Antonie van Leeuwenhoek
DOI 10.1007/s10482-016-0823-1

ORIGINAL PAPER

Enterovirga rhinocerotis gen. nov., sp. nov., isolated from *Rhinoceros unicornis* faeces

Xiu Chen · Qin-Yuan Li · Gui-Ding Li · Hui Lei · Yi Jiang · Li Han · Xue-Shi Huang · Cheng-Lin Jiang

Received: 15 August 2016 / Accepted: 19 December 2016

Abstract A novel strain, YIM 100770^T, was isolated from *Rhinoceros unicornis* faeces collected from Yunnan Wild Animal Park, China. The taxonomic status was determined based on the physiological, biochemical and phylogenetic characteristics. Strain YIM 100770^T was observed to be rod-shaped, non-motile, Gram-stain negative and aerobic. The G+C content of the genomic DNA was determined to be 68.5 mol%. The cells of strain YIM 100770^T contain ubiquinone Q-10 as the respiratory quinone. The major fatty acids (>1%) were identified as Summed feature 8 ($C_{18:1}$ $\omega 7c$ and/or $C_{18:1}$ $\omega 6c$; 78.1%), Summed feature 4 (iso-$C_{17:1}$-I and/or anteiso-$C_{17:1}$-B; 12.9%), $C_{19:0}$ cyclo $\omega 8c$ (2.8%), $C_{16:0}$ (2.2%) and $C_{18:0}$ (2.2%). Comparison of 16S rRNA gene sequences revealed the strain show high similarities with the members of the genera *Psychroglaciecola* (94.5%), *Methylobacterium* (90.5–94.1%) and *Microvirga* (92.0–93.3%) in the family *Methylobacteriaceae*. In addition, the strain also showed high similarities with the members of the genera *Chelatococcus* (93.7–94.0%) and *Pseudochelatococcus* (93.1–93.7%) in the family *Beijerinckiacea,* and the genus *Bosea* (93.1–93.8%) in the family *Bradyrhizobiaceae*. The phylogenetic analysis, combined with the chemical characteristics, suggest that the strain represents a novel genus in the order *Rhizobiales* of the class *Alphaproteobacteria*, for which the name *Enterovirga rhinocerotis* gen. nov., sp. nov. is proposed. The type strain of *E. rhinocerotis* is YIM 100770^T (=DSM 25903^T = CCTCC AB 2012048^T).

Keywords *Enterovirga rhinocerotis* gen. nov., sp. nov. · 16S rRNA gene · Faeces

Xiu Chen, Qin-Yuan Li contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s10482-016-0823-1) contains supplementary material, which is available to authorized users.

X. Chen · Q.-Y. Li · G.-D. Li · Y. Jiang (✉) · C.-L. Jiang
Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, People's Republic of China
e-mail: jiangyi@ynu.edu.cn

X. Chen · G.-D. Li · H. Lei · L. Han (✉) · X.-S. Huang
Institute of Microbial Pharmaceuticals, College of Life and Health Sciences, Northeastern University, Shenyang 110819, People's Republic of China
e-mail: hanli@mail.neu.edu.cn

Introduction

The order *Rhizobiales* in the class *Alphaproteobacteria* contains ten families (David 2005) and, in addition to these families, *Cohaesibacteraceae* (Hwang and Cho, 2008), *Roseiarcaceae* (Kulichevskaya et al. 2014) and *Xanthobacteraceae* (Lee et al. 2005) were later identified. Some species in the order *Rhizobiales* can accumulate intracellular granules of poly-β-

Published online: 28 December 2016

hydroxybutyrate (PHB) (Kim et al. 2003; Thomsen et al. 2006; Zhang et al. 2009). Large numbers of bacteria reside in animal intestines, either temporarily or for long periods, and can be beneficial to their hosts; faecal bacteria represent the bacteria of the intestine bacteria to a certain extent (Ley et al. 2008; Qin et al. 2010; Ellis et al. 2013). During an exploration of the faecal bacteria of some mammals, a novel PHB accumulating isolate, designated YIM 100770^T, was isolated from a sample of *Rhinoceros unicornis* faeces collected from Yunnan Wild Animal Park in Yunnan province, south-west China. Here we describe the phenotypic, chemotaxonomic and phylogenetic characters of the strain, and conclude that it represents a novel genus in the order *Rhizobiales* of the class *Alphaproteobacteria*, for which the name *Enterovirga rhinocerotis* gen. nov., sp. nov. is proposed.

Materials and methods

Isolation and maintenance of strains

After a *R. unicornis* defecated, fresh faecal samples from the middle of the stool were taken using a sterile plastic bag and stored at 4 °C. Air-dried samples were mixed with 0.8% NaCl solution, and then the suspension was spread onto mycose-proline agar (Cao et al. 2012) and incubated at 28 °C for 7 days. After purification, the strain was maintained on yeast extract-malt extract agar (International Streptomyces Project medium no. 2, ISP 2; Shirling and Gottlieb 1966) at 28 °C and stored in glycerol suspensions (20%, v/v) at -80 °C.

