Genome sequence and description of *Actinomyces polynesiensis* str. MS2 sp. nov. isolated from the human gut

T. Cimmino[1], S. Metidji[1], N. Labas[1], S. Le Page[1], D. Musso[2], D. Raoult[1] and J.-M. Rolain[1]

1) *Aix-Marseille Univ., URMITE UM 63 CNRS 7278 IRD 198 INSERM U1905, IHU Méditerranée Infection, Facultés de Médecine et de Pharmacie, Marseille, France and* 2) *Institut Louis Malardé, Papeete, Tahiti, French Polynesia*

Abstract

Actinomyces polynesiensis strain MS2 gen. nov., sp. nov. is a newly proposed genus within the family *Actinomycetaceae*, isolated from the stools of a healthy individual in Raiatea Island (French Polynesia, South Pacific). *Actinomyces massiliensis* is an anaerobic, Gram-positive organism. Here we describe the features of this organism, together with the complete genome sequence and annotation—2 943 271 bp with a 70.80% G+C content, assembled into 15 scaffolds and containing 2080 genes.

Keywords: *Actinomyces*, taxo-genomics, Culturomics
Original Submission: 4 February 2016; **Accepted:** 29 February 2016
Article published online: 23 March 2016

Corresponding author: J.-M. Rolain, Aix-Marseille Univ., URMITE UM 63 CNRS 7278 IRD 198 INSERM U1905, IHU Méditerranée Infection, Facultés de Médecine et de Pharmacie, 27 boulevard Jean Moulin, 13385 Marseille CEDEX 05, France
E-mail: jean-marc.rolain@univ-amu.fr

Introduction

The genus *Actinomyces* belongs to the domain Bacteria, Phylum *Actinobacteria*, Class *Actinobacteria*, Order *Actinomycetales* and Family *Actinomycetaceae*. The genus consists of a heterogeneous group of Gram-positive bacteria that have a high G+C content [1].

The genus *Actinomyces* consists of a heterogeneous group of Gram-positive bacilli, mainly facultatively anaerobic or microaerophilic rods with various degrees of branching [2]. *Actinomyces* species mainly belong to the human commensal flora of the oropharynx, gastrointestinal tract, and urogenital tract [3].

Actinomycosis is a very rare disease usually caused by one of a group of opportunistic but otherwise harmless commensals and may be complicated by one or more of another group of co-pathogens [4]. Actinomycetes are prominent among the normal flora of the oral cavity but less prominent in the lower gastrointestinal tract and female genital tract. As these microorganisms are not virulent, they require a break in the integrity of the mucous membranes and the presence of devitalized tissue to invade deeper body structures and cause human illness [4]. Many studies based on phenotypic identification of members of the genus *Actinomyces* have been performed. Several flowcharts have been proposed to enable accurate differentiation. However, during the past few years, *Actinomyces* taxonomy has undergone much improvement, primarily driven by *16S* rRNA gene sequence analysis [5]. The study of *Actinomyces polynesiensis* str. MS2 is part of a project called 'Culturomics', the comprehensive determination of the microbial composition of the gut microbiota and the relationships with health and disease, which are major challenges in the twenty-first century. Metagenomic analysis of the human gut microbiota detects mostly uncultured bacteria as reported here [6].

Materials and Methods

Growth conditions and identification

Actinomyces polynesiensis sp. nov., strain MS2 (=CSURP658=DSMZ 27066).

http://dx.doi.org/10.1016/j.nmni.2016.02.014

Actinomyces polynesiensis str. MS2 was isolated from the stools of a healthy individual in Raiatea Island (French Polynesia, South Pacific). *Actinomyces polynesiensis* MS2 was isolated for the first time using inoculation in a blood culture bottle with coconut milk and then 5% sheep blood agar. Growth was tested on Columbia agar supplemented with 5% sheep blood and chocolate agar + PolyViteX (bioMérieux, Marcy-l'Étoile, France) in aerobic and anaerobic condition using GasPak™ EZ Anaerobe Container System Sachets (BD, Franklin Lakes, NJ, USA) at 37°C and CO_2 Gen (ThermoScientific, Waltham, MA, USA). The ability of the strain to grow at different temperatures (25°C, 37°C and 45°C) was investigated.

