INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION
Chuah *et al.*, *Int J Syst Evol Microbiol*
DOI 10.1099/ijsem.0.002386

MICROBIOLOGY SOCIETY

Floricoccus tropicus gen. nov., sp. nov. and *Floricoccus penangensis* sp. nov. isolated from fresh flowers of durian tree and hibiscus

Li-Oon Chuah,[1] Kien-Pong Yap,[2] Ahamed Kamal Shamila-Syuhada,[1] Kwai Lin Thong,[2] Rosma Ahmad,[3] Min Tze Liong[3] and Gulam Rusul[1,*]

Abstract

Three strains of Gram-staining-positive, coccus-shaped, lactic acid bacteria, designated as HibF3^T, HibF2 and HibF5 were isolated from fresh flowers of hibiscus, and a fourth, DF1^T, was isolated from fresh flowers of durian tree, in Penang, Malaysia. Taxonomic characterisation was performed by polyphasic analysis. Sequence similarities of the 16S rRNA gene and the housekeeping *rpoA* and *pheS* genes of these strains with their closely-related lactococcal and streptococcal relatives were 92–94, 78 and 81 %, respectively. The results of phylogenetic analysis indicated that strains DF1^T, HibF2, HibF5 and HibF3^T were clustered together but were clearly separated from species of the genera *Streptococcus* and *Lactococcus*, indicating that they represent members of a novel genus of the family *Streptococcaceae*. Calculation of average nucleotide identity (ANI) values between the genomes of DF1^T and HibF3^T yielded values of 92.50–92.93 %. ANI values below the cut-off value and distinctive chemotaxonomic characteristics supported the hypothesis that these strains represented two novel species. Major cellular fatty acids in DF1^T, HibF2 and HibF5 were $C_{18:1}\omega7c$ and $C_{16:0}$, while $C_{12:0}$ and $C_{14:0}$ were also dominant, in addition to $C_{18:1}\omega7c$ and $C_{16:0}$, in HibF3^T. A novel genus is proposed with the name *Floricoccus* gen. nov. which consists of two species, *Floricoccus tropicus* sp. nov as the type species, and *Floricoccus penangensis* sp. nov. The respective type strains are DF1^T (=LMG 29833^T=JCM 31733^T) and HibF3^T (=LMG 29831^T=DSM 31735^T).

The family *Streptococcaceae* encompasses three genera, namely *Lactococcus*, *Lactovum* and *Streptococcus*. Members of the family *Streptococcaceae* are Gram-staining-positive, facultatively anaerobic, catalase-negative, ovoid or spherical cocci and do not form endospores. The DNA G+C contents of members of this family range from 33 to 46 mol% [1]. At present, the genera *Lactococcus* (http://www.bacterio.net/lactococcus.html), and *Lactovum* (http://www.bacterio.net/lactovum.html) have twelve and one species respectively, while the genus *Streptococcus*, which is well characterized, consists of 112 species with validly published names (http://www.bacterio.net/streptococcus.html) [2, 3]. During a study on biodiversity of culturable lactic acid bacteria (LAB) present in tropical flowers, four strains of LAB designated DF1^T, HibF2, HibF5 and HibF3^T were isolated from fresh flowers. The morphological and physiological characteristics of the strains were determined, and phylogenetic analyses using the 16S rRNA, *rpoA* and *pheS* gene sequences were performed to determine their taxonomic positions. Species delineation was performed using the average nucleotide sequence identity analysis (ANI). The results indicated that the four strains represent two species of a novel genus, for which the names *Floricoccus tropicus* gen. nov., sp. nov. (DF1^T) and *Floricoccus penangensis* sp. nov. (HibF3^T) are proposed.

Fresh flowers of durian (*Durio zibethinus*) tree were collected from an orchard in Penang (Malaysia) and hibiscus (*Hibiscus rosa-sinensis* L.) flowers from the campus grounds of Universiti Sains Malaysia, in Penang (Malaysia). Strains HibF3^T, HibF2 and HibF5 were isolated from fresh flowers