Methylobacterium organophilum DSM 18172^T and *Microvirga subterranea* DSM 14364^T were obtained from DSMZ as reference strains for comparative testing.

Molecular analysis

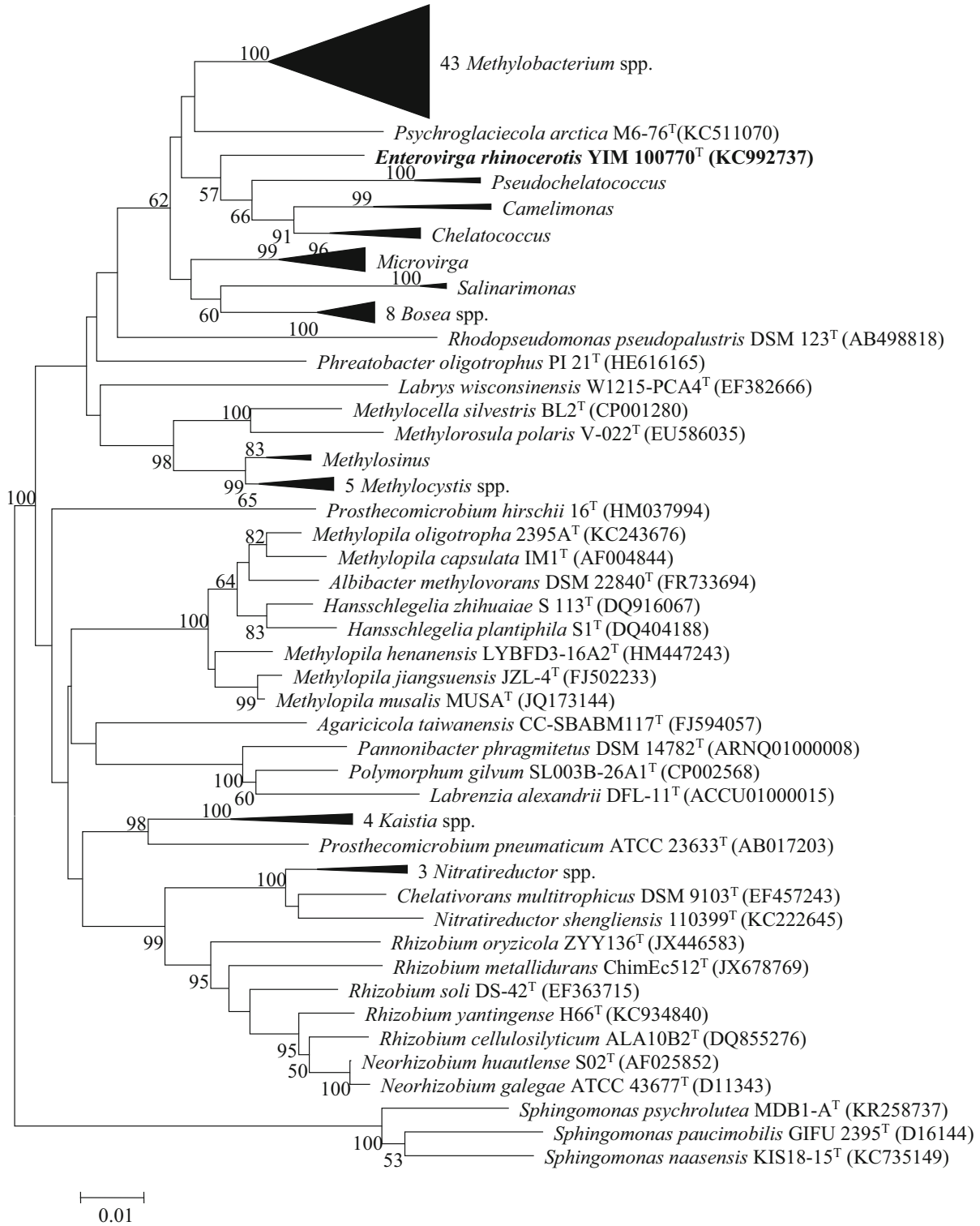
For 16S rRNA gene sequence analyses, DNA was extracted and was amplified using primers 27F and 1523R. Sequencing of the fragment was performed as described by Cui et al. (2001). Sequence data for phylogenetic trees were retrieved from GenBank/DDBJ/EMBL databases using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al. 2012) and aligned by CLUSTAL_X (Thompson et al. 1997). Phylogenetic analysis was carried out using the software MEGA version 6.0 (Tamura et al. 2013). Phylogenetic trees were constructed using the neighbour-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Fitch 1971) algorithms with bootstrap values based on 1000 replications (Felsenstein 1985). Distance matrices were calculated with Kimura's two-parameter model (Kimura 1980). Procedures described by Bouthinon and Soldano (1999) and Akutsu (2000) were used to examine the secondary structures of nine variable areas (V1–V9) of the 16S rRNA gene. The 16S rRNA gene sequences were cut using the program CLUSTAL_X and the secondary structures were evaluated and viewed via the program RNA structure 5.3. The G+C content of genomic DNA was determined as described previously (Marmur 1961; Mesbah et al. 1989).