Gram staining and electron microscopy were performed with a TechnaiG^2 Cryo (FEI Company, Hillsboro, OR, USA) at an operating voltage of 200 keV. Cells were grown on 5% sheep-blood agar for 24 h. A bacterial suspension was pre-fixed in 5% (volume/volume) glutaraldehyde in phosphate buffer (Gibco, Waltham, MA, USA) for at least 1 h at room temperature, washed in the same buffer and stained with 1% (weight/volume) ammonium molybdate 1%. Catalase activity, as determined by an ID Color catalase test kit (bioMérieux) and oxidase activity, assayed by applying the cells to moistened discs impregnated with dimethyl-*p*-phenylenediamine (bioMérieux). Biochemical tests were performed with the commercially available API ZYM and API 50 CH strips and were used to characterize the biochemical properties of the strain according to the manufacturer's instructions. The bacterium was identified by sequence analysis of the *16S* rRNA. Phylogenetic relationship with closely related species were determined using MEGA version 5.1. The evolutionary history was inferred using the maximum likelihood method based on the JTT matrix-based model [7].

Antibiotic susceptibility testing

Antibiotic susceptibility testing was conducted using the disc diffusion method on Müller–Hinton agar supplemented with 5% sheep blood medium (bioMérieux) and the results were interpreted using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [8]. The antibiotics used in this study were penicillin, oxacillin, vancomycin, teicoplanin, linezolid, gentamicin, ciprofloxacin, trimethroprim-sulphamethoxazole, fosfomycin, doxycycline, erythromycin, clindamycin, rifampicin and colistin.

Genome sequencing

DNA extracted through the BioRobot EZ 1 Advanced XL (Qiagen, Hilden, Germany) paired end library was pyrosequenced on the 454_Roche_Titanium. The global 297 979 passed filter sequences generated 85.84 Mb with a length average of 281 bp. These sequences were assembled on the gsAssembler from Roche with 90% identity and 40 bp as overlap (Roche, Basel, Switzerland). It leads to 15 scaffolds and 309 large contigs (>1500 bp), generating a genome size of 2.94 Mb, which corresponds to a coverage of 29.2 genome equivalent.

Genome annotation

The genome was annotated by Rapid Annotation using the Subsytem Technology (RAST) bioserver [9]. Blast2GO optimizes the function transfer from homologous sequences through an elaborate algorithm that considers the similarity, the extension of the homology, the database of choice, the GO hierarchy, and the quality of the original annotations [10]. The resistome was analysed with the ARG-ANNOT (Antibiotic Resistance Gene-ANNOTation) database [11] and BLASTp in GenBank. The prediction of rRNAs was carried out using the RNAmmer tools [12]. The exhaustive bacteriocin database available in our laboratories (Bacteriocins of the URMITE database; http://drissifatima.wix.com/bacteriocins) was performed by collecting all currently available sequences from the databases and from NCBI. Protein sequences from this database allowed putative bacteriocins from human gut microbiota to be identified using BLASTp methodology [13].

Results

Description of *Actinomyces polynesiensis* sp. nov., strain MS2

Phenotypic properties. Actinomyces polynesiensis (*polinesiense* masc. adj. of *πολυνησίας*, the ancient Greek name for the islands of French Polynesia, where the strain was isolated). *Actinomyces polynesiensis* grew slowly, with characteristics similar to those of the filamentous fungi; which have the ability to grow in branched filaments and to form settlers and the typical mycelium seen with other smaller beaded white colonies (Fig. 1). *Actinomyces polynesiensis* MS2 was grown anaerobically on 5% sheep-blood-enriched Columbia agar at 37°C. Optimal growth was achieved anaerobically using chocolate agar + PolyViteX with 5% CO_2 at 37°C and no growth was observed under aerobic conditions. Catalase and oxidase activity were respectively positive and negative. A motility test was positive. Acid production was observed for the following carbohydrates, with API 50 CH negative for D-arabinose, L-xylose, D-adonitol, methyl-β-D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inuline, D-tagatose, D-fucose, L-arabitol, potassium 2- and 5-gluconate; all other acid production was positive (Table 1). All activities were negative with API ZYM (Table 1).

Further identification was performed using a *16S* rRNA nucleotide sequence (GenBank accession number HF952919)

