Chryseobacterium artocarpi sp. nov., isolated from the rhizosphere soil of *Artocarpus integer*

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A bacterial strain, designated UTM-3^T, isolated from the rhizosphere soil of *Artocarpus integer* (cempedak) in Malaysia was studied to determine its taxonomic position. Cells were Gram-stain-negative, non-spore-forming rods, devoid of flagella and gliding motility, that formed yellow-pigmented colonies on nutrient agar and contained MK-6 as the predominant menaquinone. Comparative analysis of the 16S rRNA gene sequence of strain UTM-3^T with those of the most closely related species showed that the strain constituted a distinct phyletic line within the genus *Chryseobacterium* with the highest sequence similarities to *Chryseobacterium lactis* NCTC 11390^T, *Chryseobacterium viscerum* 687B-08^T, *Chryseobacterium tructae* 1084-08^T, *Chryseobacterium arthrosphaerae* CC-VM-7^T, *Chryseobacterium oncorhynchi* 701B-08^T, *Chryseobacterium vietnamense* GIMN1.005^T, *Chryseobacterium bernardetii* NCTC 13530^T, *Chryseobacterium nakagawai* NCTC 13529^T, *Chryseobacterium gallinarum* LMG 27808^T, *Chryseobacterium culicis* R4-1A^T, *Chryseobacterium flavum* CW-E2^T, *Chryseobacterium aquifrigidense* CW9^T, *Chryseobacterium ureilyticum* CCUG 52546^T, *Chryseobacterium indologenes* NBRC 14944^T, *Chryseobacterium gleum* CCUG 14555^T, *Chryseobacterium jejuense* JS17-8^T, *Chryseobacterium oranimense* H8^T and *Chryseobacterium joostei* LMG 18212^T. The major whole-cell fatty acids were iso-$C_{15:0}$ and iso-$C_{17:1}\omega9c$, followed by summed feature 4 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7t$) and iso-$C_{17:0}$ 3-OH, and the polar lipid profile consisted of phosphatidylethanolamine and several unknown lipids. The DNA G+C content strain UTM-3^T was 34.8 mol%. On the basis of the phenotypic and phylogenetic evidence, it is concluded that the isolate represents a novel species of the genus *Chryseobacterium*, for which the name *Chryseobacterium artocarpi* sp. nov. is proposed. The type strain is UTM-3^T (=CECT 8497^T=KCTC 32509^T).

The classification of members of the family *Flavobacteriaceae* is still in a state of development (Yoon *et al.*, 2013), and in the course of subdividing the genus *Flavobacterium*, Vandamme *et al.* (1994) proposed the genus *Chryseobacterium*. At the time of writing, the genus *Chryseobacterium* contains around 80 species and represents one of the genera with the fastest growing number of species. Members of this genus are widely distributed in aquatic and soil environments and some species are pathogenic to humans and animals (Bernardet *et al.*, 2006; Vaneechoutte *et al.*, 2007), indicating that the genus *Chryseobacterium* represents a group of organisms that are ubiquitous in nature. Many of the species have been found in association with plants considering their antagonistic potential against plant pathogens (Krause *et al.*, 2001; Shin *et al.*, 2007; Ramos Solano *et al.*, 2008). Recent studies of the genus *Chryseobacterium* have documented the significance of its bioactive compounds as biocontrol agents, antioxidants, prebiotics, sulfobacin A and protease producers (Scheuplein *et al.*, 2007; Wang *et al.*, 2008, 2011; Chaudhari *et al.*, 2009; Kim *et al.*, 2012), which substantiate that it is a novel source of bioactive compounds. Members of the genus *Chryseobacterium* are Gram-negative, non-motile, psychrotolerant, halotolerant with a strict respiratory metabolism and contain MK-6 as the major respiratory quinone (Bernardet *et al.*, 2006).