Chemotaxonomy

Analysis of the fatty acids of strain YIM 100770^T was performed as described by Sasser (1990) using the Microbial Identification System (MIDI) (Sherlock Version 6.1; MIDI database: TSBA6). Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), the type and length were determined using an HPLC according to the method described by Minnikin et al. (1984) and Kroppenstedt (1982). Polar lipids were extracted and analysed as described by Minnikin et al. (1984) using two dimensional TLC (silica gel 60 plates; Merck). The cells for detection of the fatty acids were havested from TSA after incubation at 28 °C for 3 days, and for other chemotaxonomic characteristics after incubation for 7 days.

Morphological, cultural, physiological, biochemical characteristics

The Gram reaction was performed using the KOH lysis test method (Cerny 1978). Cells morphology, motility and flagella were examined via transmission electron microscopy using a JEM-2100 microscope after incubation on trypticase soy agar (TSA; Difco) at 28 °C (2.5, 5 and 7 days, respectively). Growth under anaerobic conditions was tested on ISP 2 agar medium at 28 °C using the anaeropack system. Growth was also tested under a variety of conditions: 5–45 °C, pH 4.0–10.0 (at intervals of 1.0 pH units) and NaCl


100
43 Methylobacterium spp.
Psychroglaciecola arctica M6-76T(KC511070)
Enterovirga rhinocerotis YIM 100770T (KC992737)
100
Pseudochelatococcus
62
57
99
Camelimonas
66
91
Chelatococcus
99
96
Microvirga
100
Salinarimonas
60
8 Bosea spp.
100
Rhodopseudomonas pseudopalustris DSM 123T (AB498818)
Phreatobacter oligotrophus PI 21T (HE616165)
Labrys wisconsinensis W1215-PCA4T (EF382666)
100
Methylocella silvestris BL2T (CP001280)
Methylorosula polaris V-022T (EU586035)
83
98
Methylosinus
99
5 Methylocystis spp.
100
65
Prosthecomicrobium hirschii 16T (HM037994)
82
Methylopila oligotropha 2395AT (KC243676)
Methylopila capsulata IM1T (AF004844)
64
Albibacter methylovorans DSM 22840T (FR733694)
Hansschlegelia zhihuaiae S 113T (DQ916067)
100
83
Hansschlegelia plantiphila S1T (DQ404188)
Methylopila henanensis LYBFD3-16A2T (HM447243)
Methylopila jiangsuensis JZL-4T (FJ502233)
99
Methylopila musalis MUSAT (JQ173144)
Agaricicola taiwanensis CC-SBABM117T (FJ594057)
Pannonibacter phragmitetus DSM 14782T (ARNQ01000008)
100
Polymorphum gilvum SL003B-26A1T (CP002568)
60
Labrenzia alexandrii DFL-11T (ACCU01000015)
100
98
4 Kaistia spp.
Prosthecomicrobium pneumaticum ATCC 23633T (AB017203)
100
3 Nitratireductor spp.
Chelativorans multitrophicus DSM 9103T (EF457243)
Nitratireductor shengliensis 110399T (KC222645)
99
Rhizobium oryzicola ZYY136T (JX446583)
Rhizobium metallidurans ChimEc512T (JX678769)
95
Rhizobium soli DS-42T (EF363715)
Rhizobium yantingense H66T (KC934840)
Rhizobium cellulosilyticum ALA10B2T (DQ855276)
95
Neorhizobium huautlense S02T (AF025852)
50
100
Neorhizobium galegae ATCC 43677T (D11343)
Sphingomonas psychrolutea MDB1-AT (KR258737)
100
Sphingomonas paucimobilis GIFU 2395T (D16144)
53
Sphingomonas naasensis KIS18-15T (KC735149)
0.01

◀**Fig. 1** Neighbour-joining tree based on nearly full-length 16S rRNA gene sequences showing the phylogenetic position of strain YIM 100770^T among type strains of closely related genera. *Numbers* before genus names indicate numbers of type strains included in the clusters, otherwise including all the type strains in the genera. Bootstrap values (>50%) based on 1000 resamplings are showing at the branch. *Sphingomonas psychrolutea* MDB1-A^T, *Sphingomonas paucimobilis* GIFU 2395^T and *Sphingomonas naasensis* KIS18-15^T were used as outgroups

concentrations from 0 to 7% (w/v, at intervals of 0.5%). The buffer system described by Xu et al. (2005) was used in media of different pH values. Phenotypic tests performed including the following were tested as described previously (Smibert and Krieg 1994): carbon and nitrogen source utilisation; oxidase (oxidase reagent; bioMérieux) and catalase (3% H_2O_2) activities; starch, gelatin, Tween 20, Tween 40 and Tween 80 hydrolysis; nitrate reduction; milk coagulation and peptonisation; cellulase and urease activities. Other phenotypic and biochemical features were determined using API 20NE, API ZYM (bioMérieux) strips following the manufacturer's instructions.

