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SHORT GENOME REPORT

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Complete genome sequence of *Jiangella* gansuensis strain YIM 002^T (DSM 44835^T), the type species of the genus *Jiangella* and source of new antibiotic compounds

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Abstract

Jiangella gansuensis strain YIM 002^T is the type strain of the type species of the genus Jiangella, which is at the present time composed of five species, and was isolated from desert soil sample in Gansu Province (China). The five strains of this genus are clustered in a monophyletic group when closer actinobacterial genera are used to infer a 16S rRNA gene sequence phylogeny. The study of this genome is part of the *Genomic Encyclopedia of Bacteria and Archaea* project, and here we describe the complete genome sequence and annotation of this taxon. The genome of *J. gansuensis* strain YIM 002^T contains a single scaffold of size 5,585,780 bp, which involves 149 pseudogenes, 4905 protein-coding genes and 50 RNA genes, including 2520 hypothetical proteins and 4 rRNA genes. From the investigation of genome sizes of *Jiangella* species, *J. gansuensis* shows a smaller size, which indicates this strain might have discarded too much genetic information to adapt to desert environment. Seven new compounds from this bacterium have recently been described; however, its potential should be higher, as secondary metabolite gene cluster analysis predicted 60 gene clusters, including the potential to produce the pristinamycin.

Keywords: Jiangella gansuensis, Jiangellales, Desert, Genome, Taxonomic comments, GEBA

Introduction

Jiangella gansuensis strain YIM 002^T (=DSM 44835^T =CCTCC AA 204001^T =KCTC 19044^T) is the type strain of *J. gansuensis*. This organism is an aerobic, Gram-positive, haloduric filamentous actinomycete, placed within the genus *Jiangella* [1].

The genus *Jiangella* was first identified by Song et al. in 2005, including five halotolerant species listed at present by LPSN [2]. Members of this taxon isolated from different habitats, respectively, are rarely described except for their polyphasic approach based on combination of phenotypic and genotypic

characteristics [1, 3-6]. The Jiangella was originally identified as a new genus of the family Nocardioidaceae within the suborder Propionibacterineae [1] based on phenotypic and genotypic criteria. However, the reconstruction of the phylogenetic relationships of Actinobacteria at higher taxa was done later based on using the 16S rRNA genes and other related evidences, such as taxon-specific 16S rRNA gene signature nucleotides [7, 8]. After the genus Haloactinopolyspora was described by Tang et al., the genus Jiangella together with the genus Haloactinopolyspora were placed in a novel family Jiangellaceae belong to Jiangellineae subord. nov., mainly because of theirs signature nucleotide patterns, 16S rRNA gene similarity and phylogenetic criteria [9]. Presently, the *J. gansuensis* is placed in the family Jiangellaceae of the order Jiangellales within the class Actinobacteria [10].

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The capacity of *J. gansuensis* YIM 002^T to produce seven new compounds (five pyrrol-2-aldehyde compounds, jiangrines A-E; one indolizine derivative, jiangrine F; one glycolipid, jiangolide) has previously been shown [11], highlighting the importance of this bacterium and its analysis as a novel source of secondary metabolites. As part of the GEBA project and considering its phylogenetic position and biological significance, we finally decided to sequence the genome of the type strain of *J. gansuensis*. Here we present a summary classification and a set of features for *J. gansuensis* YIM 002^T, together with the description of genomic sequencing and annotation. At the same time, we will provide a brief introduction of its genome in this article.