Strain UTM-3^T was isolated from the rhizosphere soil of *Artocarpus integer* from an orchard located on the campus of Universiti Teknologi Malaysia (UTM), Malaysia in December 2012. *Artocarpus integer,* commonly known as

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain UTM-3^T is KF751867.

cempedak, is a species of tree in the family *Moraceae* native to South-east Asia. The soil sample (10 g) was placed in flasks containing 90 ml nutrient broth (Difco) and incubated at 30 °C under agitation (100 r.p.m.) for 48 h. An aliquot (0.1 ml) of the cell suspension was spread onto nutrient agar and incubated at 30 °C for 24 h. After primary isolation and purification, the strain was cultivated at 30 °C on the same medium and was preserved in nutrient broth containing 10 % (v/v) glycerol at −80 °C. The strain was subsequently analysed for 16S rRNA gene sequence similarities, fatty acid composition, phenotypic characteristics and DNA–DNA relatedness to those species most closely related on the basis of 16S rRNA gene sequence similarities. *Chryseobacterium lactis* NCTC 11390^T, *Chryseobacterium viscerum* CECT 7793^T, *Chryseobacterium tructae* CECT 7798^T, *Chryseobacterium arthrosphaerae* CCUG 57618^T, *Chryseobacterium oncorhynchi* CECT 7794^T, *Chryseobacterium vietnamense* CCTCC M 209230^T, *Chryseobacterium bernardetii* NCTC 13530^T, *Chryseobacterium nakagawai* NCTC 13529^T, *Chryseobacterium gallinarum* LMG 27808^T, *Chryseobacterium culicis* LMG 25442^T, *Chryseobacterium flavum* KCTC 12877^T, *Chryseobacterium aquifrigidense* KCTC 12894^T, *Chryseobacterium ureilyticum* CCUG 52546^T, *Chryseobacterium indologenes* LMG 8337^T, *Chryseobacterium gleum* CCUG 14555^T, *Chryseobacterium jejuense* JS17-8^T, *Chryseobacterium oranimense* H8^T and *Chryseobacterium joostei* LMG 18212^T were used as reference strains for this study.

Cultural and morphological characteristics of the strains were observed on nutrient agar. The Gram reaction was performed by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Flagella and gliding motilities were determined using the hanging drop method and gliding motility was examined further using phase-contrast microscopy on 17 h-cultures on microscopic slides coated with marine agar (MA; Difco), according to Bowman (2000). Catalase activity was tested by using 10 % (v/v) H_2O_2 and oxidase activity was tested in 1 % *N*,*N*,*N*′,*N*′tetramethyl-p-phenylenediamine solution. The presence of flexirubin-type pigments was determined by flooding the cell mass with 20 % (w/v) KOH (Bernardet *et al.*, 2002). Growth was tested on nutrient agar, brain heart infusion agar, tryptic soy agar and MacConkey agar (Difco). Growth temperature (5, 10, 15, 20, 25, 30, 37 and 40 °C) and pH (pH 3–11, at 2 increments) were tested in tryptic soy broth. Salt tolerance was determined on nutrient agar containing varying concentrations of NaCl (2–10 %, w/v, at 1 % intervals) at 30 °C.

Physiological characterization was investigated as described by Kämpfer *et al.* (1991). Biochemical tests were performed for four strains using the API 20NE, API 20E and API ZYM identification systems (bioMérieux) according to the manufacturer's instructions. Growth was checked under anaerobic and microaerobic conditions by using the GasPak^{TM} (BD BBL^{TM}) at 30 °C for 15 days. For cellular fatty acid analysis, the seven strains were grown on

Table 1. Differential characteristics between strain UTM-3^T and the type strains of closely related species of the genus *Chryseobacterium*

