# SHORT GENOME REPORT

**Open Access** 



Draft genome sequence of *Mycobacterium rufum* JS14<sup>T</sup>, a polycyclic-aromatichydrocarbon-degrading bacterium from petroleum-contaminated soil in Hawaii

Yunyoung Kwak<sup>1</sup>, Qing X. Li<sup>2</sup> and Jae-Ho Shin<sup>1\*</sup>

# Abstract

*Mycobacterium rufum* JS14<sup>T</sup> (=ATCC BAA-1377<sup>T</sup>, CIP 109273<sup>T</sup>, JCM 16372<sup>T</sup>, DSM 45406<sup>T</sup>), a type strain of the species *Mycobacterium rufum* sp. . belonging to the family *Mycobacteriaceae*, was isolated from polycyclic aromatic hydrocarbon (PAH)-contaminated soil in Hilo (HI, USA) because it harbors the capability of degrading PAH. Here, we describe the first genome sequence of strain JS14<sup>T</sup>, with brief phenotypic characteristics. The genome is composed of 6,176,413 bp with 69.25 % G + C content and contains 5810 protein-coding genes with 54 RNA genes. The genome information on *M. rufum* JS14<sup>T</sup> will provide a better understanding of the complexity of bacterial catabolic pathways for degradation of specific chemicals.

Keywords: Mycobacterium, Polycyclic aromatic hydrocarbon, Biodegradation

Abbreviations: PAHs, polycyclic aromatic hydrocarbons; SMRT, single-molecule real-time

## Introduction

Polycyclic aromatic hydrocarbons, defined as organic molecules consisting of two or more fused aromatic rings in linear, angular, or cluster arrangement, mostly result from coke production, petroleum refining, fossil fuel combustion, and waste incineration [1]. Although the physical and chemical properties of PAHs vary depending on the number of rings, the characteristics such as hydrophobicity, recalcitrance, and mutagenic and carcinogenic potentials have been considered the main factors for the toxic effects on environmental ecosystems and human beings [1, 2].

For removal of PAHs from contaminated environments, the bioremediation process based on microbial activities has attracted interest and has been actively studied [3]. Various bacteria, such as *Sphingomonas* spp., *Pseudomonas* spp., *Rhodococcus* spp., *Burkholderia* spp., and *Mycobacterium* spp., have been investigated regarding whether they can metabolize PAHs. In particular, several *Mycobacterium* species have been reported to effectively degrade high-molecular-weight PAHs [4, 5]. Moreover, genomic studies on these bacterial species have contributed to the understanding of whole regulatory mechanisms of bacterial PAH degradation, for example for *M. vanbaalenii* PYR-1 [6], *M. gilvum* Spyr1 [7], and *M. gilvum* PYR-GCK [8] as well as the most recently reported *M. aromaticivorans* JS19b1<sup>T</sup> [9].

*M. rufum* JS14<sup>T</sup> (=ATCC BAA-1377<sup>T</sup>, CIP 109273<sup>T</sup>, JCM 16372<sup>T</sup>, DSM 45406<sup>T</sup>) is the type strain of the species *Mycobacterium rufum* sp. nov. [10]. This bacterium was isolated from petroleum-contaminated soil at a former oil gasification company site in Hilo (HI, USA). The bacterium was identified because of PAH degradation activities, especially toward a four-ring-fused compound, fluoranthene [11]. Although the PAH-degrading ability has been demonstrated through metabolic and proteomic assays [12], genetic studies on the whole bacterial system with a PAH degradation pathway have not been conducted. Here, we present a brief summary of the characteristics of this strain and a genetic description of its genome sequence.



© 2016 The Author(s). **Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.

<sup>\*</sup> Correspondence: jhshin@knu.ac.kr

<sup>&</sup>lt;sup>1</sup>School of Applied Biosciences, College of Agriculture and Life Sciences, Kyungpook National University, Daegu 702-701, Republic of Korea Full list of author information is available at the end of the article



**Fig. 1** A neighbor-joining phylogenetic tree depicting the position of *M. rufum* JS14<sup>T</sup> [10] (shown in boldface with an asterisk) relative to the other species within the genus *Mycobacterium*. In this genus, species carrying the full length of 16S rRNA gene sequence were selected from the NCBI database [45]. The collected nucleotide sequences were aligned using ClustalW [46], and the phylogenetic tree was constructed using software MEGA version 6 [47] by the neighbor-joining method with 1000 bootstrap replicates [18]. The generated bootstrap values for each species are presented at the nodes, and the scale bar indicates 0.005 nucleotide changes per nucleotide position. The strains under study and their corresponding GenBank accession numbers for 16S rRNA genes are as follows: *M. chlorophenolicum* PCP-I [14, 15] (NR\_119093); *M. gilvum* Spyr1 [37, 48] (NR\_074644); *M. gilvum* PYR-GCK [37, 48] (NR\_074553); *M. rhodesiae* NBB3 [49] (NR\_102870); *M. vanbaalenii* PYR-1 [16] (NR\_074572); *M. fluoranthenivorans* FA4 [17, 50] (NR\_042224); *M. wolinskyi* 700010 [51] (NR\_119253); *M. mageritense* 938 [52] (NR\_042265); *M. smegmatis* str. MC2 155 [37, 53] (NR\_074726); *M. flavescens* ATCC 14474 [37, 54] (NR\_044815); *M. novocastrense* 73 [55] (NR\_029208); *M. insubricum* FI-06250 [56] (NR\_125525); *M. florentinum* FI-93171 [57] (NR\_042223); *M. montefiorense* ATCC BAA-256 [58, 59] (NR\_028808); *M. confluentis* 1389/90 [60] (NR\_042245); *M. holsaticum* 1406 [61] (NR\_028945); *M. elephantis* DSM 44368 [62] (NR\_025296); *M. marinum* M [37, 63] (NR\_074864); *M. alicerans* Agy99 [37, 64] (NR\_074835)