Organism information

Classification and features

Strain YIM 002^T is a free-living isolate collected from a desert soil sample of Gansu Province during an investigation into microbial diversity of extreme environments. This actinobacterium forms welldifferentiated non-sporulating aerial and substrate mycelia. Its aerial hypha was observed to have yellow-white color at the earliest and finally turns to orange-yellow after few days on NA medium, and its substrate mycelia fragmented into short or elongated rods in the early phase of the growth (Fig. 1). Growth was observed on ISP 2, ISP 3, ISP 4, ISP 5, nutrient agar and Czapek's agar [1, 12]. The type strain of this taxon is able to tolerate a pH range between 5.0 and 10.0, and able to growth at the salinity between 0 and 10% (w/v NaCl), with no growth observed at 12.5%. Optimal growth of strain YIM 002^T occurs at pH 7.0-8.0, 1-5% (w/v) NaCl and 28 °C. The diamino acid in the peptidoglycan is LL-2,6diaminopimelate. MK-9(H_4) is the predominant menaquinone. The primary phospholipids profile of strain DSM 44835^T was found to consist of phosphatidylinositol mannosides, phosphatidylinositol and diphosphatidylglycerol. Its major cellular fatty acids (>10%) are anteiso- $C_{15:0}$, anteiso- $C_{17:0}$ and iso- $C_{15:0}$. Whole cell sugar composition includes glucose and ribose, whereas the amino acids in the peptidoglycan layer were LL-A₂pm, alanine, glycine and glutamic acid [1]. The DNA G+C content of the type strain was previously determined as 70% while genome analysis showed a higher value of 70.91%.

The draft genome of *J. gansuensis* YIM 002^{T} has one almost full-length 16S rRNA gene sequence, which correspond perfectly with the original sequence from the species description (AY631071). The comparison of this 16S rRNA sequence of YIM 002^T using the EzTaxon-e server [13], showed highest similarity to Jiangella alba YIM 61503^T (98.93%), with close relationships to other species within the genus, Jiangella muralis 15-Je-017^T (98.88%), Jiangella mangrovi 3SM4-07^T (98.49%) and Jiangella alkaliphila D8-87^T (98.10%). Closest other genera are Haloactinopolyspora [9] and Phytoactinopolyspora [14]. The strains of the genus Jiangella have many 16S rRNA gene signature nucleotides compared with most of other described actinomycetes. This allows for distinguished them easily from other actinobacteria, especially in 11 unique positions, including 127:234 (G-C), 598:640 (C-G), 672:734 (G-C), 831:855 (U-A), 833:853 (G-C), 840:846 (A-U), 950:1231 (G-C), 952:1229 (G-C), 955:1225 (G–U), 986:1219 (U–G) and 987:1218 (C–G) [9].

Phylogenetic analyses were performed using both neighbor-joining (NJ) and maximum-likehood (ML) algorithms. The NJ phylogenetic tree of the genus *Jiangella* based on 16S rRNA genes provide an evidence of its independent taxon (Figs. 2 and Additional file 1: Figure S1), together with the genera *Haloactinopolyspora* and *Phytoactinopolyspora*, which arouse ours reflection on the relationship of three families among *Jiangellaceae*,

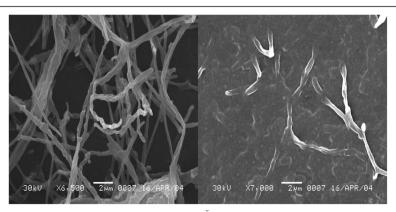


Fig. 1 Scanning electron micrograph of Jiangella gansuensis strain YIM 002^T grown on ISP medium 2 for 14d at 28 °C. Bar size: 2 µm