Strains: 1, UTM-3^T; 2, *C. lactis* NCTC 11390^T; 3, *C. viscerum* CECT 7793^T; 4, *C. tructae* CECT 7798^T; 5, *C. arthrosphaerae* CCUG 57618^T; 6, *C. oncorhynchi* CECT 7794^T; 7, *C. vietnamense* CCTCC M 209230^T; 8, *C. bernardetii* NCTC 13530^T; 9, *C. nakagawai* NCTC 13529^T; 10, *C. gallinarum* LMG 27808^T; 11, *C. culicis* LMG 25442^T; 12, *C. flavum* KCTC 12877^T; 13, *C. aquifrigidense* KCTC 12894^T; 14, *C. ureilyticum* CCUG 52546^T; 15, *C. indologenes* LMG 8337^T; 16, *C. gleum* CCUG 14555^T; 17, *C. jejuense* JS17-8^T; 18, *C. oranimense* H8^T; 19, *C. joostei* LMG 18212^T. All data are from this study. All strains produced acids from D-glucose and trehalose, hydrolysed starch, and grew with 3 % NaCl and at 30 °C. None of the strains produced acid from L-arabinose, cellobiose, ethanol, lactose or D-xylose. +, Positive; (+), weakly positive; −, negative.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Production of:																			
Indole	−	−	+	+	−	+	−	+	+	−	−	+	+	+	+	−	+	+	+
H_2S	+	+	−	+	+	−	+	−	+	−	+	(+)	−	−	−	+	−	−	−
Nitrate reduction	+	−	−	−	−	−	+	−	−	+	−	−	+	+	+	+	−	−	−
Acid production from:																			
D-Fructose	+	+	−	+	−	−	+	−	+	+	(+)	+	+	−	+	+	−	−	+
Glycerol	+	+	(+)	+	−	+	−	+	−	(+)	−	−	+	−	+	−	−		(+)
D-Maltose	−	+	−	+	+	+	−	−	−	−	−	+	+	+	+	−	+	−	+
D-Mannitol	−	−	+	−	+	−	+	−	+	−	+	−	+	−	−	−	+	−	−
Raffinose	+	−	+	+	−	−	+	−	−	+	−	+	+	−	−	−	−	−	−
Hydrolysis of:																			
Tyrosine	+	+	+	+	−	+	+	−	+	−	−	−	−	−	+	+	−	+	+
Urea	−	−	+	−	+	+	−	+	−	+	+	−	(+)	+	+	+	+	−	(+)
Growth at:																			
5 °C	−	−	−	+	−	−	−	−	−	+	+	+	−	−	−	−	+	+	+
40 °C	−	+	−	−	+	−	+	+	−	+	−	+	+	−	−	−	+	−	−

Table 2. Cellular fatty acid profiles of strain UTM-3^T and related species of the genus *Chryseobacterium*

Strains: 1, UTM-3^T; 2, *C. lactis* NCTC 11390^T; 3, *C. viscerum* CECT 7793^T; 4, *C. tructae* CECT 7798^T; 5, *C. arthrosphaerae* CCUG 57618^T; 6, *C. oncorhynchi* CECT 7794^T; 7, *C. vietnamense* CCTCC M 209230^T; 8, *C. bernardetii* NCTC 13530^T; 9, *C. nakagawai* NCTC 13529^T; 10, *C. gallinarum* LMG 27808^T; 11, *C. culicis* LMG 25442^T; 12, *C. flavum* KCTC 12877^T; 13, *C. aquifrigidense* KCTC 12894^T; 14, *C. ureilyticum* CCUG 52546^T; 15, *C. indologenes* LMG 8337^T; 16, *C. gleum* CCUG 14555^T; 17, *C. jejuense* JS17-8^T; 18, *C. oranimense* H8^T; 19, *C. joostei* LMG 18212^T. All data are from this study. Fatty acids that account for <1 % of the total fatty acids in all the strains studied are not shown. TR, Trace (<1 %); ND, not detected; ECL, equivalent chain-length (identity of the fatty acid is unknown).