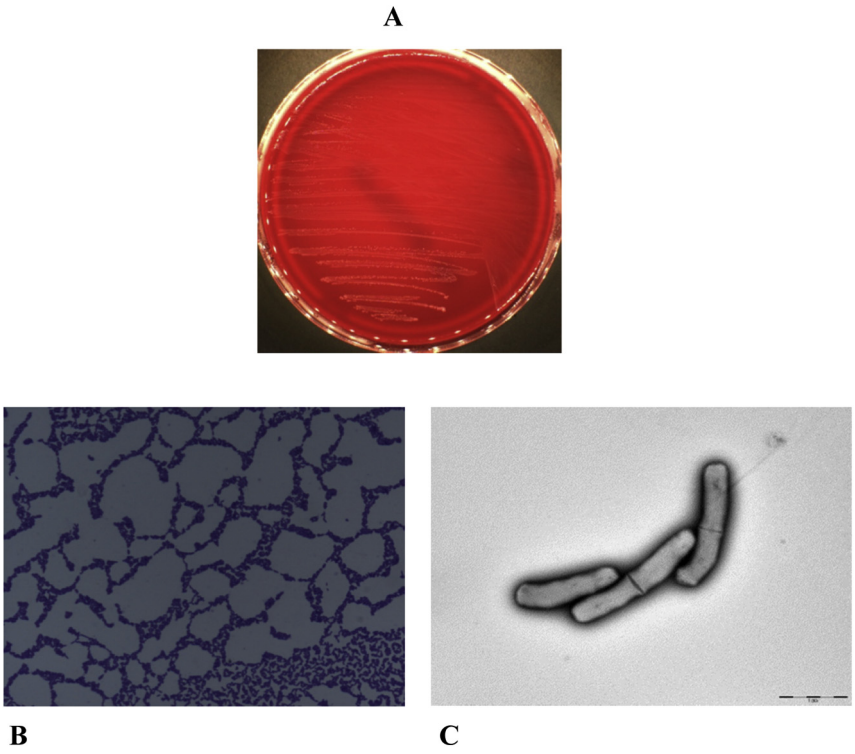

FIG. 1. (a) *Actinomyces polynesiensis* str. MS2 isolated on Müller–Hinton agar supplemented with 5% sheep blood medium (bioMérieux); (b) Gram staining of *Actinomyces polynesiensis* str. MS2; (c) electron microscopy of *Actinomyces polynesiensis* str. MS2 performed with a TechnaiG2 Cryo (FEI Company) at an operating voltage of 200 keV.

TABLE 1. Acid production compared between four *Actinomyces* species

Test	1	2	3	4
Acid production				
Glycerol	+	–	nd	nd
L-Arabinose	+	–	+	+
D-Ribose	+	–	nd	nd
D-Xylose	+	–	nd	nd
D-Galactose	+	+	nd	nd
D-Glucose	+	+	+	+
D-Fructose	+	+	nd	nd
D-Mannose	+	–	nd	–
Inositol	+	–	nd	nd
D-Mannitol	+	–	–	+
Methyl β-D-glucopyranoside	+	–	–	–
N-Acetylglucosamine	+	–	nd	nd
Amygdalin	+	–	nd	nd
Aesculin	+	–	+	+
Salicin	+	–	nd	nd
Cellobiose	+	–	nd	nd
Lactose	+	+	–	+
Melibiose	+	–	+	–
Raffinose	+	–	+	–
Gentiobose	+	–	nd	nd
Turanose	+	–	nd	nd
D-Lyxose	+	–	nd	nd
API ZYM				
Esterase	–	–	–	–
Valine arylamidase	–	+	–	–
α-Galactosidase	–	–	+	–
β-Galactosidase	–	+	+	+
α-Glucosidase	–	+	–	+
β-Glucosidase	–	–	+	+

1, *Actinomyces polynesiensis* str. MS2; 2, *Actinomyces israelii* CIP 103259; 3, *Actinomyces suimastitidis* DSM15538; 4, *Actinomyces vaccimaxillae* DSM 15804

gene closely related (99.8%) to *Actinomyces* 152R-3 (GenBank accession number DQ278863.1) (Fig. 2). This value was lower than the 95% 16S rRNA gene sequence threshold recommended by Stackebrandt and Elbers to delineate a new genus without carrying out DNA-DNA hybridization [14]. The spectrum from MS2 was added to our MALDI-TOF mass spectra database.

Phenotypically, *A. polynesiensis* str. MS2 was resistant to oxacillin, second-generation (cefoxitine) and third-generation cephalosporins (ceftriaxone), trimethroprim-sulphamethoxazole, fosfomicin, erythromycin and clindamycin but was susceptible to vancomycin, teicoplanin, linezolid, gentamicin, ciprofloxacin, doxycycline, rifampicin and colistin (Fig. 2).

Genome features. The genome size of *Actinomyces polynesiensis* str. MS2 is 2 943 271 bp with a 70.80% G+C content assembled into 15 scaffolds (309 contig). A total of 2080 genes (91%) were assigned to putative functions (by clusters of orthologous groups) and 216 (9%) genes were identified as unknown function and 110 were RNAs, ten genes were 5S rRNA, nine genes were 16S rRNA and four genes were 23S rRNA. The distribution of genes into clusters of orthologous group functional categories is presented in Table 2 and the properties and statistics of the genome are summarized in Table 3.