# Organism information

# Classification and features

The 16S ribosomal RNA gene sequence of M. rufum JS14<sup>T</sup> was compared with those from other *Mycobacter*ium species using the BLAST software of NCBI [13]. The highest similarity was found with M. chlorophenolicum PCP-1 (99 % identity) [14, 15] followed by M. gilvum Spyr1 (99 % identity) [7], M. gilvum PYR-GCK (99 % identity) [8], M. vanbaalenii PYR-1 (98 % identity) [16], and *M. fluoranthenivorans* FA4T (97 % identity) [17]. Species identified by the BLAST search and represented by full-length 16S rRNA gene sequences were included in the phylogenetic analysis. The phylogenetic tree was generated by the neighbor-joining method [18], and bootstrapping was set to 1000 times for random replicate selections. The consensus phylogenetic neighborhood of *M. rufum* JS14<sup>T</sup> within the genus *Mycobac*terium is shown in Fig. 1.

*M. rufum*  $JS14^{T}$  is a non-motile, aerobic, Gram-positive bacterium belonging to the family *Mycobacteriaceae* [10].



**Fig. 2** A scanning electron micrograph of *M. rufum* JS14<sup>1</sup>. The image was taken using a Field Emission Scanning Electron Microscope (SU8220; Hitachi, Japan) at an operating voltage of 10.0 kV. The scale bar represents 5.0  $\mu$ m

The cell shape is medium-to-long thin rods, and cell size is approximately 1.0–2.0 µm in length with the width of 0.4–0.6 µm as shown in Fig. 2. Generally, large, round, raised, smooth orange-pigmented colonies form within 7 days [10]. As one of the rapidly growing members of the genus *Mycobacterium*, the strain grows optimally at 28 °C, reduces nitrate, but does not tolerate salinity (over 2.5 % NaCl, w/v) [10]. Strain JS14<sup>T</sup> shows positive reactions in tests for catalase,  $\alpha$ -glucosidase, aesculin hydrolysis, and urease, but negative reactions regarding  $\beta$ -glucuronidase,  $\beta$ -galactosidase, *N*-acetyl- $\beta$ -glucosaminidase, gelatin hydrolysis, alkaline phosphatase, and pyrrolidonyl arylamidase activities [10]. Substrate oxidation was noticed for Tween 40, Tween 80, D-gluconic acid, D-glucose, D-fructose, D-xylose, D-mannose, D-psicose, trehalose, dextrin, glycogen, and D-mannitol, but not for  $\alpha$ -/ $\beta$ -cyclodextrin, D-galactose,  $\alpha$ -D-lactose, maltose, sucrose, mannan, or maltotriose [10]. When cultured in the minimal medium (per liter: 8.8 g of Na<sub>2</sub>HPO<sub>4</sub>°2H<sub>2</sub>O, 3.0 g of KH<sub>2</sub>PO<sub>4</sub>, 1.0 g of NH<sub>4</sub>Cl, 0.5 g of NaCl, 1.0 mL of 1 M MgSO<sub>4</sub>, and 2.5 mL of a trace element solution [per liter: 23 mg of MnCl<sub>2</sub>°2H<sub>2</sub>O, 30 mg of MnCl<sub>4</sub>·H<sub>2</sub>O, 31 mg of H<sub>3</sub>BO<sub>3</sub>, 36 mg of CoCl<sub>2</sub>°6H<sub>2</sub>O, 10 mg of CuCl<sub>2</sub>°2H<sub>2</sub>O, 20 mg of NiCl<sub>2</sub>°6H<sub>2</sub>O, 30 mg of Na<sub>2</sub>MoO<sub>4</sub>°2H<sub>2</sub>O, and 50 mg of ZnCl<sub>2</sub>]) [11] supplemented with the four-aromatic ring-fused PAH compound fluoranthene (final

