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SHORT GENOME REPORT

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Improved-high-quality draft genome sequence of *Rhodococcus* sp. JG-3, a eurypsychrophilic *Actinobacteria* from Antarctic Dry Valley permafrost

Jacqueline Goordial¹, Isabelle Raymond-Bouchard¹, Jennifer Ronholm¹, Nicole Shapiro², Tanja Woyke², Lyle Whyte^{1*} and Corien Bakermans³

Abstract

The actinobacterium *Rhodococcus* sp. JG-3 is an aerobic, eurypsychrophilic, soil bacterium isolated from permafrost in the hyper arid Upper Dry Valleys of Antarctica. It is yellow pigmented, gram positive, moderately halotolerant and capable of growth from 30 °C down to at least -5 °C. The 5.28 Mb high-quality-draft genome is arranged into 6 scaffolds, containing 9 contigs and 4998 protein coding genes, with 64 % GC content. Increasing the availability of genome sequences from cold-adapted species is crucial to gaining a better understanding of the molecular traits of cold adaptation in microbes.

Keywords: *Rhodococcus* sp. JG-3, Permafrost, Eurypsychrophile, Dry valleys, Antarctica

Introduction

Actinobacteria is a ubiquitous phylum in the biosphere, including many environments that exist predominantly and perennially at sub-zero temperatures (cryoenvironments) such as massive ground ice, polar and alpine saline springs and lakes, cryopegs, and permafrost, where it is often a dominant phylum [1]. The molecular traits which allow *Actinobacteria* to predominate in cryoenvironments remains largely unknown. *Actinobacteria* may be protected in the permafrost environment by cyst-like resting forms or arthrospores, as observed in *Arthrobacter* and *Micrococcus* species isolated from permafrost [2]. It is also possible that dominance of *Actinobacteria* are due to increased viability and activity in this phylum, as *Actinobacteria* that can metabolize at sub-zero temperatures have been found [3, 4]. Though Antarctic permafrost has generally been found to harbor orders of magnitude lower culturable microorganisms (0-10⁵ cells/g) than Arctic permafrost, *Rhodococcus* spp. have been readily isolated from both Antarctic and Arctic permafrost [5]. The

genome sequence of *Rhodococcus* sp. JG-3 is also of interest since species within the genus *Rhodococcus* are known to have versatile degradative metabolisms for recalcitrant xenobiotics [6], including the capability to degrade halogenated organics [7], short and long chain alkanes [8], and petroleum hydrocarbons [9]. Several reports have investigated the catabolic potential of *Rhodococcus* spp. for contaminant removal at cold temperatures [8, 10, 11]. The public availability of other mesophilic *Rhodococcus* genomes, in addition to other cryophilic bacterial isolates will enable identification of genes and molecular traits which enable cryophilic organisms like *Rhodococcus* sp. JG-3 to thrive in cold and extreme environments.

Organism information

Classification and features

Rhodococcus sp. JG-3 is a yellow pigmented strain capable of growth from 30 °C down to at least -5 °C. It does not require salt, but is moderately halotolerant up to 7 % NaCl. It is a Gram positive short rod (Fig. 1), and grows well on TSB and R2A media. *Rhodococcus* sp. JG-3 was isolated from University Valley, a small hanging valley (1650–1800 m.a.s.l) above Beacon Valley in the upper elevation McMurdo Dry Valleys, Antarctica.

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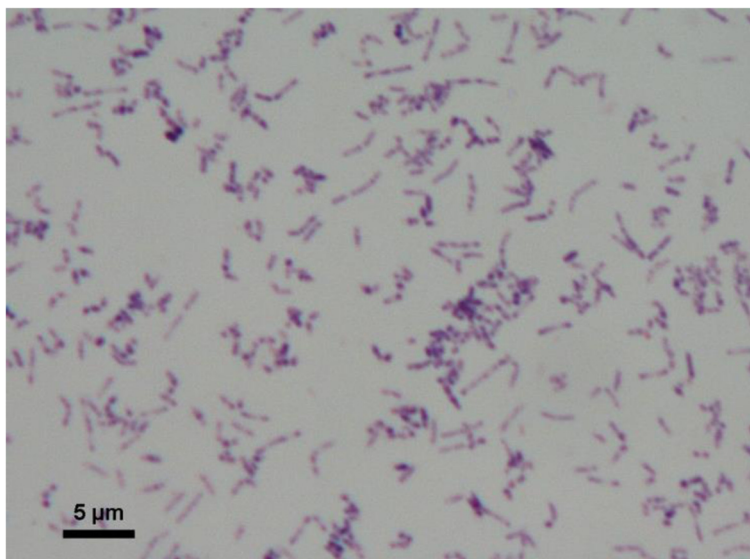


