that stabilizes the PHB granule and prevents its coalescence in the cytoplasm [40].

4. Discussion

The genome of *Delftia* sp. strain JD2 was sequenced, and annotation and gene prediction were performed using two different services (RAST and PGAAP). Results using both programs were in agreement, showing that the genome size (6.76 Mb), GC content (66.4%) and number of predicted CDS (6,051) were similar to those reported for other *Delftia* strains [41,42], except for *D. tsuruhatensis* strain MTQ3 and *D. lacustris* strain LZ-C that showed a smaller genome and lower number of CDS [9,43]. JD2 showed two copies of the 16S, 5S and 23S rRNA genes, but both Cs1-4 and SPH-1 have five copies of rRNA. Thus, it is likely that the number of rRNA genes in JD2 were underestimated due to the lack of a complete genome sequence.

Phylogenetic relationships showed that JD2 clustered together as a sister group to the genus *Comamonas*, and the tree was in agreement with a previous phylogenetic analysis of the group [44]. In addition, species of the genus *Acidovorax* showed as a polyphyletic group, as previously reported [45]; however, our study was performed using a larger number of sequences, strongly supporting the result.

The estimated ANI values among *Delftia* genomes were above 98%. Usually, a threshold range of 95–96% is considered a robust measurement of genomic similarity between strains [46]. Thus, the

results strongly suggest that the strains JD2, Cs1-4 and SPH-1 belong to the same species, *Delftia acidovorans*. As the JD2 genome is still in draft, the number of genes and homology families has been probably underestimated. However, the high percentage of core gene families suggests that most of the JD2 genes have been included in the current assembly. The results from the pan- and core-genome indicate that there are highly conserved sequences and gene functions within the genus. However, this study was performed with only three strains, and more genomes have to be included in this study until a conclusive decision.

During the search of singletons, 875 CDS were identified as JD2-specific genes, with non-homologous in neither SPH-1 nor Cs1-4 genomes. The genetic information involved in HM-resistance, virulence, IAA production, synthesis and uptake of siderophores, lactic fermentation and L-fucose catabolism were identified. Some of these functions might be involved in the independent evolution of JD2 in its natural environment (HM-contaminated soil). This result supports the JD2 might be a versatile microbe that easily adapt to different lifestyles in distinct ecological niches. The comparative study of *Delftia* strains provides the basis of future functional works. In addition, this study also suggests that the bioremediation and agronomic traits previously reported in JD2 are rather not so common in *Delftia* strains.

Among the HM-resistance genes, the *chrBACF* cluster was found. This is a well-studied operon [47–49]. ChrB is the transcriptional regulator (pfam09828 family) of a specific efflux system that pumps chromate out of the cell. This pumping is done by the action of the membrane-bounded and chromate efflux protein ChrA (COG 2059 family), while other proteins (ChrC and ChrF) help in the detoxification [48]. The proposed activity of ChrC is as a scavenger of superoxide radicals originated when chromate interacts with cellular compounds or during the reduction of Cr(VI) to Cr(III) [50–53]. ChrF has been described as a repressor for chromate-dependent induction of *chrB*[50]. In *Ochrobactrum tritici* strain 5bvl1, *chrBACF* was located in the chromosomally integrated transposable element TnOtChr, suggesting that this bacterium has the ability to transfer the genetic information related to chromate resistance [47].

Down- and up-stream to the *chrBACF* cluster, Tnp genes and syntenic mobile genetic (Tnp+Int) elements were found. Potentially, this *chr* cluster might be a key feature that allows *Delftia* sp. JD2 to survive in chromate-contaminated environments. Since Int and Tnp are all recombinases that could be involved in events of genome rearrangements [53,54], a hypothesis of a potential acquisition of *chr* genes by an horizontal gene transfer event was analyzed, studying the phylogeny of the genes (Figure 5b). The data showed that the *chr* genes of JD2, *Delftia* sp. 670 and other members of the *Comamonadaceae* clustered together, suggesting that more than an acquisition by JD2, the *chr* cluster was probably lost by SPH-1 and Cs1-4.