**Table 1** Classification and general features of *M. rufum* JS14<sup>T</sup> [22]

MIGS ID	Property	Term	Evidence code <sup>a</sup>
	Classification	Domain Bacteria	TAS [33]
		Phylum Actinobacteria	TAS [34]
		Class Actinobacteria	TAS [35]
		Order Actinomycetales	TAS [36–38]
		Family Mycobacteriaceae	TAS [37–39]
		Genus Mycobacterium	TAS [37, 40, 41]
		Species Mycobacterium rufum	TAS [37, 39]
		(Type) strain: JS14 <sup>T</sup> (=ATCC BAA-1377 <sup>T</sup> , CIP 109273 <sup>T</sup> , JCM 16372 <sup>T</sup> , DSM 45406 <sup>T</sup> )	TAS [10]
	Gram stain	Positive: weak uptake of Gram stain	TAS [10]
	Cell shape	Medium to long thin rods	TAS [10]
	Colony pigmentation	Orange	TAS [10]
	Motility	Non-motile	TAS [10]
	Sporulation	Not reported	NAS
	Temperature range	Mesophile	NAS
	Optimum temperature	28 °C	TAS [10]
	pH range; Optimum	7.0–8.0; 7.5	NAS
	Carbon source	Fluoranthene, glucose, fructose, mannitol, trehalose, xylose, others	TAS [10–12]
	Energy source	Fluoranthene	TAS [11, 12]
MIGS-6	Habitat	Soil	TAS [10]
MIGS-6.3	Salinity	Not tolerant salinity (2.5–5.0 % NaCl, w/v)	TAS [10]
MIGS-22	Oxygen requirement	Aerobic	TAS [10]
MIGS-15	Biotic relationships	Free living	NAS
MIGS-14	Pathogenicity	None	NAS
MIGS-4	Geographic location	Hawaii, United States	TAS [10]
MIGS-5	Sample collection	February, 2003	NAS
MIGS-4.1	Latitude	19° 49' 20" N	TAS [11]
MIGS-4.2	Longitude	155° 05' 01" W	TAS [11]
MIGS-4.3	Depth	Not reported	
MIGS-4.4	Altitude	Not reported	

<sup>a</sup>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 the Gene Ontology project [42]

MIGS ID	Property	Term
MIGS-31	Finishing quality	Draft
MIGS-28	Libraries used	20 kb SMRT-bell library
MIGS-29	Sequencing platforms	PacBio RS II
MIGS-31.2	Fold coverage	113.03×
MIGS-30	Assemblers	RS HGAP Assembly Protocol [24] in SMRT analysis pipeline v.2.2.0
MIGS-32	Gene-calling method	NCBI Prokaryotic Genome Annotation Pipeline [43]; GeneMarkS+ [44]
	Locus Tag	EU78
	INSDC ID	JROA0000000
	GenBank Date of Release	October 2, 2014
	GOLD ID	Gi0074119
	BIOPROJECT	PRJNA247390
MIGS-13	Source Material Identifier	atcc baa-1377 <sup>T</sup> , CIP 109273 <sup>T</sup> , JCM 16372 <sup>T</sup> , DSM 45406 <sup>T</sup>
	Project relevance	Environmental

concentration of 40 mg/L), *M. rufum*  $JS14^{T}$  showed an effective degrading action on the added compound by utilizing it completely during 10 days as a sole source of carbon and energy [11].

#### Chemotaxonomic data

The main cellular fatty acids of *M. rufum* JS14<sup>T</sup> are C18:1 $\omega$ 9c (36.72 %), C16:0 (26.24 %), C16:1 $\omega$ 7c + C16:1 $\omega$ 6c (9.40 %), C17:1 $\omega$ 7c (8.44 %), C14:0 (5.27 %), C18:0 (3.14 %), and C17:0 (1.94 %), respectively [10]. The profile of whole-cell fatty acids showed a pattern similar to that of the other representative of *Mycobacter-ium* species [10, 19–21]. The strain showed bright red color under a microscope after acid-fast staining. A gas chromatogram of fatty acid methyl esters from the transmethylated cells of *M. rufum* JS14<sup>T</sup> revealed a major C24:0 peak and a trace of a C22:0 peak. The general characteristics of the strain are summarized in Table 1.

### Genome sequencing information

#### Genome project history

Strain *M. rufum* JS14<sup>T</sup> was selected for sequencing because of its effective ability to degrade PAH, as a model organism for a recalcitrant organic-pollutant-degrading bacterium. The genome sequencing was performed in September, 2014, and the Whole Genome Shotgun project was deposited in the DDBJ/EMBL/GenBank databases under the accession number JROA00000000. The version described in this study is the first version, labeled JROA00000000.1. The sequencing project information and its association with the Minimum Information about a Genome Sequence version 2.0 compliance [22] are described in Table 2.

#### Growth conditions and genomic DNA preparation

*M. rufum* JS14<sup>T</sup> from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (strain accession number DSM 45406<sup>T</sup>) was used for preparation of genomic DNA. The strain was cultured aerobically in a 250-mL Erlenmeyer flask containing 50 mL of tryptic soy broth (Difco Laboratories Inc., Detroit, MI), on a rotary shaker at 200 rpm and 30 °C. Genomic DNA was isolated from 50 mL of culture using the QIAamp<sup>®</sup> DNA Mini Kit (Qiagen, Valencia, CA) following the standard protocol recommended by the manufacturer. The quantity and purity of the extracted genomic DNA were assessed with a Picodrop Microliter UV/Vis Spectrophotometer

Table 3	Genome	statistics
---------	--------	------------

Attribute	Value	% of Total
Genome size (bp)	6,176,413	100.00
DNA coding (bp)	5,622,516	91.03
DNA G + C (bp)	4,277,025	69.25
DNA scaffolds	4	100.00
Total genes	5864	100.00
Protein-coding genes	5810	99.08
RNA genes	54	0.92
Pseudogenes	367	6.26
Genes in internal clusters	944	16.10
Genes with function prediction	4498	76.71
Genes assigned to COGs	3669	62.57
Genes with Pfam domains	4544	77.49
Genes with signal peptides	314	5.35
Genes with transmembrane helices	1227	20.92
CRISPR repeats	0	0.00

(Thermo Fisher Scientific Inc., Waltham, MA) and Qubit<sup>®</sup> 2.0 Fluorometer (Fisher Scientific Inc.), respectively. Finally, a DNA concentration of 780.0 ng/ $\mu$ L and OD  $_{260}$ /OD  $_{280}$  of 1.87 was determined.