Fig. 1 Gram stain of *Rhodococcus* JG-3

Table 1 Classification and general features of *Rhodococcus* sp. JG-3 [13]

MIGS ID	Property	Term	Evidence code ^a
	Classification	Domain <i>Bacteria</i>	TAS [14]
		Phylum <i>Actinobacteria</i>	TAS [15]
		Class <i>Actinobacteria</i>	TAS [15]
		Order <i>Actinomycetales</i>	TAS [14]
		Family <i>Nocardiaceae</i>	TAS [16]
		Genus <i>Rhodococcus</i>	TAS [14]
		Species <i>Rhodococcus</i>	
		Strain JG-3	
	Gram stain	positive	IDA
	Cell shape	Rod	IDA
	Motility	Not reported	IDA
	Sporulation	Not reported	NAS
	Temperature range	<-5 °C to 30 °C	NAS
	Optimum temperature	~20 °C	IDA
	pH range; Optimum	no data; 7	IDA
	Carbon source	R2A, TSA complex media	IDA
MIGS-6	Habitat	Terrestrial, permafrost soil	IDA
MIGS-6.3	Salinity	0-7 % NaCl	IDA
MIGS-22	Oxygen requirement	aerobic	IDA
MIGS-15	Biotic relationship	free-living	IDA
MIGS-14	Pathogenicity	Non-pathogen	NAS
MIGS-4	Geographic location	University Valley, Dry Valleys, Antarctica	IDA
MIGS-5	Sample collection	December, 2009	IDA
MIGS-4.1	Latitude	77d 51.817 s S	IDA
MIGS-4.2	Longitude	160d43.524 s E	IDA
MIGS-4.4	Altitude	37-42 cm below soil surface, in ice-cemented permafrost	IDA

^aEvidence 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 derived from the Gene Ontology project [17]

This bacterium was isolated from ice-cemented permafrost soils aged ca. 150,000 years old [12] which experience permanent darkness, hyper oligotrophy (0.013 % total carbon), low water activity (<1 % gravimetric soil moisture content) and constant cold temperature (mean annual soil temperature -24°C). The classification and general features of *Rhodococcus* sp. JG-3 are summarized in Table 1.

The 16S rRNA gene sequence of *Rhodococcus* sp. JG-3 was compared using NCBI nucleotide BLAST [18] against the nucleotide collection database (nr/nt) under default parameters, and excluding uncultured microorganisms. *Rhodococcus* sp. JG-3 showed 99 % similarity to that of *R. cercidiphylli* str. BZ22 [19] (GenBank accession: HQ588861.1), a cold adapted isolate from an industrial site contaminated with heavy oil and heavy metals, and which has demonstrated low temperature degradation of petroleum hydrocarbons [9], and 99 % similarity to *Rhodococcus* sp. K4-07B (GenBank accession: EF612291) isolated from a semi-arid lead-zinc mine tailing site [20]. Phylogenetic analysis based on the 16S rRNA gene of taxonomically classified type strains of the family *Nocardiaceae* placed *Rhodococcus fascians* DSM 20669 [21] as the closest validly named species to *Rhodococcus* sp. JG-3 (Fig. 2). *R. fascians* DSM 20669 was originally isolated from sweet peas and has an optimum growth temperature of 24 to 27 $^{\circ}\text{C}$ [21].

Genome sequencing information

Genome project history

Rhodococcus sp. JG-3 was selected for sequencing in 2012 as part of a DOE Joint Genome Institute (JGI) Community Sequencing Program (Quarterly) project to sequence 12 cryophilic isolates from permafrost and cryoenvironments. The Improved Quality Draft assembly and annotation were completed on May 30, 2013. The complete genome sequence of strain JG-3 is available for public access in DDBJ/EMBL/GenBank under accession numbers AXVF01000001- AXVF01000009. The date of Release was December 12, 2013. Table 2 presents the main project information and its association with MIGS version 2.0 compliance [23]. The MIGS record associated with this strain is found in Additional file 1: Table S1.

Growth conditions and DNA isolation

Rhodococcus JG-3 was grown to stationary phase on TSB medium at room temperature. Genomic DNA was isolated using the Epicentre MasterPure Gram Positive DNA Purification Kit (Epicentre, Madison, Wisconsin) as per the manufacturer's instructions. Purified DNA was evaluated with the NanoDrop 1000 (Thermoscientific, Wilmington, Delaware), according to the standards of the DOE Joint Genome Institute.