Results and discussion

The analysis of its nearly complete 16S rRNA sequence (GenBank accession number KC992737) indicated that strain YIM 100770^T showed high similarities with the members of the genera *Psychroglaciecola* (94.5%), *Methylobacterium* (90.5–94.1%) and *Microvirga* (92.0–93.3%) in the family *Methylobacteriaceae, Chelatococcus* (93.7–94.0%) and *Pseudochelatococcus* (93.1–93.7%) in the family *Beijerinckiacea,* and *Bosea* (93.1–93.8%) in the family *Bradyrhizobiaceae*. Phylogenetic analysis using the neighbor-joining method (Fig. 1) revealed that strain YIM 100770^T formed a cluster with the members of the genera *Chelatococcus, Camelimonas* and *Pseudochelatococcus* in the family *Beijerinckiaceae* with a bootstrap value of 57. The topological relationship of this cluster was maintained in the maximum-likelihood tree but with a lower bootstrap value of 43 (Fig. S1). However, strain YIM 100770^T formed a cluster with the members of the genera *Methylobacterium* and *Psychroglaciecola* in the family *Methylobacteriaceae* with a bootstrap value of 47 when a maximum-parsimony tree was constructed (Fig. S1). In addition, the topologies of the trees were variable when the strains were added or removed. Thus, the family level affiliation of strain YIM 100770^T remains unclear. Secondary structures of the V1 and V3 regions in the 16S rRNA gene are similar in patterns to those of the type species (except *Chelatococcus caeni* EBR-4-1^T for the sequence of the type species is short) in closely related genera, but the structures of the V2, V4 and V9 regions are unique to strain YIM 100770^T (Fig. S2). Yarza et al. (2014) proposed a threshold of 94.5% sequence identity for delineation of a new genus based on 16S rRNA gene analyses. Thus, strain YIM 100770^T can be considered to represent a novel genomic genus in the order *Rhizobiales* of the class *Alphaproteobacteria*. The G+C content of genomic DNA of strain YIM 100770^T was determined to be 68.5 mol%.

Ubiquinone 10 was detected as the predominant respiratory quinone, which in accordance with the characteristics of closely related genera. The polar lipids profile was determined to contain diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylmonomethylethanolamine (PME), four unidentified phospholipids ($PL_{1–4}$) and seven unidentified polar lipids ($L_{1–7}$). The polar lipids were similar to those of the type species of closely related genera in the family *Methylobacteriaceae*, but the amounts of the unidentified polar lipids and phospholipids were different from each other (Fig. S3; Qu et al. 2014). The presence of DPG, PE and PME, and the presence of unidentified phospholipids and absence of unidentified glycolipid and unidentified aminolipids in the strain YIM 100770^T lipid profile could distinguish the strain from the type species of the genera *Chelatococcus*, *Pseudochelatococcus* and *Camelimonas* of the family *Beijerinckiacea*, respectively (Yoon et al. 2008; Kämpfer et al. 2010, 2015). The presence of PE, $PL_{1–4}$ and $L_{1–7}$, and the absence of unidentified aminolipids in strain YIM 100770^T can differentiate it from the members of the genus *Bosea* (Das et al. 1996). The cellular fatty acids (>1.0%) of strain YIM 100770^T were found to contain Summed feature 8 ($C_{18:1}$ $\omega7c$ and/or $C_{18:1}$ $\omega6c$; 78.1%), Summed feature 4 (iso-$C_{17:1}$-I and/or anteiso-$C_{17:1}$-B; 12.9%), $C_{19:0}$ cyclo $\omega8c$ (2.8%), $C_{16:0}$ (2.2%) and $C_{18:0}$ (2.2%). The fatty acid profiles of strain YIM 100770^T and members of closely related genera are given in Table 1. Notably, the presence of Summed feature 4 (iso-$C_{17:1}$-I and/or anteiso-$C_{17:1}$-B; 12.9%) is a discriminative characteristic compared with the type species of closely related genera. This characteristic supports the

Table 1 Cellular fatty acid content (%) of strain YIM 100770^T and type strains of closely related genera