Author affiliations: [1]Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, Minden, Penang, Malaysia; [2]Institute of Biological Sciences, Faculty of Science Building, University of Malaya, Kuala Lumpur, Malaysia; [3]Bioprocess Technology Division, School of Industrial Technology, Universiti Sains Malaysia, Minden, Penang, Malaysia.
***Correspondence:** Gulam Rusul, rusulg@gmail.com
Keywords: *Floricoccus*; *Floricoccus tropicus*; *Floricoccus penangensis*; flower; lactic acid bacteria; *Streptococcaceae*.
Abbreviations: ANI, Average nucleotide identity; DDH, DNA–DNA hybridization; FAME, fatty acid methyl ester; FESEM, Field Emission Scanning Electron Microscope; LAB, lactic acid bacteria; RDP, Ribosomal Database Project; WGS, whole-genome sequence.
The 16S rRNA gene sequences for DF1^T, HibF2, HibF3^T and HibF5, have been deposited in the GenBank/EMBL/DDBJ databases with the accession numbers KX185706–KX185709, respectively, the *rpoA* gene sequences for DF1^T, HibF2, HibF3^T and HibF5, have been deposited as KY971277–KY971280, respectively, the pheS gene sequences for DF1^T, HibF2, HibF3^T and HibF5 as KY971281–KY971284, respectively and the Whole Genome Shotgun projects for DF1^T and HibF3^T as MKIR00000000 and MKIQ00000000, respectively.
Three supplementary tables and two supplementary figures are available with the online Supplementary Material.

002386 © 2017 IUMS

Downloaded from www.microbiologyresearch.org by
IP: 163.152.24.7
On: Tue, 31 Oct 2017 01:01:53

of hibiscus and DF1^T was isolated from fresh flowers of a durian tree, by using all purpose Tween (APT) media supplemented with 1 % sodium pyruvate incubated at 30 °C for 24–48 h. Motility of cells was observed by inoculating the cultures into motility agar using the streak/stab technique. Light microscopy was used to observe Gram staining reaction, cell shape, cell arrangement and endospore formation. Cell shape and size were also determined using a Leo Supra 50 VP Field Emission Scanning Electron Microscope (FESEM; Carl Zeiss), equipped with an INCA-X energy dispersive microanalysis system (Oxford Instruments). Sample preparation for SEM was performed as previously described by Choi and Lee [4], with minor modifications. Briefly, overnight cultures were fixed in McDowell–Trump fixative [4 % formaldehyde (Ajax UNIVAR) and 1 % glutaraldehyde (TAAB Laboratory)] for 2 h and post-fixed in 1 % osmium tetroxide solution (Electron Microscope Sciences) for 1 h. The fixed cells were serially dehydrated in ethanol (50, 75, 90 and 99.98 %), kept in hexamethyldisilazane (Electron Microscope Sciences), decanted and allowed to dry. Catalase activity, oxidase activity (Microbact Oxidase Strips, Oxoid) and the lactic acid configuration produced from glucose (D- and L-lactate dehydrogenase test kits, Megazyme) were determined after 24 h. Gas production was assessed as described by Leisner *et al.* [5], but using APT broth supplemented with 1 % (w/v) sodium pyruvate. Salt tolerance (growth in the presence of 4.0, 5.0 and 6.5 % NaCl), pH tolerance (pH 3, 5, 6, 7.5 and 9) and temperature grow range (7, 15, 25, 30, 35, 40 and 45 °C) were determined as described previously [6], with minor modifications. In this study, brain–heart infusion (BHI) broth supplemented with 0.1 % (w/v) bromocresol purple was used as the basal medium and growth was evaluated after 1, 2 and 5 days. Acidifying activity in milk was determined by measuring the pH after 6 and 24 h [7]. Haemolysis testing was performed by streaking overnight cultures on blood agar base (Oxoid) supplemented with 5 % (v/v) sheep blood (Oxoid) and incubating them under anaerobic condition at 37 °C for 24–48 h. The carbohydrate fermentation profile was determined using API 50 CHL kits (bioMérieux), enzyme production using API ZYM (bioMérieux) and biochemical characteristics were analysed using API 20 Strep (bioMérieux), according to the manufacturer's instructions. Determinations of the cellular fatty acids composition were performed by the BCCM/LMG (Belgian Coordinated Collections of Microorganisms/Laboratory of Microbiology, Department of Biochemistry and Microbiology, Faculty of Sciences of Ghent University) identification service. Cells were grown for 4 days at 28 °C under micro-aerophilic conditions on BHI medium (Oxoid). Inoculation and harvesting of the cells, and the extraction and analysis were performed according to the standard protocols of the Microbial Identification System (MIDI), except that cells were harvested from a liquid culture by centrifugation to obtain a sufficient concentration of fatty acids in the extract.