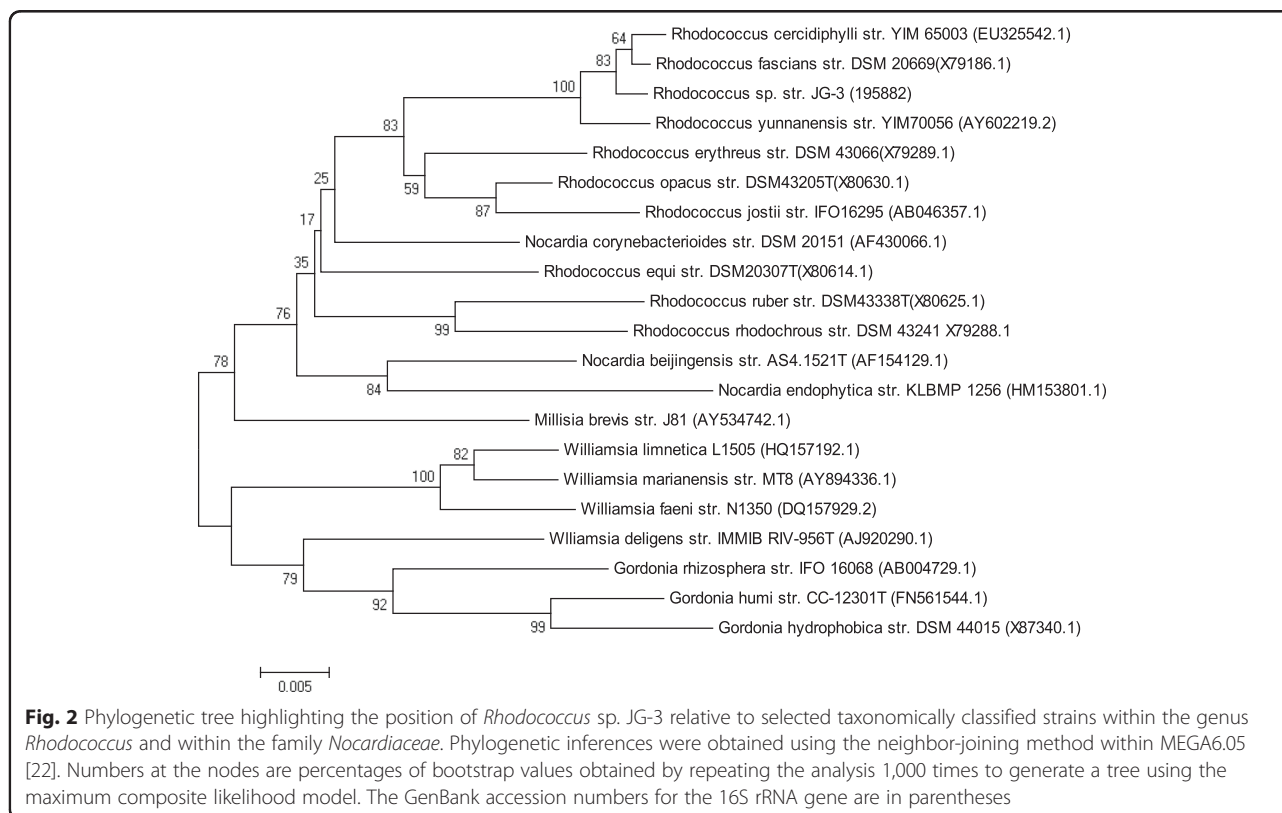


Table 2 Project information

MIGS ID	Property	Term
MIGS 31	Finishing quality	Improved-high-quality draft
MIGS-28	Libraries used	Illumina Std. PE, Illumina Clip PE
MIGS 29	Sequencing platforms	Illumina HiSeq 2000
MIGS 31.2	Fold coverage	1298.1x Illumina coverage
MIGS 30	Assemblers	AllpathsLG
MIGS 32	Gene calling method	Prodigal, GenePrimp
	Locus Tag	K414
	Genbank ID	AXVF00000000
	GenBank Date of Release	December 12, 2013
	GOLD ID	Gi22490
	BIOPROJECT	PRJNA195882
MIGS 13	Source Material Identifier	ARS Culture collection, NRRL: B-65292)
	Project relevance	Permafrost, adaptation to cold, carbon metabolism

Genome sequencing and assembly

The draft genome of *Rhodococcus sp. JG-3* was generated at the DOE Joint Genome Institute (JGI) using the Illumina technology. An Illumina std shotgun library and long insert mate pair library was constructed and sequenced using the Illumina HiSeq 2000 platform [24]. 20,820,738 reads totaling 3,123.1 Mb were generated from the std shotgun sequence and 41,292,560 reads totaling 3,757.6 Mb were generated from the long insert mate pair library. All general aspects of library construction and