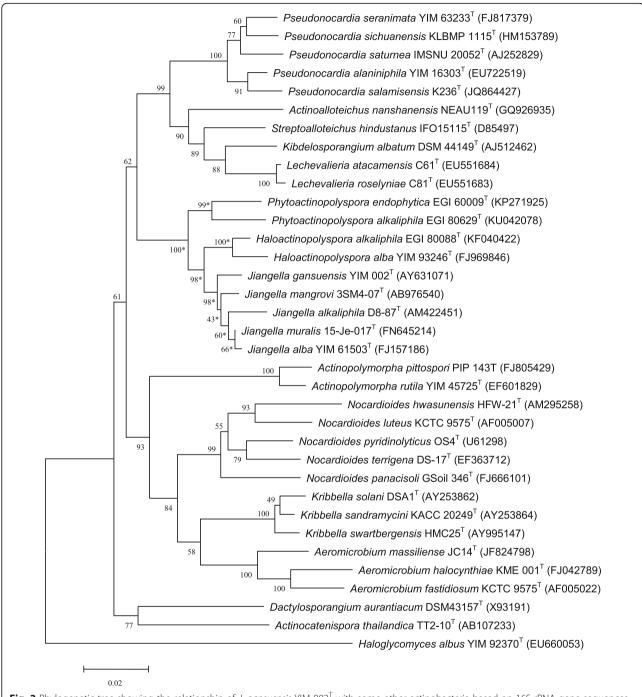


Fig. 2 Phylogenetic tree showing the relationship of *J. gansuensis* YIM 002^T with some other actinobacteria based on 16S rRNA gene sequences. The Neighbour-joining tree was built using MEGA 5 [39] with the Kimura 2-parameter model. Bootstrap values (percentages of 1000 replicates) are shown at branch points. *Asterisks* denote nodes that were also recovered using the Maximum Likelihood method in the branch of *Jiangellaceae*. The *Haloglycomyces albus* act as the outgroup

Nocardioidaceae and Pseudonocardiaceae. The ML tree (Additional file 1: Figure S1) demonstrates the same positions in Jiangellaceae compared with the NJ tree. Minimum Information about the Genome Sequence is provided in Table 1.

Genome sequencing information Genome project history

This organism was selected for sequencing on the basis of its important phylogenetic position and biological significance [15, 16], and for a better

Table 1 Classification and general features of *Jiangella gansuensis* strain YIM 002^T in accordance with the MIGS recommendations [20], List of Prokaryotic names with Standing in Nomenclature [40] and the Names for Life database [41]

MIGS ID	Property	Term	Evidence code ^a
	Current classification	Domain <i>Bacteria</i>	TAS [42]
		Phylum Actinobacteria	TAS [43]
		Class Actinobacteria	TAS [7]
		Order Jiangellales	TAS [44]
		Family Jiangellaceae	TAS [9]
		Genus Jiangella	TAS [1]
		Species Jiangella gansuensis	TAS [1]
		Type strain YIM 002 ^T (=DSM 44835 ^T)	TAS [1]
	Gram stain	Positive	IDA
	Cell shape	Filamentous	IDA
	Motility	Non motile	IDA
	Sporulation	Non-sporulating	IDA
	Temperature range	10−45 °C	IDA
	Optimum temperature	28 ℃	IDA
	pH range; Optimum	7.0-8.0	TAS [1]
	Carbon source	Various	IDA
	Energy source	Chemoorganotroph	IDA
MIGS-6	Habitat	Desert soil	IDA
MIGS-6.3	Salinity	Halotolerant	IDA
MIGS-22	Oxygen requirement	Aerobic	IDA
MIGS-15	Biotic relationship	Free living	IDA
MIGS-14	Pathogenicity	None	IDA
MIGS-4	Geographic location	Gansu Province, China	IDA
MIGS-5	Sample collection time	2005 or before	NAS
MIGS-4.1	Latitude	Not reported	NAS
MIGS-4.2	Longitude	Not reported	
MIGS-4.4	Altitude	Not reported	

^a Evidence codes - IDA: Inferred from Direct Assay; TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from of the Gene Ontology project [45]

understanding of the school of 'evolutionary taxonomy' [17]. Sequencing of *J. gansuensis* YIM 002^T is part of Genomic Encyclopedia of Bacteria and Archaea pilot project [18], which aims for generating high quality draft genomes for bacterial and archaeal strains. The genome project is deposited in the Genomes OnLine Database (GOLD) [19], and the finished genome sequence was deposited in Gen-Bank. Genome sequencing, finishing and annotation were performed by the Department of Energy, Joint Genome Institute (JGI) using state of the art genome sequencing technology [20]. A summary of project information is shown in Table 2, compliance with MIGS version 2.0 [21].