#### Genome sequencing and assembly

The genome of *M. rufum* JS14<sup>T</sup> was sequenced using the single-molecule real-time DNA sequencing platform on the Pacific Biosciences RS II sequencer with P5 polymerase - C3 sequencing chemistry (Pacific Biosciences, Menlo Park, CA) [23]. A 20-kb insert SMRT-bell library was prepared from the sheared genomic DNA and loaded onto two SMRT cells. During the single 180-min run-time, 1,020,750,498 read bases were generated with 300,584 reads. Reads of less than 100 bp or with low accuracy (below 0.8) were removed. In total, 111,515 reads produced 823,795,879 bases with a read quality of 0.831.

All post-filtered reads were assembled *de novo* using the RS hierarchical genome assembly process, version 3.3 in SMRT analysis software, version 2.2.0 (Pacific Biosciences) [24] and resulted in 4 contigs corresponding to 4 scaffolds, with 113.03-fold coverage. The maximal contig length and N50 contig length had the same size of 5,760,162 bp. The whole genome was found to be 6,176,413 bp long.

#### Genome annotation

The protein-coding sequences were predicted by Prokaryotic Genome Annotation Pipeline, version 2.8, on the NCBI website (rev. 447580) [25]. Additional gene prediction and functional annotation were performed in the Rapid Annotation using Subsystems Technology server [26] and Integrated Microbial Genomes-Expert Review pipeline [27], respectively.

#### **Genome properties**

The genome size of *M. rufum* JS14<sup>T</sup> was found to be 6,176,413 bp with the average G + C content of 69.25 %. The genome was predicted to contain a total of 5864 genes, which include 5810 protein-coding genes with 54 RNA genes (6 rRNAs, 47 tRNAs, and 1 ncRNA). Of these, 4498 genes were assigned to putative functions, and 3669 genes (approximately 62.57 %) were assigned to the COG functional categories. The genome statistics are presented in Table 3 and Fig. 3, respectively. The gene distribution within the COG functional categories is presented in Table 4.

## Insights from the genome sequence

Regarding the specific degradation capability toward the four-aromatic-ring-fused compound, fluoranthene [10–12],



Code	Value	% age	Description
J	181	4.25	Translation, ribosomal structure and biogenesis
А	1	0.02	RNA processing and modification
К	353	8.29	Transcription
L	118	2.77	Replication, recombination and repair
В	0	0.00	Chromatin structure and dynamics
D	32	0.75	Cell cycle control, cell division, chromosome partitioning
V	98	2.30	Defense mechanisms
Т	173	4.06	Signal transduction mechanisms
Μ	210	4.93	Cell wall/membrane/envelope biogenesis
Ν	12	0.28	Cell motility
U	22	0.52	Intracellular trafficking, secretion, and vesicular transport
0	142	3.34	Post-translational modification, protein turnover, chaperones
С	312	7.33	Energy production and conversion
G	245	5.76	Carbohydrate transport and metabolism
E	333	7.82	Amino acid transport and metabolism
F	89	2.09	Nucleotide transport and metabolism
Н	266	6.25	Coenzyme transport and metabolism
I	422	9.91	Lipid transport and metabolism
Р	224	5.26	Inorganic ion transport and metabolism
Q	264	6.20	Secondary metabolites biosynthesis, transport and catabolism
R	516	12.12	General function prediction only
S	209	4.91	Function unknown
W	2	0.05	Extracellular structures
Х	33	0.78	Mobilome: prophages, transposons
-	2195	37.43	Not in COGs

Table 4 Numbers of genes associated with general COG functional categories

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

the genome of *M. rufum*  $JS14^{T}$  was found to contain corresponding genes encoding proteins for the aromatic-compound degradation.

Generally, it is known that an initial step of the bacterial degradation of PAHs is mainly catalyzed by multicomponent dioxygenases that produce dihydrodiols [28, 29]. In the genome, multiple genes encoding various dioxygenases such as aromatic-ring-hydroxylating dioxygenase (EU78\_28655, 28730, 29130), extradiol dioxygenase (EU78\_24090, 26390), protocatechuate 3,4-dioxygenase alpha subunit (EU78\_29035), protocatechuate 3,4-dioxygenase beta subunit (EU78\_29030), phthalate 3,4-dioxygenase ferredoxin reductase subunit (EU78\_29090), and extradiol ring-cleavage dioxygenase (EU78\_16970, 28720) were predicted. In addition, the genes coding for such enzymes as cytochrome P450 (EU78\_02320, 09230, 14085, 14465, 20055, 26160), methyltransferase (EU78\_01005), flavin-dependent oxidoreductase (EU78\_19900), and 3,4-dihydroxyphthalate decarboxylase (EU78\_28715) were also identified as functional genes on the Kyoto Encyclopedia of Genes and Genomes map [30] for the PAH degradation. Nonetheless, when compared with the complete genome sequences of PAH-degrading organisms [6–9], several genes coding for representative functional enzymes with relevance to PAH degradation such as *nidA* (PAH dioxygenase large subunit), *nidB* (PAH dioxygenase small subunit), *phtAa* (phthalate 3,4-dioxygenase alpha subunit), *phtAb* (phthalate 3,4-dioxygenase beta subunit), *phtB* (phthalate 3,4-dioxygenase beta subunit), *phtB* (phthalate 3,4-dioxygenase beta subunit), *phtB* (phthalate 3,4-cis-dihydrodiol dehydrogenase), *phdE* (cis-3,4-dihydrophenanthrene-3,4,-diol dehydrogenase), and *phdK* (2-formylbenzoate dehydrogenase) were not identified (shown in Table 5).