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
iso-$C_{13:0}$	1.4	1.2	1.3	1.6	TR	TR	TR	1.1	TR	1.3	TR	TR	1.4	1.3	TR	TR	1.6	TR	TR
Unknown ECL 13.566	3.3	2.1	TR	3.1	2.2	5.4	2.3	3.2	7.4	3.6	8.7	1.9	1.2	TR	2.2	8.9	8.5	3.6	1.1
iso-$C_{15:0}$	41.2	36.8	40.9	38.5	37.6	33.7	34.4	37.2	37.4	36.1	33.9	35.1	37.1	40.2	34.8	29.6	32.6	41.2	34.6
iso-$C_{15:0}$ 3-OH	3.5	3.2	2.9	3.2	2.4	3.5	3.7	3.1	6.8	3.2	2.5	3.7	3.1	7.6	3.1	2.6	2.5	2.4	2.9
iso-$C_{15:1}$	TR	TR	1.1	TR	TR	TR	TR	TR	TR	1.1	TR	1.4	1.2	1.2	1.8	1.2	TR	TR	TR
anteiso -$C_{15:0}$	TR	1	TR	TR	TR	TR	TR	1.1	TR	TR	1.2	TR	1.1	TR	TR	TR	TR	TR	TR
$C_{16:0}$	1.3	1.4	1.8	1.8	1.9	1.4	2.6	1.8	1.9	1.4	1.8	1.1	2.8	1.2	1.2	1.1	1.8	1.6	1.1
$C_{16:0}$ 3-OH	TR	1.6	TR	1.1	TR	TR	TR	TR	1.5	TR	1.3	2	2.2	1.8	1.6	1.2	1.1	2.2	1.4
Iso-$C_{16:0}$ 3-OH	1.4	1.6	1.9	1.8	TR	TR	1.1	1.8	1.6	1.7		1.2	1.6	1.2	TR	TR	TR	TR	1.2
Unknown ECL 16.580	1.7	2.1	1.1	1.4	1.5	1.1	1.4	1.7	2	3.1	1.3	1.5	-	TR	1.6	1.4	1.3	1.1	1.6
iso-$C_{17:0}$	3	2.6	2.5	3.8	14.1	1.2	3.2	2.8	1.8	2.2	1.4	TR	2.1	4.4	1.1	1.5	1.5	2.8	TR
iso-$C_{17:0}$ 3-OH	10.3	22.4	14.7	14.7	24.1	21.6	25.7	23.6	20.8	16.4	15.5	18.5	17.3	14.7	20.5	18.4	15.4	22.4	20.2
iso-$C_{17:1}\omega 9c$	20.1	8.6	21.6	23.4	2.8	24.6	9.4	12.4	8.2	17.2	20.7	16.8	23.4	17.2	19.8	22.2	21.9	12.6	22.9
Summed feature*																			
4	12.1	14.8	8.2	4.8	12.8	11.2	15.3	8.4	10.4	12.1	11	13.3	3.8	8.4	11.6	11.3	11.0	8.6	12.1
5	ND	ND	1.1	ND	ND	1.2	ND	1.2	TR	ND	ND	2.4	1.2	TR	ND	TR	ND	1.1	TR

*Summed features represent groups or two or more fatty acids that cannot be separated by the Microbial Identification System. Summed feature 4 contains iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega 7t$; summed feature 5 contains iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B.