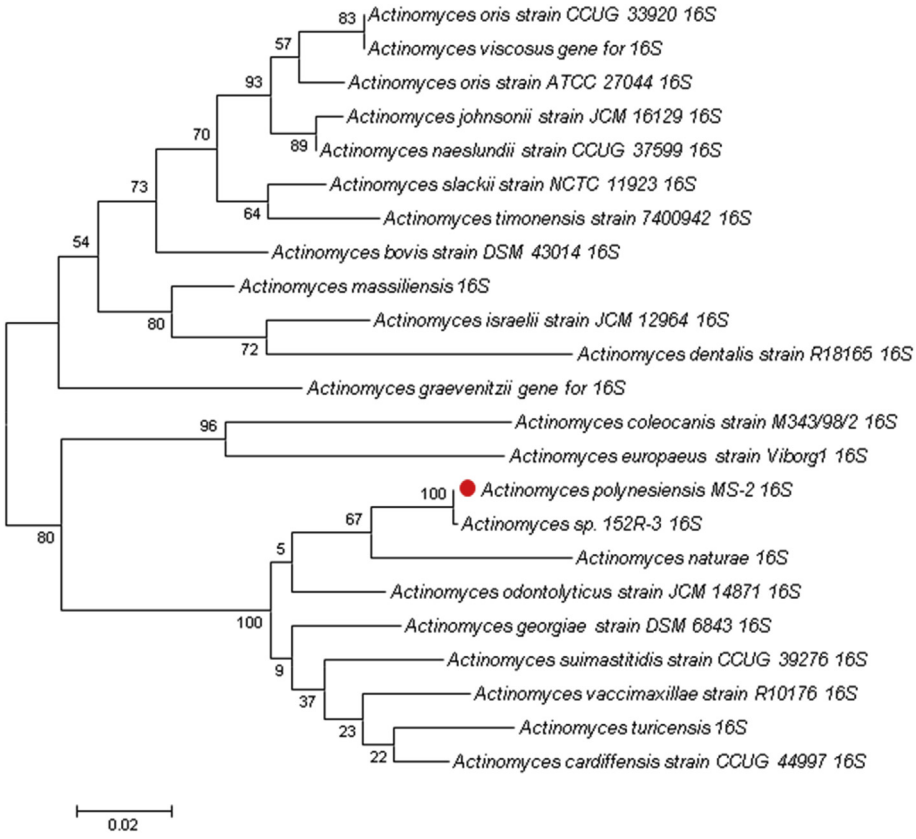

FIG. 2. Molecular phylogenetic analysis by maximum likelihood method of representatives of genus *Actinomyces* inferred from 16SrRNA gene sequence.

Resistome. The resistome of this multidrug-resistant *Actinomyces polynesiensis* str. MS2 includes penicillin-binding protein (296 amino acids), metallo-hydrolase enzyme (239 amino acids) and the major facilitator superfamily (Table 4).

TABLE 2. Number of genes associated with 25 general functional categories of clusters of orthologous groups (COGs)

COG class	Calue	Description
A	**32**	RNA processing and modification
C	**126**	Energy production and conversion
D	**30**	Cell cycle control, cell division, chromosome partitioning
E	**204**	Amino acid transport and metabolism
F	**80**	Nucleotide transport and metabolism
G	**239**	Carbohydrate transport and metabolism
H	**70**	Coenzyme transport and metabolism
I	**61**	Lipid transport and metabolism
J	**156**	Translation, ribosomal structure and biogenesis
K	**183**	Transcription
L	**135**	Replication, recombination and repair
M	**120**	Cell wall/membrane/envelope biogenesis
N	**2**	Cell motility
O	**68**	Post-translational modification, protein turnover, chaperones
P	**132**	Inorganic ion transport and metabolism
Q	**24**	Secondary metabolites biosynthesis, transport and catabolism
R	**245**	General function prediction only
S	**216**	Function unknown
T	**87**	Signal transduction mechanisms
U	**37**	Intracellular trafficking, secretion, and vesicular transport
V	**49**	Defence mechanisms

Specific features. Toxin–antitoxin loci encode a stable toxin that is neutralized by a metabolically unstable antitoxin [15]. The analysis of the genome has not demonstrated the presence of bacteriocin and non-ribosomal polyketide synthase.