Generally, research on bacteria degrading PAHs holds great promise for biotechnological applications to decontamination of pollutants [10]. In this regard, understanding of PAH degradation by indigenous microbes is important for evaluation of ecological effects of these microbes [31]. On Hawaiian islands, PAH contamination has occurred through various activities such as the petroleum industry, waste incineration, and fossil fuel

Function ID	Name	M. van <sup>a</sup>	<i>M. gil</i> GCK <sup>a</sup>	<i>M. gil</i> Sp1ª	M. aro <sup>b</sup>	M. ruf <sup>b</sup>
KO:K00448	protocatechuate 3,4-dioxygenase, alpha subunit [EC:1.13.11.3] (pcaG)	1	1	1	1	3
KO:K00449	protocatechuate 3,4-dioxygenase, beta subunit [EC:1.13.11.3] ( <i>pcaH</i> )	1	1	1	1	2
KO:K18253	phthalate 3,4-dioxygenase ferredoxin subunit (phtAc)	0	2	1	2	1
KO:K18254	phthalate 3,4-dioxygenase ferredoxin reductase subunit [EC:1.18.1.3] (phtAd)	1	2	1	0	1
KO:K00517	E1.14 (cytochrome P450)	12	10	10	5	6
KO:K18256	3,4-dihydroxyphthalate decarboxylase [EC:4.1.1.69] (phtC)	1	2	1	0	1
KO:K11943	PAH dioxygenase large subunit [EC:1.13.11] (nidA)	1	2	1	1	0
KO:K11944	PAH dioxygenase small subunit [EC:1.13.11] (nidB)	2	4	2	4	0
KO:K11948	1-hydroxy-2-naphthoate dioxygenase [EC:1.13.11.38] (phdl)	1	2	1	0	0
KO:K11949	4-(2-carboxyphenyl)-2-oxobut-3-enoate aldolase [EC:4.1.2.34] (phdJ)	1	2	1	1	0
KO:K18251	phthalate 3,4-dioxygenase alpha subunit [EC:1.14.12] (phtAa)	1	2	1	0	0
KO:K18252	phthalate 3,4-dioxygenase beta subunit [EC:1.14.12] (phtAb)	1	2	1	1	0
KO:K18255	phthalate 3,4-cis-dihydrodiol dehydrogenase [EC:1.3.1] (phtB)	1	2	1	1	0
KO:K18257	cis-3,4-dihydrophenanthrene-3,4-diol dehydrogenase [EC:1.3.1.49] (phdE)	1	2	1	1	0
KO:K18275	2-formylbenzoate dehydrogenase [EC:1.2.1.78] (phdK)	1	1	1	1	0

Table 5 Comparison of the functional gene counts in the function profile of genome sequences

Comparison of the selected five genome sequences was conducted using function profile categories in the IMG-ER pipeline [27], and the genome sequences analyzed are as follows: *M. van, M. vanbaalenii* PYR-1 (IMG Genome ID 639633044) [6]; *M. gil* GCK, *M. gilvum* PYR-GCK (IMG Genome ID 640427122) [8]; *M. gil* Sp1, *M. gilvum* Spyr1 IMG Genome ID 649633070) [7]; *M. aro, M. aromaticivorans* JS19b1 (whole Genome Sequencing) (IMG Genome ID 2558309009) [9]; *M. ruf, M. rufum* JS14 (whole Genome Sequencing) (IMG Genome ID 2593339261)

Reported sequencing status for the individual genome set: <sup>a</sup> Complete genome sequence; <sup>b</sup> Draft whole-genome sequence

combustion, even via natural causes such as volcanic activity [10]. *Mycobacterium* is a well-known genus capable of mineralizing PAHs [12]. Considering the Hawaiian delicate island ecosystem, several native bacteria belonging to the genus *Mycobacterium* were isolated, *M. rufum* JS14<sup>T</sup> is one of them [10].

One of native isolates from the petroleum-contaminated Hawaiian soil in Hilo (HI, USA), M. aromaticivorans JS19b1<sup>T</sup> [10], is known to have rapid degrading capabilities toward various PAHs such as fluorene, phenanthrene, pyrene, and fluoranthene [10, 11, 29]. Similarly, M. rufum JS14<sup>T</sup> was found as an effective degrader of a fouraromatic-ring-fused compound, fluoranthene, not showing degrading capacity toward other high-molecularweight PAHs (e.g., pyrene, benzo[a]pyrene) or toward low-molecular-weight PAHs (e.g., fluorene, phenanthrene) [11, 12]. The gene annotation profiles for the genome of *M. rufum* JS14<sup>T</sup> may provide important clues to the identity of the whole metabolic pathway for fluoranthene degradation. Just as a recent study on the functional pangenome analysis of the genus Mycobacterium capable of degrading PAHs [32], our data can also help to explain the complexity of bacterial catabolic pathways for degradation of specific chemicals, from the standpoint of microbial ecology.