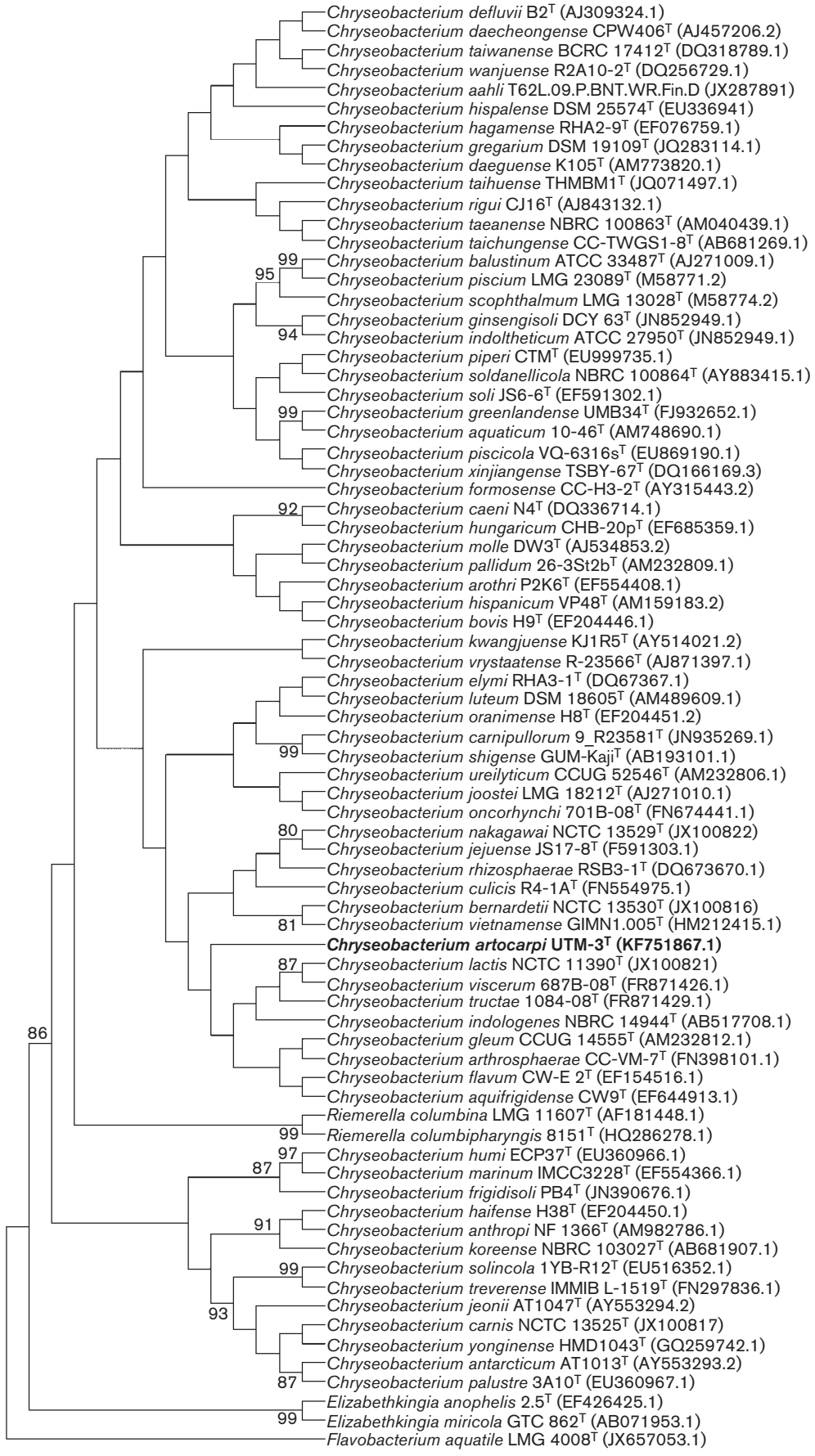

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the position of strain UTM-3^T within the radiation of species of the genus *Chryseobacterium*. Percentage bootstrap values >70 % (based on 1000 replications) are shown at branch points.

Columbia II agar (BBL) with 5 % horse blood for 48 h at 30 °C using the standard protocol of the Sherlock Microbial Identification System (MIDI); the polar lipid profile was also detected (Ventosa *et al.*, 1993). Extraction of genomic DNA, PCR amplification of 16S rRNA gene and sequencing of purified PCR products were carried out according to Miranda-Tello *et al.* (2004). First, DNA sequences (1381 bp) were aligned and trimmed using MEGA 6 software (Tamura *et al.*, 2013) and an initial neighbour-joining tree using Kimura's two-parameter method was generated. Next, a maximum-parsimony analysis using Tree-Bisection-Reconnection (TBR) with 10 initial trees was done with 1000 bootstrap replications. MODELTEST (Posada & Crandall, 1998) was then performed in TOPALi v2 (Milne *et al.*, 2009) to calculate the most suitable substitution model for the maximum-likelihood analysis. Based on the MODELTEST scores, it was found that the General Time Reversible model together with Gamma distribution and Invariant sites (GTR+G+I) most fit the data. These parameters were used for the maximum-likelihood analysis in the MEGA 6 software package. Branch support for all phylogenetic trees were assessed via 1000 bootstrap replications.