Table 3 Nucleotide content and gene count levels of the genome

Attribute	Value	% of Total
Genome size (bp)	5,286,918	100.00
DNA coding (bp)	4,884,848	92.40
DNA G + C (bp)	3,405,333	64.41
DNA scaffolds	6	100.00
Total genes	5,067	100.00
Protein coding genes	4,998	98.64
RNA genes	69	1.36
Pseudo genes	60	1.18
Genes in internal clusters	NA	
Genes with function prediction	3,977	24.18
Genes assigned to COGs	3,805	75.09
Genes with Pfam domains	4,134	81.59
Genes with signal peptides	370	7.30
Genes with transmembrane helices	1,192	23.52
CRISPR repeats	1	-

sequencing performed at the JGI. All raw Illumina sequence data was passed through DUK, a filtering program developed at JGI, which removes known Illumina sequencing and library preparation artifacts [25]. Filtered Illumina reads were assembled using AllpathsLG (PrepareAllpathsInputs: PHRED 64 = 1 PLOIDY = 1 FRAG COVERAGE = 75 JUMP COVERAGE = 25; RunAllpathsLG: THREADS = 8 RUN = std pairs TARGETS = standard VAPI WARN ONLY = True OVERWRITE = True) [26]. The final draft assembly contained 9 contigs in 6 scaffolds. The total size of the genome is 5.3 Mb. The final assembly is based on 3,122.6 Mb of Illumina Std PE, 3,757.6 Mb of Illumina CLIP PE post filtered data, which provides an average 1298.1X Illumina coverage of the genome.

Genome annotation

Genes were identified using Prodigal [27], followed by a round of manual curation using GenePRIMP [28] for

Table 4 Number of genes associated with general COG functional categories

Code	Value	% age	Description
J	176	4.17	Translation, ribosomal structure and biogenesis
A	1	0.02	RNA processing and modification
K	443	10.50	Transcription
L	173	4.10	Replication, recombination and repair
B	1	0.02	Chromatin structure and dynamics
D	31	0.76	Cell cycle control, Cell division, chromosome partitioning
V	53	1.26	Defense mechanisms
T	213	5.05	Signal transduction mechanisms
M	179	4.24	Cell wall/membrane biogenesis
N	6	0.14	Cell motility
U	43	1.02	Intracellular trafficking and secretion
O	133	3.15	Posttranslational modification, protein turnover, chaperones
C	275	6.52	Energy production and conversion
G	288	6.83	Carbohydrate transport and metabolism
E	380	9.01	Amino acid transport and metabolism
F	97	2.3	Nucleotide transport and metabolism
H	184	4.36	Coenzyme transport and metabolism
I	233	5.52	Lipid transport and metabolism
P	244	5.78	Inorganic ion transport and metabolism
Q	166	3.93	Secondary metabolites biosynthesis, transport and catabolism
R	566	13.42	General function prediction only
S	333	7.89	Function unknown
-	1262	24.91	Not in COGs

The total is based on the total number of protein coding genes in the genome

finished genomes and Draft genomes in fewer than 10 scaffolds. The predicted CDSs were translated and used to search the National Center for Biotechnology Information nonredundant database, UniProt, TIGRFam, Pfam, KEGG, COG, and InterPro databases. The tRNAScanSE tool [29] was used to find tRNA genes, whereas ribosomal RNA genes were found by searches against models of the ribosomal RNA genes built from SILVA [30]. Other non-coding RNAs such as the RNA components of the protein secretion complex and the RNase P were identified by searching the genome for the corresponding Rfam profiles using INFERNAL [17]. Additional gene prediction analysis and manual functional annotation was performed within the Integrated Microbial Genomes platform [1] developed by the Joint Genome Institute, Walnut Creek, CA, USA [31].

Genome properties

The improved high quality draft genome includes 9 contigs in 6 scaffolds, for a total size of 5286918 bp, 64.41 % GC content. Most of the genome (96 %, 5092715 bp) assembled into one scaffold. For the genome, 5067 genes were predicted, 4998 of which are protein-coding genes; 3977 protein coding genes were assigned to a putative function with the remaining annotated as hypothetical proteins. The properties and statistics of the genome are summarized in Tables 3 and 4.

Conclusion

The genome sequence of *Rhodococcus* sp. JG-3 will be used for examination of the molecular traits of cold adaptation and to aid understanding of carbon metabolism in cryoenvironments. This is the first reported genome of a bacterium isolated from the Upper Dry Valley permafrost and will provide insight into how microbes survive such extreme conditions. As the availability of genomes from cryophilic strains increases, it may be possible to infer if there is a phylogenetic basis for some cold adaptive traits, as well as identify novel molecular mechanisms for cold adaptation.

Additional file

Additional file 1: Table S1. Associated MIGS record. (DOC 73 kb)

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Overall development and writing of the manuscript was carried out by JG. Isolation of strain was done by JG. Growth and extraction of DNA was carried by JR. Sequencing and annotation of the strain and support was provided by NS, TW IRB, LGW and CB provided critical editing and contributed to the writing of the manuscript.

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