Growth conditions and genomic DNA preparation

J. gansuensis strain YIM $002^{\rm T}$ (=DSM $44835^{\rm T}$) was grown in DSMZ medium 65 (GYM *Streptomyces* medium) at 28 °C. Genomic DNA was isolated using Qiagen

Table 2 Genome sequencing project information

	1 31 7	
MIGS ID	Property	Term
MIGS 31	Finishing quality	Non-contiguous Finished
MIGS-28	Libraries used	Illumina Std shotgun library
MIGS 29	Sequencing platforms 454-GS-FLX-Titanium Illumina G	
MIGS 31.2	Fold coverage Unknown	
MIGS 30	Assemblers	ALLPATHS v. R37654
MIGS 32	Gene calling method	Prodigal 1.4, GenePRIMP
	Locus Tag	JIAGA
	GenBank ID	AZXT00000000
	GenBank Date of Release	15-08-2013
	GOLD ID	Gp0001209
	BIOPROJECT	PRJNA224116, PRJNA63165
MIGS 13	Source Material Identifier	YIM 002, DSM 44835
	Project relevance	Tree of Life, GEBA

Genomic 500 DNA Kit (Qiagen, Hilden, Germany) following the standard protocol provided by the manufacturer. Some modifications were included for cell lysis, first freezing for 20 min (-70 °C), then heating 5 min (98 °C), and cooling 15 min to 37 °C; adding 1.5 ml lysozyme (standard: 0.3 ml, only), 1.0 ml achromopeptidase, 0.12 ml lysostaphine, 0.12 ml mutanolysine, 1.5 ml proteinase K (standard: 0.5 ml, only), followed by overnight incubation at 35 °C.

Genome sequencing and assembly

All general aspect of library construction and sequencing performed can be found at the JGI website. The complete sequence in one scaffold was obtained from

9 contigs with the assembly method ALLPATHS v. R37654, obtaining a total size of 5.5 Mbp from a total volume data of 4 Gbases (Fig. 3).

Genome annotation

Prodigal [22] was used to identify genes as part of the JGI genome annotation pipeline [23, 24] followed by a round of manual curation using the JGI GenePRIMP pipeline [25]. The National Center for Biotechnology Information non-redundant database, UniProt, TIGR/Fam, Pfam, PRIAM, KEGG, COG, and InterPro databases were used to analyse the predicted CDSs after translation. RNA genes identification was done using HMMER

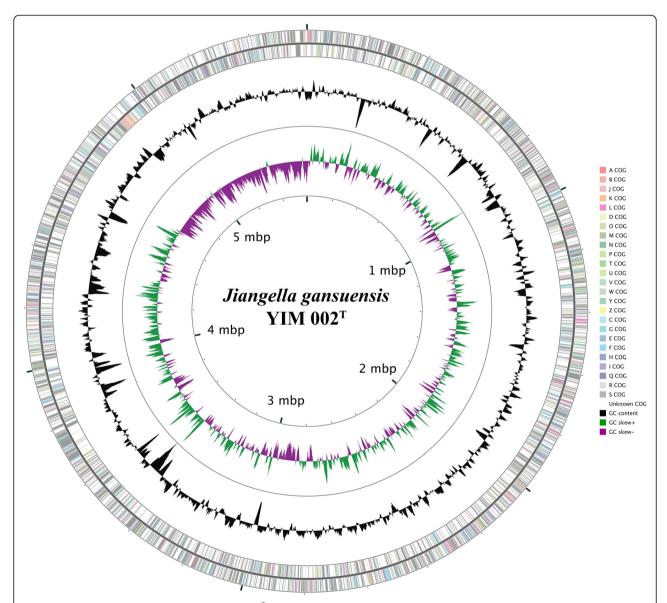


Fig. 3 Graphical map of the *J. gansuensis* strain YIM 002^T chromosome. The genome circular map was set up by the CGView Server [46]. From the outside to the center: Genes on forward strand (colored by COG categories), Genes on reverse strand (colored by COG categories), GC content, GC skew, where *green* indicates positive values and *magenta* indicates negative values