Cellular fatty acid	1	2	3	4	5	6	7	8
Unidentified	–	–	–	–	–	–	–	0.8
Iso-$C_{13:0}$	–	–	–	–	–	–	0.2	–
$C_{14:0}$	–	–	–	–	–	–	0.3	–
Summed feature 1	–	–	–	–	–	–	0.4	–
iso-$C_{15:0}$	–	–	–	–	–	–	0.6	–
anteiso-$C_{15:0}$	–	–	–	–	–	–	0.4	–
$C_{15:0}$	–	–	–	–	2.3	–	–	–
Summed feature 2	0.5	1.2	1.3	4.0	–	4.8	1.7	4.6
Summed feature 3	0.2	22.1	0.6	1.1	3.7	–	2.5	0.6
$C_{16:0}$	2.2	6.4	4.1	8.5	7.6	4.3	7.9	7.9
Sum feature 4	12.9	–	–	–	–	–	–	–
$C_{15:0}$ 3-OH	–	–	–	–	0.5	–	0.7	–
$C_{16:0}$ 3-OH	–	–	–	–	3.3	–	–	–
$C_{17:1}$ $\omega 8c$	–	–	–	–	3.5	–	1.9	0.8
$C_{17:1}$ $\omega 6c$	–	–	–	–	–	–	1.1	–
$C_{17:0}$	0.5	–	–	0.4	4.8	–	7.9	–
$C_{17:0}$ cyclo	–	–	–	3.2	2.1	–	–	–
$C_{17:0}$ 3-OH	–	–	–	–	0.9	–	–	–
$C_{18:1}$ $\omega 9c$	–	1.0	–	–	–	–	0.2	–
Summed feature 8	78.1	64.3	86.2	47.4	61.6	86.0	66.9	11.5
$C_{18:0}$	2.2	5.0	6.3	0.9	0.7	–	3.4	1.3
11-Methyl $C_{18:1}$ $\omega 7c$	0.3	–	–	–	–	–	–	1.4
$C_{19:\ 0}$ cyclo $\omega 8c$	2.8	–	–	29.6	8.3	4.9	3.2	67.3
10-Methyl $C_{19:0}$	–	–	–	–	0.7	–	0.4	1.0
$C_{18:1}$ 2-OH	–	–	–	0.9	–	–	–	–
$C_{18:0}$ 3-OH	–	–	1.6	0.8	–	–	0.3	1.7
$C_{20:1}$ $\omega 7c$	0.4	–	–	1.4	–	–	–	–
$C_{20:2}$ $\omega 6,9c$	–	–	–	–	–	–	–	1.0

(1) *Enterovirga rhinocerotis* YIM 100770^T; (2) *Psychroglaciecola arctica* M6-76^T (Qu et al. 2014); (3) *Methylobacterium organophilum* DSM 18172^T; (4) *Chelatococcus asaccharovorans* DSM 6462^T (Yoon et al. 2008); (5) *Bosea thiooxidans* DSM 9653^T (Das et al. 1996); (6) *Pseudochelatococcus lubricantis* MPA 1113^T (major; Kämpfer et al. 2015); (7) *Microvirga subterranea* DSM 14364^T; (8) *Camelimonas lactis* M 2040^T (Kämpfer et al. 2010). – Not detected or no data available. Data in 1, 3 and 7 are from this study

In MIDI analyses, Summed feature 2 contains iso-$C_{15:1}$-H and/or $C_{13:0}$ 3-OH; Summed feature 2 contains $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$-I; Summed feature 3 contains $C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$; Summed feature 4 contains iso-$C_{17:1}$- I and/or anteiso-$C_{17:1}$-B; Summed feature 8 contains $C_{18:1}\omega 7c$ and/or $C_{18:1}$ $\omega 6c$

conclusion that strain YIM 100770^T represents a novel genus.

Strain YIM 100770^T was determined to be aerobic, negative short rod-shaped cells lacking flagella that can accumulate PHB granules (Fig. S4). However, the PHB accumulation was only observed after 2.5 days culture. The colonies of strain YIM 100770^T were observed to be milk-white with a diameter of 0.5–1.5 mm on both ISP2 and TSA. Strain YIM 100770^T was found to be able to grow at 8–37 °C (optimum 28 °C) but no colonies were observed at 5 °C or 40 °C. The absence of flagella distinguishes strain YIM 100770^T from the members of genera *Psychroglaciecola, Methylobacterium, Bosea* and *Microvirga*; the colony colour (milk-white rather than pink, pink to red or light pink) of strain YIM 100770^T

Table 2 Characteristics that differentiate strain YIM 100770^T from the type strains of closely related genera

Characteristic	1	2	3	4	5	6	7	8
Colony colour	Milk-white	Pink	Pink to red	White to beige	Creamy	Beige	Light pink	Beige
Colony size (mm)	0.5–1.5	0.5–1.5	1.0–3.0	3.0–4.0	1.0–1.5	2.0	3.0–5.0	2.0
Cell shape (μm)	Oval to rods, 0.8–1.0 × 1.5–2.5	Rods, 0.4–0.7 × 0.8–2.0	Rods, 0.8–1.0 × 1.5–2.0[a]	Coccoid to oval, 1.5–2.0 × 1.2–1.5	Rods, 0.85 × 1.4–1.6	Rods, 1.0 × 2.0	Rods, 1.0 × 1.5–4.0[b]	Rods, 1.0 × 2.0
Flagella	None	Single polar	Single polar[a]	None	Single polar	None	Single polar[b]	None
Range for growth								
Temperature (°C)	8–37	4–28	25–31[a]	Optimum 36	Optimum 30–32	4–50	28–40	15–37
Nacl (w/v; %)	0.0–3.0	0.0–1.0	0.0–1.0	0.0–2.5	ND	1.5–5.5	0.0–0.5	ND
pH	6.0–8.0	5.0–8.0	6.0–8.0	Optimum 7.0–8.0	6.0–9.0	5.5–10.5	6.0–9.0	5.5–10.5
Nitrate reduction	+	−	−	+	+	ND	+	ND
Urease activity	−	+	+	+	−	ND	−	+
Oxidase activity	−	−	+	+	+	+	−	+
Acid from glucose fermentation	−	−	−	−	ND	−	−	ND
Indole production	−	−	−	−	−	ND	−	ND
Hydrolysis of gelatin	+	−	−	+	−	ND	+	ND
Utilization								
Methanol	−	+	+	ND	−	ND	+	ND
Glucose	+	−	+	+	+	−	+	+
Arginine	−	−	−	−	+	ND	−	ND
Aesculin	+	+	−	+	ND	−	−	+
Arabinose	+	−	+	+	+	−	+	−
Mannose	+	−	+	+	ND	−	−	−
Mannitol	+	−	−	−	−	−	−	−
N-acetyl-glucosamine	+	−	−	−	ND	−	−	−
Maltose	+	−	−	−	ND	−	−	−
Gluconate	+	−	−	+	+	−	−	−
Adipic acid	−	−	−	+	+	+	−	+
Malic acid	+	−	−	+	+	+	−	−