## Conclusions

*M. rufum*  $JS14^{T}$  was isolated from PAH-contaminated soil of a former oil gasification company site in Hilo (HI,

USA) and was designated as a novel species that was named *Mycobacterium rufum* (ru'fum. L. neut. adj. *rufum* ruddy or red, pertaining to the colony pigmentation of the type strain) [10]. In this study, we presented the genome sequence of the strain. This genetic information may provide new insights that will help to extend the application potential of bacterial bioremediation of various toxic compounds and to elucidate the features of metabolic degradation pathways for PAHs.

#### Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (NRF-2015R1C1A2A01055308).

#### Authors' contributions

QXL isolated and characterized the *M. rufum* JS14<sup>T</sup> and drafted the manuscript. YK and JHS performed all the experiments on the genome sequencing and drafted the manuscript. All authors read and approved the final manuscript.

#### **Competing interests**

The authors declare that they have no competing interests.

#### Author details

 <sup>1</sup>School of Applied Biosciences, College of Agriculture and Life Sciences, Kyungpook National University, Daegu 702-701, Republic of Korea.
 <sup>2</sup>Department of Molecular Biosciences and Bioengineering, University of Hawaii, Honolulu, HI 96822, USA.

Received: 12 June 2015 Accepted: 13 July 2016 Published online: 02 August 2016

#### References

- 1. Cerniglia C. Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation. 1992;3:351–68.
- Lors C, Damidot D, Ponge JF, Perie F. Comparison of a bioremediation process of PAHs in a PAH-contaminated soil at field and laboratory scales. Environ Pollut. 2012;165:11–7.
- Lu XY, Zhang T, Fang HH. Bacteria-mediated PAH degradation in soil and sediment. Appl Microbiol Biotechnol. 2011;89:1357–71.
- Kanaly RA, Harayama S. Advances in the field of high-molecular-weight polycyclic aromatic hydrocarbon biodegradation by bacteria. Microb Biotechnol. 2010;3:136–64.
- Peng RH, Xiong AS, Xue Y, Fu XY, Gao F, Zhao W, et al. Microbial biodegradation of polyaromatic hydrocarbons. FEMS Microbiol Rev. 2008;32:927–55.
- Kim SJ, Kweon O, Jones RC, Edmondson RD, Cerniglia CE. Genomic analysis of polycyclic aromatic hydrocarbon degradation in *Mycobacterium* vanbaalenii PYR-1. Biodegradation. 2008;19:859–81.
- Kallimanis A, Karabika E, Mavromatis K, Lapidus A, Labutti KM, Liolios K, et al. Complete genome sequence of *Mycobacterium* sp. strain (Spyr1) and reclassification to *Mycobacterium gilvum* Spyr1. Stand Genomic Sci. 2011;5:144–53.
- Badejo AC, Badejo AO, Shin KH, Chai YG. A gene expression study of the activities of aromatic ring-cleavage dioxygenases in *Mycobacterium gilvum* PYR-GCK to changes in salinity and pH during pyrene degradation. PLoS One. 2013;8:e58066.
- Kwak Y, Park GS, Lee SE, Li QX, Shin JH. Genome sequence of Mycobacterium aromaticivorans JS19b1<sup>T</sup>, a novel isolate from Hawaiian soil. J Biotechnol. 2014;186:137–8.
- Hennessee CT, Seo JS, Alvarez AM, Li QX. Polycyclic aromatic hydrocarbondegrading species isolated from Hawaiian soils: *Mycobacterium crocinum* sp. nov., *Mycobacterium pallens* sp. nov., *Mycobacterium rutilum* sp. nov., *Mycobacterium rufum* sp. nov. and *Mycobacterium aromaticivorans* sp. nov. Int J Syst Evol Microbiol. 2009;59:378–87.
- Seo JS, Keum YS, Harada RM, Li QX. Isolation and characterization of bacteria capable of degrading polycyclic aromatic hydrocarbons (PAHs) and organophosphorus pesticides from PAH-contaminated soil in Hilo. Hawaii J Agric Food Chem. 2007;55:5383–9.
- Lee SE, Seo JS, Keum YS, Lee KJ, Li QX. Fluoranthene metabolism and associated proteins in *Mycobacterium* sp. JS14. Proteomics. 2007;7:2059–69.
- 13. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215:403–10.
- Briglia M, Eggen RI, Van Elsas DJ, De Vos WM. Phylogenetic evidence for transfer of pentachlorophenol-mineralizing *Rhodococcus chlorophenolicus* PCP-I<sup>T</sup> to the genus *Mycobacterium*. Int J Syst Bacteriol. 1994;44:494–8.
- Haggblom MM, Nohynek LJ, Palleroni NJ, Kronqvist K, Nurmiaho-Lassila EL, Salkinoja-Salonen MS, et al. Transfer of polychlorophenol-degrading *Rhodococcus chlorophenolicus* (Apajalahti et al. 1986) to the genus *Mycobacterium as Mycobacterium chlorophenolicum* comb. nov. Int J Syst Bacteriol. 1994;44:485–93.
- Khan AA, Kim SJ, Paine DD, Cerniglia CE. Classification of a polycyclic aromatic hydrocarbon-metabolizing bacterium, *Mycobacterium* sp. strain PYR-1, as *Mycobacterium vanbaalenii* sp. nov. Int J Syst Evol Microbiol. 2002;52:1997–2002.
- Hormisch D, Brost I, Kohring GW, Giffhorn F, Kroppenstedt RM, Stackebrandt E, et al. *Mycobacterium fluoranthenivorans* sp. nov., a fluoranthene and aflatoxin B1 degrading bacterium from contaminated soil of a former coal gas plant. Syst Appl Microbiol. 2004;27:653–60.
- 18. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4:406–25.
- Derz K, Klinner U, Schuphan I, Stackebrandt E, Kroppenstedt RM. Mycobacterium pyrenivorans sp. nov., a novel polycyclic-aromatic-hydrocarbon-degrading species. Int J Syst Evol Microbiol. 2004;54:2313–7.
- 20. Willumsen P, Karlson U, Stackebrandt E, Kroppenstedt RM. *Mycobacterium frederiksbergense* sp. nov., a novel polycyclic aromatic hydrocarbon-degrading *Mycobacterium* species. Int J Syst Evol Microbiol. 2001;51:1715–22.
- Kleespies M, Kroppenstedt RM, Rainey FA, Webb LE, Stackebrandt E. Mycobacterium hodleri sp. nov., a new member of the fast-growing Mycobacteria capable of degrading polycyclic aromatic hydrocarbons. Int J Syst Evol Microbiol. 1996;46:683–7.
- Field D, Garrity G, Gray T, Morrison N, Selengut J, Sterk P, et al. The minimum information about a genome sequence (MIGS) specification. Nat Biotech. 2008;26:541–7.
- Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, et al. Real-time DNA sequencing from single polymerase molecules. Science. 2009;323:133–8.