Conclusion

On the basis of the phylogenetic analysis, the novel species *Actinomyces polynesiensis* str. MS2 sp. nov. is proposed with the accession number CCXH01000000 in the GenBank database.

TABLE 3. Genome properties of *Actinomyces polynesiensis* str. MS2

	Size Mb	GC%	Genes	Protein
Actinomyces polynesiensis str. MS2	2.87	70.80	2514	2566
Actinomyces israelii CIP 103259	4.03	71	3262	3125
Actinomyces odontolyticus ATCC 17982	2.39	65.4	2054	1982
*Actinomyces georgiae*DSM 6843	2.5	69.80	2102	2031
Actinomyces suimastitidis DSM15538	2.29	56.4	1960	1891
Actinomyces vaccimaxillae DSM 15804	2.34	57.6	2067	1991

TABLE 4. List of genes associated with antibiotic resistant in *Actinomyces polynesiensis* str. MS2

Open reading frame	Gene name	GC%	Size aa	Function	Best blast hit in GenBank	% aa coverage	% aa identity
WP_043535219.1	MBL	74.4	239	metallo-hydrolase	*Actinomyces* SD1	100	65
WP052450410.1	MFS	71.3	510	MFS transporter	*Leifsonia aquatica*	96	58
WP_043535826.1	PBP	73	269	penicillin-binding protein	*Actinomyces vaccimaxillae*	99	58

aa, amino acids; MBL, metallo-hydrolase enzyme; MFS, the major facilitator superfamily; PBP, penicillin-binding protein.

Genome Sequence Accession Number

The genome of *Actinomyces polynesiensis* str. MS2 has been submitted to the EBI database under bioproject ID: PRJEB1958 with accession number on GenBank database CCXH01000000 and 16S rRNA accession number HF952919.

Acknowledgements

We are very grateful to Linda Hadjadj for her technical assistance.

Funding

This work was founded by the French Centre Nationale de la Recherche Scientifique (CNRS) and Infectiopole Sud Fundation.

Transparency Declaration

There are no conflicts of interest to declare.

References

[1] Renvoise A, Raoult D, Roux V. *Actinomyces massiliensis* sp. nov., isolated from a patient blood culture. Int J Syst Evol Microbiol 2009;59:540–4.
[2] Schaal KP. Genus actinomyces. Bergey's Man Syst Bacteriol 1986;2:1383–418.
[3] Valour F, Sénéchal A, Dupieux C, Karsenty J, Lustig S, Breton P, et al. Actinomycosis: etiology, clinical features, diagnosis, treatment, and management. Infect Drug Resist 2014;7:183–97.
[4] Moniruddin ABM, Begum H, Nahar K. Actinomycosis : an update. Med Today 2010;22:43–7.
[5] Nikolaitchouk N, Hoyles L, Falsen E, Grainger JM, Collins MD. Characterization of *Actinomyces* isolates from samples from the human urogenital tract: description of *Actinomyces urogenitalis* sp. nov. Int J Syst Evol Microbiol 2000;50(Pt 4):1649–54.
[6] Lagier J-C, Armougom F, Million M, Hugon P, Pagnier I, Robert C, et al. Microbial culturomics: paradigm shift in the human gut microbiome study. Clin Microbiol Infect 2012;18:1185–93.
[7] Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 2007;24:1596–9.
[8] Leclercq R, Cantón R, Brown DFJ, Giske CG, Heisig P, MacGowan AP, et al. EUCAST expert rules in antimicrobial susceptibility testing. Clin Microbiol Infect 2013;19:141–60.
[9] 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.
[10] Conesa A, Götz S. Blast2GO: a comprehensive suite for functional analysis in plant genomics. Int J Plant Genomics 2008;2008:619832.
[11] Gupta SK, Padmanabhan BR, Diene SM, Lopez-Rojas R, Kempf M, Landraud L, et al. ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. Antimicrob Agents Chemother 2014;58:212–20.
[12] Lagesen K, Hallin P, Rødland EA, Staerfeldt H-H, Rognes T, Ussery DW. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 2007;35:3100–8.
[13] Drissi F, Buffet S, Raoult D, Merhej V. Common occurrence of antibacterial agents in human intestinal microbiota. Front Microbiol 2015;6:441.
[14] Stackebrandt E, Elbers J. Taxonomic parameters revisited: tarnished gold standards. Microbiol Today 2006;33:152–5.
[15] Roux V, Robert C, Gimenez G, Gharbi R, Raoult D. Draft genome sequence of *Actinomyces massiliensis* strain 4401292T. J Bacteriol 2012;194:5121.