Table 2 continued

Characteristic	1	2	3	4	5	6	7	8
Citrate	+	−	−	−	+	ND	−	+
Phenylacetic acid	−	−	−	+	+	ND	−	−
Enzyme								
Alkaline phosphatase	+	+	−	+	ND	ND	−	ND
Esterase(C4)	−	+	+	+	ND	ND	+	ND
Leucine arylamidase	+	+	+	+	−	−	+	−
Valine arylamidase	+	+	−	−	ND	ND	−	ND
Cystine arylamidase	+	+	−	−	ND	ND	−	ND
Trypsin	+	+	−	−	ND	ND	−	ND
α-Chymotrypsin	+	−	−	−	ND	ND	−	ND
β-Galactosidase	−	+	−	−	ND	ND	−	ND
α-Glucosidase	+	+	−	−	ND	ND	−	ND
Polar lipids	PC, PE, PME, DPG, PG, $PL_{1–4}$ and $L_{1–7}$	PC, PE, PME, DPG, PG and $L_{1–2}$	PC, PE, PME, DPG, PG, $PL_{1–2}$ and $L_{1–7}$	PC, PE, PG, $APL_{1–2}$ and $PL_{1–2}$	PC, PME, DPG, PG and AL	PC, PME, DPG, PG, $GL_{1–2}$, $AL_{1–5}$ and $L_{1–5}$	PC, PE, PME, DPG, PG, $PL_{1–2}$ and $L_{1–4}$	PC, PE, DPG, PG, GL, $AL_{1–3}$ and $L_{1–3}$
DNA G+C content (mol%)	68.5	67.0	66.0[a]	63.3–63.5	68.2	58.2 ± 0.5	63.5 ± 0.5[b]	65.0

(1) *Enterovirga rhinocerotis* YIM 100770^T; (2) *Psychroglaciecola arctica* M6-76^T (Qu et al. 2014); (3) *Methylobacterium organophilum* DSM 18172^T; (4) *Chelatococcus asaccharovorans* DSM 6462^T (Auling et al.1993; Yoon et al. 2008; Panday and Das 2010); (5) *Bosea thiooxidans* DSM 9653^T (Das et al. 1996); (6) *Pseudochelatococcus lubricantis* MPA 1113^T (Kämpfer et al. 2015); (7) *Microvirga subterranea* DSM 14364^T; (8) *Camelimonas lactis* M 2040^T (Kämpfer et al. 2010). *ND* no data available. *DPG* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PME* phosphatidylmonomethylethanolamine, *PL* unidentified phospholipid, *GL* acidic glycolipid, *AL* unidentified aminolipids, *L* unidentified polar lipid

[a] Patt et al. (1976)

[b] Kanso and Patel (2003)

is different from that of members of the genera in the family *Methylobacteriaceae*. Oxidase was found to be negative for strain YIM 100770^T but the members of the genus *Chelatococcus, Pseudochelatococcus* and *Camelimonas* are positive. Differences in phenotypic traits between the strain YIM 100770^T and the type species of the closely related genera are shown in Table 2. In addition, strain YIM 100770^T was found to be able to utilise raffinose, glucose, arabinose, mannose, mannitol, *N*-Acetyl-glucosamine, maltose, gluconate, malic acid and citrate, but not methanol, ethanol, capric acid, adipic acid or phenylacetic acid. Strain YIM 100770^T was found to be positive for catalase, alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-glucosidase and naphthol-AS-BI-phosphohydrolase, but negative for oxidase, esterase (C4), lipase (C14), α-galactosidase, *β*-galactosidase, *β*-glucuronidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase, α-mannosidase and α-fucosidase.

In conclusion, the results of examination of the phylogenetic, chemotaxonomic and some morphologic and physiological properties of the isolate, and the low levels of 16S rRNA gene sequence similarity with its close relatives, suggest that strain YIM 100770^T represents a novel genus in the order *Rhizobiales* of the class *Alphaproteobacteria*, for which the name *Enterovirga rhinocerotis* gen. nov., sp. nov. is proposed.