- Chin C-S, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, et al. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Meth. 2013;10:563–9.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Ciufo S, Li W. Prokaryotic Genome Annotation Pipeline, in The NCBI Handbook. Bethesda: National Center for Biotechnology Information; 2013.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, et al. The RAST Server: rapid annotations using subsystems technology. BMC Genomics. 2008;9:75.
- Markowitz VM, Mavromatis K, Ivanova NN, Chen I-MA, Chu K, Kyrpides NC. IMG ER: a system for microbial genome annotation expert review and curation. Bioinformatics. 2009;25:2271–8.
- Moody JD, Fu PP, Freeman JP, Cerniglia CE. Regio- and stereoselective metabolism of 7,12-dimethylbenz[a]anthracene by *Mycobacterium* vanbaalenii PYR-1. Appl Environ Mirobiol. 2003;69:3924–31.
- Seo JS, Keum YS, Li QX. Mycobacterium aromativorans JS19b1<sup>T</sup> degrades phenanthrene through C-1,2, C-3,4 and C-9,10 dioxygenation pathways. Int Biodeterior Biodegradation. 2012;70:96–103.
- Kanehisa M, Goto S. KEGG:kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000;28:27–30.
- Ren G, Ren W, Teng Y, Li Z. Evident bacterial community changes but only slight degradation when polluted with pyrene in a red soil. Front Microbiol. 2015;6:22.
- 32. Kweon O, Kim SJ, Blom J, Kim SK, Kim BS, Baek DH, et al. Comparative functional pan-genome analyses to build connections between genomic dynamics and phenotypic evolution in polycyclic aromatic hydrocarbon metabolism in the genus *Mycobacterium*. BMC Evol Biol. 2015;15:21.
- Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci U S A. 1990;87:4576–9.
- Garrity GM, Holt JG. The Road Map to the Manual. In: Garrity GM, Boone DR, Castenholz RW, editors. Bergey's Manual of Systematic Bacteriology. Volume 1. 2nd ed. New York: Springer; 2001. p. 119.
- Stackebrandt E, Rainey FA, Ward-rainey NL. Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. Int J Syst Bacteriol. 1997;47: 479–91.
- Buchanan RE. Studies in the nomenclature and classification of the bacteria: II. The primary subdivisions of the schizomycetes. J Bacteriol. 1917;2:155–64.
- Skermen VBD, Mcgowan V, Sneath PHA. Approved lists of bacterial names. Int J Syst Bacteriol. 1980;30:225–420.
- Zhi X-Y, Li W-J, Stackebrandt E. An update of the structure and 16S rRNA gene sequence-based definition of higher ranks of the class Actinobacteria, with the proposal of two new suborders and four new families and emended descriptions of the existing higher taxa. Int J Syst Evol Microbiol. 2009;59:589–608.
- Chester FD. Report of mycologist: bacteriological work. Del Agr Exp Sta Bull. 1897;9:38–145.
- Lehmann KB, Neumann R. Atlas und Grundriss der Bakteriologie und Lehrbuch der speziellen bakteriologischen Diagnostik. 1st ed. München: J. F. Lehmanns; 1896. p. 1–448.
- Runyon EH, Wayne LG, Kubica GP, Genus I. *Mycobacterium* Lehmann and Neumann. 1896, 363. In: Buchanan RE, Gibbons NE, editors. Bergey's Manual of Determinative Bacteriology. 8th ed. Baltimore: The Williams and Wilkins Co; 1974. p. 682.
- 42. Kodaka H, Armfield AY, Lombard GL, Dowell Jr VR. Practical procedure for demonstrating bacterial flagella. J Clin Microbiol. 1982;16:948–52.
- Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity G, et al. Toward an online repository of standard operating procedures (SOPs) for (meta)genomic annotation. Omics. 2008;12:137–41.
- Lukashin AV, Borodovsky M. GeneMark.hmm: new solutions for gene finding. Nucleic Acids Res. 1998;26:1107–15.
- Benson DA, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. GenBank. Nucleic Acids Res. 2014;42(Database issue):D32–7.
- Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, et al. Clustal W and Clustal X version 2.0. Bioinformatics. 2007;23:2947–8.
- 47. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 2013;30:2725–9.
- Stanford JL, Gunthorpe WJ. A study of some fast-growing scotochromogenic mycobacteria including species descriptions of *Mycobacterium gilvum* (new species) and *Mycobacterium duvalii* (new species). Br J Exp Pathol. 1971;52:627–37.