3.0 [26] (rRNAs) and tRNAscan-SE 21.23 [27] (tRNAs). INFERNAL 1.0.2 [28] was used for prediction of other non-coding genes. Integrated Microbial Genomes Expert Review platform [29] permitted the additional gene prediction analysis and functional annotation. CRISPR elements were detected with CRT [30] and PILER-CR [31]. General statistics are shown in Table 3.

Genome properties

The assembly of the draft genome sequence consists of one scaffold for the strain YIM 002^T (Fig. 1), with 70.9% GC content (Table 3) in 5,585,780 nucleotides. From a total of 5104 genes, there were 4905 protein-coding genes, 149 pseudogens and 50 RNA genes. Numbers of the genes were assigned a putative function (48.86%), while the remaining protein-coding genes were annotated as hypothetical proteins. COGs categories distributions for the genes are presented in Table 4.

Insights from the genome sequence

The genome of YIM 002^{T} with a high G + C content and the smallest size within the *Jiangella* genomes (Table 3) may be the result of selection and mutation [32], which could involve several factors, such as environment, aerobiosis and others [33]. Generally speaking, a larger genome size may correlate with more complex habitat, suggesting that the genome encodes a large metabolic and stress-tolerance potential [34]. However, after we investigated the genome size of other type strains of *Jiangella* species, we found the size of the other three strains sequenced of this genus, *J. alkaliphila*, *J. alba*

Table 3 Genome Statistics

Attribute	Value	% of total ^a	
Genome size (bp)	5,585,780	100.00	
DNA coding (bp)	4,761,339	85.24	
DNA G+C (bp)	3,960,974	70.91	
DNA scaffolds	1	-	
Total genes	5,104	-	
Protein-coding genes	4,905	98.03	
RNA genes	50	0.98	
Pseudo genes	149	2.98	
Genes in internal clusters	1763	34.54	
Genes with function prediction	2,504	48.86	
Genes assigned to COGs	2,156	42.07	
Genes with Pfam domains	1,734	33.97	
Genes with signal peptides	456	8.69	
Genes with trandmembrane helices	1230	23.43	
CPISPR repeats	0	-	

^a The total is based on either the size of genome in base pairs or the total number of genes in the predicted genome

Table 4 Number of genes associated with the general COG functional categories

Code Value % age a Description					
Value	% age ^a	Description			
160	3.18	Translation, ribosomal structure and biogenesis			
1	0.02	RNA processing and modification			
230	4.58	Transcription			
116	2.31	Replication, recombination and repair			
1	0.02	Chromatin structure and dynamics			
21	0.42	Cell cycle control, cell division, chromosome partitioning			
60	1.19	Defence mechanisms			
75	1.49	Signal transduction mechanisms			
96	1.91	Cell wall/membrane biogenesis			
0	0.00	Cell motility			
18	0.36	Intracellular trafficking, secretion, and vesicular transport			
69	1.37	Posttranslational modification, protein turnover, chaperones			
160	3.18	Energy production and conversion			
223	4.44	Carbohydrate transport and metabolism			
298	5.93	Amino acid transport and metabolism			
56	1.11	Nucleotide transport and metabolism			
114	2.27	Coenzyme transport and metabolism			
111	2.21	Lipid transport and metabolism			
179	3.56	Inorganic ion transport and metabolism			
84	1.67	Secondary metabolites biosynthesis, transport and catabolism			
311	6.19	General function prediction only			
151	3.00	Function unknown			
2868	57.09	Not in COGs			
	Value 160 1 230 116 1 21 60 75 96 0 18 69 160 223 298 56 114 111 179 84 311 151	Value % age a 160 3.18 1 0.02 230 4.58 116 2.31 1 0.02 21 0.42 60 1.19 75 1.49 96 1.91 0 0.00 18 0.36 69 1.37 160 3.18 223 4.44 298 5.93 56 1.11 114 2.27 111 2.21 179 3.56 84 1.67 311 6.19 151 3.00			