Five Trp-dependent pathways for the biosynthesis of IAA have been reported in bacteria, being the most common ones the indole-3-acetamide (IAM), indole-3-pyruvic (AIP) and the tryptamine (TAM) pathways [55]. In addition, it has been reported that some bacteria produce IAA through a tryptophan-independent pathways [55,56]. In general, many bacterial strains possess several IAA biosynthetic pathways with redundant activities. Among relevant agronomic traits, the IAM-pathway for the biosynthesis of IAA was found in the JD2 genome. This is a common path found in PGPB [57,58], but it has been mainly linked to phytopathology fitness [59]. However, JD2 did not induce plant defense response, at least in alfalfa plant (unpublished data; by determination of peroxidase, glucanase and glucosaminidase activities, and malondialdehyde content). Instead, the addition of JD2 cells to alfalfa plants inoculated with rhizobia induces IAA secretion to the

rhizosphere, and promotes plant growth [11]. Further experiments are essential to show if the production of this phytohormone by JD2 is responsible for the positive effect in the microbe-plant interaction. However, the information supports that the production of IAA by JD2, thought the IAM-pathway, might be involved in the plant growth promotion phenotype of this bacterium.

We also analyzed the occurrence of Trp biosynthetic pathways, the precursor of IAA biosynthesis. The entirely set of genes involved in the Shikimate pathway were detected. It has been reported that the genetic information involved in Trp biosynthesis can be organized in dispersed genes as in *Acinetobacter boumannii* [60], in a single operon (*trpE/GDFBA*) as in *Escherichia coli* and *Chlamydia psittaci* [60,61] or in several operons as in *Pseudomonas* spp. and *Methylococcus capsulatus* [62,63]. Our results support that JD2 possesses a similar structure as in *Pseudomonas* strains. The information was organized in minor clusters following the structure *trpBA, trpE* and *trpGDC*. Overall, the information suggests that JD2 may produce IAA using erythrose-4-phosphate and phosphoenolpyruvate as precursor for the biosynthesis of Trp (through the Shikimate pathway), followed by the IAM-pathway.

Siderophores are ferric chelating chemical structures that improve Fe availability of plants and microbes, being microbes more competitive than plants. Microbes compete for scarce resources in their natural environments [64]. For example, microbes compete for the acquisition of ferric ion, mainly be the production of high affinity siderophores that capture and uptake Fe, suppressing the growth of non-siderophore producing microbes [65]. Siderophores also scavenge for other metallic ions, providing an extracellular protection, maintaining toxic HM in a non-diffusible state and avoiding their diffusion through porins. Thus, siderophores might be beneficial for bacteria and plants in an environment contaminated with toxic metals [66–68]. The production of siderophores by JD2 was already shown by [4], and during the present work it was found that JD2 has the genetic machinery for the synthesis and uptake of pyoverdines. These are dihydroquinoline-type chromophores linked to a peptide and their biosynthesis involve at least 12 different proteins. The secretion of pyoverdine involves a recently identified ATP-dependent efflux pump that also uptakes molecules of pyoverdine that already chelated iron, into the bacterial periplasm [69]. All these genetic information was found throughout the JD2 genome. The information suggests that JD2 produces pyoverdine for ferric iron chelation and uptake, but the bacterium also has the ability to use ferric ion by the uptake of pyochelin and enterobacterin (probably produced by other microbes). The ability of using different kinds of siderophores probably represents a competitive advantage in their natural environment.

Among JD2-specific genes, we also found the genetic information for the production of a putative bifunctional enzyme, chitinase/lysozyme. The chitinase and lysozyme activities, both relevant to peptidoglycan degradation, were first described in *Delftia lacustris* strain 332 [70]. The chitinase enzyme (EC 3.2.1.14), a glycosyl hydrolase, has received attention in regard to its role as microbial biocontrol agent. The result suggests that JD2 may have antagonistic activity against phytopathogenic microorganisms, such as *D. tsuruhatensis* HR4 [10] and *D. tsuruhatensis* WGR–UOM–BT1 [71].