- 49. Tsukamura M, Mizuno S, Tsukamura S. Numerical analysis of rapidly growing scotochromogenic mycobacteria, including *Mycobacterium obusense* sp. nov., nom. rev., *Mycobacterium rhodesiae* sp. nov., nom. rev., *Mycobacterium aichiense* sp. nov., nom. rev., *Mycobacterium chubuense* sp. nov., nom. rev., and *Mycobacterium tokaiense* sp. nov., nom. rev. Int J Syst Bacteriol. 1981;31:263–75.
- Validation List No. 110. List of new names and new combinations previously effectively, but not validly, published. Int J Syst Evol Microbiol. 2006; 56:1459-1460.
- 51. Brown BA, Springer B, Steingrube VA, Wilson RW, Pfyffer GE, Garcia MJ, et al. Mycobacterium wolinskyi sp. nov. and Mycobacterium goodii sp. nov., two new rapidly growing species related to Mycobacterium smegmatis and associated with human wound infections: a cooperative study from the international working group on mycobacterial taxonomy. Int J Syst Bacteriol. 1999;49:1493–511.
- Domenech P, Jimenez MS, Menendez MC, Bull TJ, Samper S, Manrique A, et al. Mycobacterium mageritense sp. nov. Int J Syst Bacteriol. 1997;47:535–40.
- Lehmann KB, Lehmann's NR, Medizin HX. Atlas und Grundriss der Bakteriologie und Lehrbuch der speziellen bakteriologischen Diagnostik. 2nd ed. München: J. F. Lehmann; 1899. p. 1–497.
- Bojalil LF, Cerbon J, Trujillo A. Adansonian classification of mycobacteria. J Gen Microbiol. 1962;28:333–46.
- Shojaei H, Goodfellow M, Magee JG, Freeman R, Gould FK, Brignall CG. Mycobacterium novocastrense sp. nov., a rapidly growing photochromogenic mycobacterium. Int J Syst Bacteriol. 1997;47:1205–7.
- Tortoli E, Baruzzo S, Heijdra Y, Klenk HP, Lauria S, Mariottini A, et al. Mycobacterium insubricum sp. nov. Int J Syst Evol Microbiol. 2009;59:1518–23.
- Tortoli E, Rindi L, Goh KS, Katila ML, Mariottini A, Mattei R, et al. Mycobacterium florentinum sp. nov., isolated from humans. Int J Syst Evol Microbiol. 2005:55:1101–6.
- Levi MH, Bartell J, Gandolfo L, Smole SC, Costa SF, Weiss LM, et al. Characterization of *Mycobacterium montefiorense* sp. nov., a novel pathogenic *Mycobacterium* from moray eels that is related to *Mycobacterium triplex*. J Clin Microbiol. 2003;41:2147–52.
- Validation List No. 94. Validation of publication of new names and new combinations previously effectively published outside the IJSEM. Int J Syst Evol Microbiol. 2003; 53:1701–1702.
- Kirschner P, Teske A, Schröder KH, Kroppenstedt RM, Wolters J, Böttger EC. Mycobacterium confluentis sp. nov. Int J Syst Bacteriol. 1992;42:257–62.
- Richter E, Niemann S, Gloeckner FO, Pfyffer GE, Rüsch-Gerdes S. Mycobacterium holsaticum sp. nov. Int J Syst Evol Microbiol. 2002;52:1991–6.
- Shojaei H, Magee JG, Freeman R, Yates M, Horadagoda NU, Goodfellow M. Mycobacterium elephantis sp. nov., a rapidly growing nonchromogenic Mycobacterium isolated from an elephant. Int J Syst Evol
- Microbiol. 2000;50:1817–20.
  Aronson JD. Spontaneous tuberculosis in salt water fish. J Infect Dis. 1926;
- Aronson JD. Spontaneous tuberculosis in sait water inst. J intect Dis. 1920; 39:315–20.
- MacCallum P, Tolhurst JC, Buckle G, Fenner F. The significance of the incubation period in infectious diseases. Med J Aust. 1950;2:813–8.
- 65. Karlson AG, Lessel EF. *Mycobacterium bovis* nom. nov. Int J Syst Bacteriol. 1970;20:273–82.
- van Soolingen D, Hoogenboezem T, de Haas PE, Hermans PW, Koedam MA, Teppema KS, et al. A novel pathogenic taxon of the *Mycobacterium tuberculosis* complex, Canetti: characterization of an exceptional isolate from Africa. Int J Syst Bacteriol. 1997;47:1236–45.
- Castets M, Rist N, Boisvert H. La variété africaine du bacille tuberculeux humain. Med Afr Noire. 1969;16:321–2.
- Alikhan N-F, Petty N, Ben Zakour N, Beatson S. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics. 2011;12:402.

# Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at www.biomedcentral.com/submit