^aThe total is based on the total number of protein-coding genes in the genome

and *J. muralis* greater than 7 Mbp based on the genome data from NCBI. This result could implicate that the tight packing and small size of *J. gansuensis* is likely an adaptation for reproductive efficiency or competitiveness [35]. As a halotolerant actinobacterium, solute and ion transporter were predicted in its genome. At the same time, the genome shows properties related to solution of nitrate and sulfonate transport systems. Moreover, nitrite reductase and nitrogen fixation protein NifU were also detected.

The capacity of this microorganism to produce antibiotics has been recently proved with the description of seven new compounds (five pyrrol-2-aldehyde compounds, jiangrines A-E; one indolizine derivative, jiangrine F; one glycolipid, jiangolide) [11]. However, its potential should be higher, taken account the 45 biosynthetic clusters found within the JGI tool [36] and the 497 genes implicated in these clusters. As most of the clusters appear to be putative genes in this analysis, a

second approach was carried out to detect the variety of biosynthetic types and enhance manual genome annotations of secondary metabolite biosynthesis. The software pipeline antiSMASH for secondary metabolite gene cluster identification, annotation and analysis was used [37, 38]. From this analysis, 60 gene clusters were identified, including 20 gene clusters in which the most similar clusters were still unknown (Additional file 2: Table S1). The result of the analysis shown the potential of *J. gansuensis* to produce pristinamycin, an antibiotic derived from *Streptomyces pristinaespiralis* effective against staphylococcal infections, and other antibiotics.

Conclusions

The genome sequence and annotation of J. gansuensis YIM 002^T were presented. This draft genome possess a smaller size (5.59 Mb) compared with other Jiangella species, and contents 2504 function predicted proteins, indicating that J. gansuensis possibly discarded many genes to adapt to the extreme desert conditions during its evolution. Although the processes of nitrous metabolism and secondary metabolism need further investigation to fully understand the related pathways, we believe that J. gansuensis participates in nitrogen cycling and has an important ability to produce secondary metabolites. This genome will contribute to further studies on phylogenetics and the mechanisms of environmental adaptation. A combined study together with genomes of other members in the family Jiangellaceae will help us to better understand the ecological role of this taxon and its relationships to other actinobacteria.

Additional files

Additional file 1: Figure S1. Phylogenetic tree showing the relationship of *J. gansuensis* YIM 002^T with some other actinobacteria based on 16S rRNA sequences. The maximum-likelihood tree was built using MEGA 5 [39]. Bootstrap values (percentages of 1000 replicates) are shown at branch points. *Haloglycomyces albus* was used as outgroup. (PDF 92 kb)

Additional file 2: Table S1. Number of gene clusters associated with antiSMASH. (DOCX 73 kb)

Abbreviations

CRISPR: Clustered regularly interspaced short palindromic repeats; GEBA: Genomic encyclopedia of bacteria and archaea; IMG-ER: Integrated microbial genomes – expert review; JGI: Joint Genome Institute; LPSN: List of prokaryotic names with standing in nomenclature; ML: Maximum likelihood; NJ: Neighbour joining

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Authors' contributions

JYJ, NCK, WJL, MG and HPK designed research and project outline. MG selected and prepared the samples. JYJ, LC, LL and XYG performed comparative genomics and 165 rRNA genes analyses. JYJ, LC, XTZ and AL analysed bioclusters and secondary metabolites. WNH, JYJ and WJL provided the background information on the current taxonomy in relationship to monophyletic groups. JYJ, LC, XYG, WJL and HPK drafted the manuscript. MH, TBKR, NY, MP, MH, NNI, JAE and TW performed genome sequencing, assembly and annotation. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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