It has been suggested that aromatic-degrading soil bacteria are able to break down lignin [72] and bacteria of the genus *Delftia* are known for their ability to degrade aromatic compounds (see Introduction). Although the biochemistry of bacterial lignin breakdown is not well understood, peroxidases and laccases could be probably involved [72]. Our findings of some peroxidases, and a multi-cooper polyphenol oxidoreductase with putative laccase activity, and the identification of the

PCA ring cleavage pathway (*lig* genes, [33]) suggest that JD2, SPH-1 and Cs1-4 might mineralize aromatic compounds, including lignin-derived ones. The ability to grow on lignin-derived compounds, as sole carbon and energy source, has not been studied in JD2, but the cleavage of the aromatic β-aryl ether by *D. acidovorans* was reported by [73]. PCA is one of the most important intermediate metabolites in the bacterial catabolic pathways for the degradation of lignin-derived aromatic compounds and it could be further degraded through its ring cleavage [35]. In summary, the genetic information supports that JD2 could grow on various lignin-derived mono- and bi-aryls via the PCA 4,5-cleavage pathway. These kind of microbes are commonly used for the biosynthesis of 2-pyrone-4,6-dicarboxylate (PDC) polyester, using lignin as a bioresource [74]. Thus, JD2 might be a useful bacterium for the biopolymer, food and flavor industries, and for fine chemicals and materials synthesis.

The genetic information also suggests that JD2 is able to conduct the L-lactic acid fermentation process using L-fucose as a carbon source. In recent years, the demand for lactic acid has increased considerably, due to its novel application in the food, pharmaceutical, leather, and textile industries [75]. Its biotechnological production offers several advantages such as low cost of substrates, among others. The best selection of the material to be fermented is a critical factor in the efficient production, accounting for the largest proportion of industrial costs. The relevance of lactic acid fermentation using raw material has focused on the utilization of land plants and seaweed biomass as carbon sources (mainly composed by D-glucose and D-galactose, [76]), and the so called 'rare' sugars, also known as 'good' sugars, such as L-fucose [75].

Although the ability of JD2 to incorporate monomers into PHB has not been evaluated, the genomic information supports that this bacterium may produce and accumulate this carbon storage polymer. Other *Delftia* strains, such as *D. acidovorans* and *D. tsuruhatensis* Bet002 have been reported as polyhydroxyalkanoates (PHA) producing microbes [77–80]. PHB production and degradation could be important in JD2 endurance and competition in the rhizosphere. However, the importance of PHB production and accumulation resides in the potential of PHA producing bacteria as a genetic resource for the industry of bioplastic production (biodegradable polymers).

5. Conclusion

During this work we searched for genes, or clusters of genes, with potential biotechnological applications using a genomic approach. We studied the draft genome of *Delftia* sp. strain JD2, identified as a HM-resistant and, IAA and siderophore producing microbe [4,12]. As expected for an environmental isolate, JD2 has a large genome (6.76 Mbp). By comparative genomics with Cs1-4 and SPH-1, many genes were identified as JD2-specific ones: a) the *chrBACF* cluster involved in chromium resistance. The topology of the trees (Figure 5b) and the presence of this cluster in other members of the *Comamonadaceae*, suggest that this cluster could has been lost during the evolution of Cs1-4 and SPH-1; b) genes involved in copper, zinc, arsenic, cobalt-cadmium-zinc and multidrug homeostasis and resistance, including multidrug efflux pumps; c) the IAM-pathway for the biosynthesis of IAA, a phytohormone implicated in the JD2 plant growth promotion phenotype; and d) genes involved in the synthesis, maturation and exportation of pyoverdin, but also in the uptake of different classes of siderophores. Other JD2-specific gene was the chitinase/lysozyme CDS, a glycosyl hydrolase that has received attention in regards to their role as a microbial biocontrol agent; suggesting that JD2 may have antagonistic activity against phytopathogenic microorganisms.

In addition, genes involved in the production of novel bio-based products were detected. On this regard, the genomic information suggests that this bacterium has many potential biotechnological applications: the production of bio-polymers through the utilization of lignin-derived aromatic compounds as bioresource; the production of lactic acid by the fermentation of L-fucose rich polysaccharides, such as fucoidan, a polymer mainly found in the cell-wall matrix of brown seaweed; and the eco-friendly production of biodegradable PHB based plastics, as biodegradable polymeric packaging materials.

In summary, the genetic information suggests that JD2 may be useful as a microbial model for many biotechnological purposes, such as HM-bioremediation, growth promotion of crops and the production of industrial relevant molecules.

Although further sequencing steps (filling gaps) and comparative genomics have to be done, the results allow us to better understand the physiology of JD2 and show the potential of this bacterium as genetic resource for the production of biotechnological products.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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