The DNA base content (G+C) of strain UTM-3^T was determined by HPLC as described by Tamaoka & Komagata (1984) using non-methylated λ DNA (Sigma) as a standard. DNA–DNA hybridization experiments were performed to confirm the taxonomic status of the novel strain. DNA–DNA hybridization experiments were performed between strain UTM-3^T and the type strains of the most closely related species of the genus *Chryseobacterium* (*C. lactis* NCTC 11390^T, *C. viscerum* CECT 7793^T, *C. tructae* CECT 7798^T, *C. arthrosphaerae* CCUG 57618^T, *C. oncorhynchi* CECT 7794^T, *C. vietnamense* CCTCC M 209230^T, *C. bernardetii* NCTC 13530^T, *C. nakagawai* NCTC 13529^T, *C. gallinarum* LMG 27808^T, *C. culicis* LMG 25442^T, *C. flavum* KCTC 12877^T, *C. aquifrigidense* KCTC 12894^T, *C. ureilyticum* CCUG 52546^T, *C. indologenes* LMG 8337^T, *C. gleum* CCUG 14555^T, *C. jejuense* JS17-8^T, *C. oranimense* H8^T and *C. joostei* LMG 18212^T). DNA was isolated by chromatography on hydroxyapatite (Cashion *et al.*, 1977), and hybridization was performed (De Ley *et al.*, 1970) with the modifications of Escara & Hutton (1980) and Huss *et al.* (1983).

Strain UTM-3^T was Gram-stain-negative, devoid of flagella and gliding motility, non-spore-forming and rod-shaped after 24 h at 30 °C. The colonies were yellowish, translucent and shiny with entire edges becoming mucoid after 72 h of incubation. No growth was observed at 5 °C and above 40 °C. Flexirubin-type pigment was produced on nutrient agar. Strain UTM-3^T grew well on nutrient agar, tryptic soy agar and brain heart infusion agar, but was unable to grow on MacConkey agar. The physiological and other biochemical characteristics of strain UTM-3^T are summarized in Table 1 and in the species description. The predominant respiratory quinone was menaquinone MK-6 with a minor amount of MK-5 present. The polar lipids were composed of phosphatidylethanolamine, an unknown aminolipid, an unknown phospholipid and several unknown lipids. Cellular fatty acid analysis showed that iso-$C_{15:0}$ and iso-$C_{17:1}\omega 9c$ were the most abundant fatty acids followed by summed feature 4 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega 7t$) and iso-$C_{17:0}$ 3-OH. The complete fatty acid pattern of strain UTM-3^T is shown in Table 2 in comparison to the most closely related species of the genus *Chryseobacterium*.

The 16S rRNA gene sequence of strain UTM-3^T consisted of 1381 bp. Seventy bacterial strains were used to reconstruct the phylogenetic trees. MODELTEST results indicated that the General Time Reversible model together with Gamma distribution and Invariant sites (GTR+G+I) most fit the data and this substitution model was used to reconstruct the maximum-likelihood tree (Fig. 1). Sequence database searches revealed that the strain UTM-3^T was most closely related to species of the genus *Chryseobacterium*. Phylogenetic analysis confirmed that strain UTM-3^T associated with the genus *Chryseobacterium*, showing highest sequence similarities to *C. lactis* NCTC 11390^T, *C. viscerum* 687B-08^T, *C. tructae* 1084-08^T, *C. arthrosphaerae* CC-VM-7^T, *C. oncorhynchi* 701B-08^T, *C. vietnamense* GIMN1.005^T, *C. bernardetii* NCTC 13530^T, *C. nakagawai* NCTC 13529^T, *C. gallinarum* LMG 27808^T, *C. culicis* R4-1A^T, *C. flavum* CW-E2^T, *C. aquifrigidense* CW9^T, *C. ureilyticum* CCUG 52546^T, *C. indologenes* NBRC 14944^T, *C. gleum* CCUG 14555^T, *C. jejuense* JS17-8^T, *C. oranimense* H8^T and *C. joostei* LMG 18212^T. DNA–DNA hybridization values of strain UTM-3^T with *C. lactis* NCTC 11390^T, *C. viscerum* CECT 7793^T, *C. tructae* CECT 7798^T, *C. arthrosphaerae* CCUG 57618^T, *C. oncorhynchi* CECT 7794^T, *C. vietnamense* CCTCC M 209230^T, *C. bernardetii* NCTC 13530^T, *C. nakagawai* NCTC 13529^T, *C. gallinarum* LMG 27808^T, *C. culicis* LMG 25442^T, *C. flavum* KCTC 12877^T, *C. aquifrigidense* KCTC 12894^T, *C. ureilyticum* CCUG 52546^T, *C. indologenes* LMG 8337^T, *C. gleum* CCUG 14555^T, *C. jejuense* JS17-8^T, *C. oranimense* H8^T and *C. joostei* LMG 18212^T were 48.3 %, 25.4 %, 33.9 %, 41.2 %, 35.7 %, 29.6 %, 46.8 %, 42.3 %, 25.4 %, 36.8 %, 38.5 %, 24.8 %, 41.6 %, 37.2 %, 35.4 %, 38.8 %, 41.2 % and 38.5 %, respectively. All these values clearly confirm that strain UTM-3^T belongs to a distinct novel genomic species when recommendations by Wayne *et al.* (1987) are considered.