Description of *Enterovirga* gen. nov

Enterovirga (En.te.ro.vir'ga. Gr. n. *enteron*, intestine; L. fem. n. *virga*, twig, rod; *Enterovirga* intestinal rod-shaped bacterium)

Cells stain Gram-stain negative, are non-motile, and lack flagella. Aerobic. Nitrate reduction is positive. Catalase positive and oxidase negative. Respiratory quinone is Q-10. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, four unidentified phospholipids and seven unidentified polar lipids. The main cellular fatty acids are Summed feature 8 ($C_{18:1}$ $\omega7c$ and/or $C_{18:1}$ $\omega6c$) and Summed feature 4 (iso-$C_{17:1}$- I and/or anteiso-$C_{17:1}$-B). The G+C content of the DNA of the type strain of the type species is approximately 68.5 mol%. *Enterovirga* belongs to the order *Rhizobiales* of the class *Alphaproteobacteria* and the type species is *Enterovirga rhinocerotis*.

Description of *Enterovirga rhinocerotis* sp. nov

Enterovirga rhinocerotis (rhi.no.ce.ro'tis, L. masc. gen. n., *rhinocerotis* referring to the isolation of the organism from *Rhinoceros unicornis* faeces)

In addition to the properties given in the genus description, cells are small, rod-shaped, can accumulate poly-*β*-hydroxybutyrate granules and produce milk-white colonies. Growth occurs at 8–37 °C, and the pH range for growth is pH 6–8 and the NaCl range is 0–3% (w/v). Negetive result for indole production; hydrolyses gelatin, Tween 20 and aesculin, but not Tween 40, Tween 80, starch, cellulose or urea. The type strain YIM 100770^T (=DSM 25903^T = CCTCC AB 2012048^T) was isolated from *R. unicornis* faeces. The partial 16S rRNA gene sequence (1472-bp) of strain YIM 100770^T was deposited in GenBank with the accession number of KC992737.

Acknowledgements This research was supported by the National Natural Science Foundation of China (Nos. 31270001, 31460005 and 81573327), and supported by Program for New Century Excellent Talents in University and Yunnan Provincial Society Development Project (2014BC006). Thanks for the assistance of Yunnan Wild Animal Park, and thanks for the assistance from Mr. Jiang Liu and You-Long Li.

References

Akutsu T (2000) Dynamic programming algorithms for RNA secondary structure prediction with pseudoknots. Discret Appl Math 104:45–62

Auling G, Busse HJ, Egli T, El-Banna T, Stackebrandt E (1993) Description of the gram-negative, obligately aerobic, nitrilotriacetate (nta)-utilizing bacteria as *Chelatobacter heintzii*, gen. nov. sp. nov. and *Chelatococcus asaccharovorans*, gen. nov. sp. nov. Syst Appl Microbiol 16:104–112

Bouthinon D, Soldano H (1999) A new method to predict the consensus secondary structure of a set of unaligned RNA sequences. Bioinformatics 15:785–798

Cao YR, Jiang Y, Jin RX, Han L, He WX, Li YL, Huang XS, Xue QH et al (2012) Enteractinococcus coprophilus gen. nov., sp. nov., of the family Micrococcaceae, isolated from *Panthera tigris* amoyensis faeces, and transfer of *Yaniella*

fodinae Dhanjal. 2011 to the genus *Enteractinococcus* as *Enteractinococcus fodinae* comb. nov. Int J Syst Evol Microbiol 62:2710–2716

Cerny G (1978) Studies on the aminopeptidase test for the distinction of gram-negative from gram-positive bacteria. Appl Microbiol Biotechnol 5:113–122

Cui XL, Mao PH, Zeng M, Li WJ, Zhang LP, Xu LH, Jiang CL (2001) *Streptomonospora salina* gen. nov., sp. nov., a new member of the family *Nocardiopsaceae*. Int J Syst Evol Microbiol 51:357–363

Das SK, Mishra AK, Tindall BJ, Rainey FA, Stackebrandt E (1996) Oxidation of thiosulfate by a new bacterium, *Bosea thiooxidans* (strain BI-42) gen. nov. sp. nov.: analysis of phylogeny based on chemotaxonomy and 16S ribosomal DNA sequencing. Int J Syst Evol Microbiol 46:981–987

David KL (2005) Class I. *Alphaproteobacteria* class. In: Brenner JD, Krieg NR, Staley JT (eds) Bergey's manual of systematic bacteriology, vol 2, 2nd edn. New York, Springer, pp 324–574

Ellis RJ, Bruce KD, Jenkins C, Stothard JR, Ajarova L, Mugisha L, Viney ME (2013) Comparison of the distal gut microbiota from people and animals in africa. PLoS ONE 8:570

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. Syst Zool 20:406–416

Hwang CY, Cho BC (2008) *Cohaesibacter gelatinilyticus* gen. nov. sp. nov. a marine bacterium that forms a distinct branch in the order *Rhizobiales*, and proposal of *Cohaesibacteraceae* fam. nov. Int J Syst Evol Microbiol 58:267–277