Based on the genotypic and phenotypic characteristics, strain UTM-3^T represents a novel species of the genus *Chryseobacterium*, for which the name *Chryseobacterium artocarpi* sp. nov. is proposed.

Description of *Chryseobacterium artocarpi* sp. nov.

Chryseobacterium artocarpi (ar.to.car′pi. N.L. gen. n. *artocarpi* of the tree *Artocarpus integer* from whose rhizosphere soil the type strain was isolated).

Cells are Gram-stain-negative, non-spore-forming rods approximately 1.8 μm in length and 0.8 μm in diameter, and are devoid of flagella and gliding motility. Colonies are smooth, yellowish, translucent and shiny with entire edges. Colonies become mucoid and cannot be identified as a singly colony after prolonged incubation. Flexirubin-type pigments are produced. Growth is good at 20–30 °C (optimum, 30 °C); no growth at 5 or 40 °C. Growth occurs at pH 5–8 (optimum, pH 7) and in the presence of 0–6 % (w/v) NaCl (optimum, 3 %). Catalase- and oxidase-positive. In the API ZYM gallery, alkaline phosphatase, acid phosphatase, esterase (C4), lipase (C14), leucine arylamidase, valine arylamidase, trypsin and *N*-acetyl-β-glucosaminidase activities are present, but esterase lipase (C8), naphthol-AS-BI-phosphohydrolase, cysteine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase and α-mannosidase activities are absent. With the API 20NE and API 20E kits, nitrate is reduced but nitrite is not reduced. Indole production, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are negative. Aesculin, casein, gelatin, tyrosine and starch are hydrolysed, but Tweens 20 and 80 and urea are not. No precipitation occurs on egg-yolk agar. Hydrogen sulphide is produced on Kligler's iron agar slants. No haemolysis occurs on 5 % horse blood agar. Acid is produced from D-glucose, D-fructose, glycerol, raffinose and trehalose, but not from L-arabinose, cellobiose, ethanol, lactose, D-maltose, D-mannitol or D-xylose. Dextrin, D-glucose, glycogen, D-maltose, D-mannose, sucrose, Tween 40, acetate, propionate, methyl pyruvate, L-alanine, L-asparagine, L-asparate, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-threonine and glycerol are utilized as sole carbon sources. Cells contain MK-6 as the major respiratory quinone and phosphatidylethanolamine as the major polar lipid. The major whole-cell fatty acids are iso-$C_{15:0}$, iso-$C_{17:1}\omega 9c$ followed by summed feature 4 (iso-$C_{15:0}$ 2-OH and/or$C_{16:1}\omega 7t$) and iso-$C_{17:0}$ 3-OH.

The type strain, UTM-3^T (=CECT 8497^T=KCTC 32509^T) was isolated from the rhizosphere soil of *Artocarpus integer* (cempedak) grown in an orchard located on the campus of Universiti Teknologi Malaysia, Malaysia. The DNA G+C content of the type strain is 34.8 mol%.

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