Kämpfer P, Scholz HC, Langer S, Wernery U, Wernery R, Johnson B, Joseph M, Lodders N, Busse HJ (2010) *Camelimonas lactis* gen. nov., sp. nov., isolated from the milk of camels. Int J Syst Evol Microbiol 60:2382–2386

Kämpfer P, Glaeser SP, Gräber M, Rabenstein A, Kuever J, Busse HJ (2015) *Pseudochelatococcus lubricantis* gen. nov. sp. nov. and *pseudochelatococcus contaminans* sp. nov. from coolant lubricants. Int J Syst Evol Microbiol 65:147–153

Kanso S, Patel BKC (2003) *Microvirga subterranea* gen. nov., sp. nov., a moderate thermophile from a deep subsurface Australian thermal aquifer. Int J Syst Evol Microbiol 53:401–406

Kim SG, Joung SH, Ahn CY, Ko SR, Boo SM, Oh HM (2003) *Microvirga subterranea* gen. nov. sp. nov. a moderate thermophile from a deep subsurface australian thermal aquifer. Int J Syst Evol Microbiol 53:401–406

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA Gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequence. J Mol Evol 16:111–120

Kroppenstedt RM (1982) Separation of bacterial menaquinones by HPLC using reverse phase (RP18) and a silver loaded ion exchanger as stationary phases. J Liq Chromatogr 5:2359–2367

Kulichevskaya IS, Danilova OV, Tereshina VM, Kevbrin VV, Dedysh SN (2014) Descriptions of *Roseiarcus fermentans* gen. nov. sp. nov. a bacteriochlorophyll a-containing fermentative bacterium related phylogenetically to alphaproteobacterial methanotrophs, and of the family *Roseiarcaceae* fam. nov. Int J Syst Evol Microbiol 64:2558–2565

Lee KB, Liu CT, Anzai Y, Kim H, Aono T, Oyaizu H (2005) The hierarchical system of the '*Alphaproteobacteria*': description of *Hyphomonadaceae* fam. nov. *Xanthobacteraceae* fam. nov. and *Erythrobacteraceae* fam. nov. Int J Syst Evol Microbiol 55:1907–1919

Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, Schlegel ML, Tucker TA, Schrenzel MD, Knight R, Gordon JI (2008) Evolution of mammals and their gut microbes. Science 320:1647–1651

Marmur J (1961) A procedure for the isolation of deoxyribonucleic acid from microorganisms. J Mol Biol 3:208–218

Mesbah M, Premachandran U, Whitman WB (1989) Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. Int J Syst Bacteriol 39:159–167

Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, Schaal A, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241

Panday D, Das SK (2010) *Chelatococcus sambhunathii* sp. nov. a moderately thermophilic alphaproteobacterium isolated from hot spring sediment. Int J Syst Evol Microbiol 60:861–865

Patt TE, Cole GC, Hanson RS (1976) *Methylobacterium*, a new genus of facultatively methylotrophic bacteria. Int J Syst Bacteriol 26:226–229

Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, YamadaT, Mende, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Paslier DL, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz PT, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Dore J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Consortium M, Bork P, Ehrlich D, Wang J (2010) A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464:59–65

Qu ZH, Jiang F, Chang XL, Qiu X, Ren LZ, Fang CX, Peng F (2014) *Psychroglaciecola arctica* gen. nov. sp. nov. isolated from arctic glacial foreland soil. Int J Syst Evol Microbiol 64:1817–1824

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic tree. Mol Biol Evol 4:406–425

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. USFCC Newsl 20:16

Shirling EB, Gottlieb D (1966) Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol 16:313–340

Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Gerhart P, Murray RGE, Wood WA, Krieg NR (eds)

Methods for general and molecular bacteriology. American Society for Microbiology, Washington, DC, pp 607–654

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Thomsen TR, Blackall LL, Muro MA, Nielsen JL, Nielsen PH (2006) *Meganema perideroedes* gen. nov., sp. nov., a filamentous alphaproteobacterium from activated sludge. Int J Syst Evol Microbiol 56:1865–1868

Xu P, Li WJ, Tang SK, Zhang YQ, Chen GZ, Chen HH, Xu LH, Jiang CL (2005) *Naxibacter alkalitolerans* gen. nov., sp. nov., a novel member of the family '*Oxalobacteraceae*' isolated from China. Int J Syst Evol Microbiol 55:1149–1153

Yarza P, Yilmaz P, Pruesse E, Glöckner FO, Ludwig W, Schleifer KH, Whitman WB, Euzéby J, Amann R, Rosselló-Móra R (2014) Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. Nat Rev Microbiol 12:635–645

Yoon JH, Kang SJ, Im WT, Lee ST, Oh TK (2008) *Chelatococcus daeguensis* sp. nov. isolated from wastewater of a textile dye works, and emended description of the genus *Chelatococcus*. Int J Syst Evol Microbiol 58:2224–2228

Zhang JL, Song F, Xin YH, Zhang J, Fang CY (2009) *Microvirga guangxiensis* sp. nov. a novel alphaproteobacterium from soil, and emended description of the genus *Microvirga*. Int J Syst Evol Microbiol 59:1997–2001