16S rRNA PCR amplification was performed as described by Endo and Okada [8], using two primers: 8F (5′-AGAG TTTGATCMTGCTCAG-3′) and 15R (5′-AAGGAGGTGA TCCARCCGCA-3′). The housekeeping genes *rpoA* and *pheS* were amplified by PCR using primers rpoA-21-F (5′-A TGATYGARTTTGAAAAACC-3′), rpoA-23-R (5′-ACH GTR TTR ATD CCD GCR CG-3′), pheS-21-F (5′-CAYCC NGCHCGYGAYATC-3′) and pheS-23-R (5′-GGRTGRAC-CATVCCNGCHCC-3′) [9]. PCR was performed using a TProfessional Standard Gradient 96 Thermocycler (Biometra). DNA sequencing was performed by 1st BASE Laboratories (Selangor, Malaysia). The similarities between the isolates based on 16S rRNA, *rpoA* and *pheS* genes were calculated by using the pairwise nucleotide sequence alignments of the BioEdit software [10]. The 16S rRNA gene sequences were compared with sequences from type strains deposited in Ribosomal Database Project (RDP) database (http://rdp.cme.msu.edu/) [11]. Classification of the selected sequences was performed using RDP Naive Bayesian rRNA Classifier Version 2.10 [12], with a confidence threshold of 80 %. The Jukes–Cantor corrected distance model was employed to generate the distance matrix. The phylogenetic tree was reconstructed using Weighbor, a weighted neighbour-joining algorithm, with bootstrap analysis based on 100 replicates.

DNA extraction of the overnight cultures were performed using the DNeasy Blood and Tissue Kit (Qiagen). The extracted genomic DNA was sent to Science Vision (Selangor, Malaysia) for library construction and sequenced on a MiSeq platform (Illumina). Sequence trimming, QC, *de novo* assembly and annotation of the whole-genome sequence (WGS) were performed as previously described [13]. The DNA G+C contents for the type strains were obtained from the WGS. DNA–DNA relatedness was determined by calculating the percentage ANI as previously described [14]. The representative full-genome sequences of all available closely related genera and species (*Streptococcus* and *Lactococcus*) to Hib3^T and DF1^T were downloaded from the NCBI FTP site (ftp://ftp.ncbi.nlm.nih.gov/). An ANI value of $\geq$94–95 % corresponds to 70 % DNA–DNA hybridization (DDH), a cut-off points for species delineation [14, 15].

DF1^T, HibF2, HibF5 and HibF3^T are Gram-staining-positive, catalase- and oxidase-negative, non-endospore-forming and non-motile cocci. Scanning electron microscopy (SEM) revealed that the cell division occurred in one plane, producing diplococcus or streptococcus arrangement (Fig. S1, available in the online Supplementary Material). 16S rRNA gene sequence (1359–1373 bp) similarities of the four strains to type strains of their closest lactococcal and streptococcal relatives in GenBank were 92–94 % (results not shown), which are lower than the cut-off value of 93–95 % which has been conventionally applied as the threshold for genera delineation [16–20]. Comparison of the partial DNA sequences of housekeeping genes i.e. *rpoA* (722–745 bp) and *pheS* (392–396 bp) to those of type strains of closely-related relatives in GenBank revealed low similarity between the novel LAB strains and their closest relatives

Downloaded from www.microbiologyresearch.org by
IP: 163.152.24.7
On: Tue, 31 Oct 2017 01:01:53

(*rpoA* 78 % and *pheS* 81 %; results not shown). Based on the 70 % DNA–DNA relatedness, the cut-off values for *rpoA* and *pheS* gene sequences similarities for species delineation were 95 and 90 %, respectively [21]. Other research groups have also reported on a cut-off value of 92.3 % for species delineation based on *rpoA* gene similarity among species of the genus *Streptococcus* [22]. In this study, however, these housekeeping gene sequences were too distant (dissimilar) for phylogenetic analysis, mainly due to the very low maximum score and percentages of similarity between the novel LAB strains and their closest relatives, which were members of a various genera of LAB. Hence, phylogenetic analysis was performed by using the 16S rRNA gene sequence. The results (Fig. 1) indicated that all four strains (DF1^T, HibF2, HibF5 and HibF3^T) belong to a novel line of descent in the family *Streptococcaceae*. They were clustered together and were clearly separated from members of the genera *Streptococcus*, *Lactococcus* and *Lactovum*, another member of the family *Streptococcaceae*. The percentages of similarity between these LAB strains and their close relatives, which were below the cut-off values, and the topology of the phylogenetic tree conclusively indicated that they represented members of a novel genus. In addition to morphological observations and phylogenetic analysis, a series of phenotypic analyses were conducted. Differences in phenotypic characteristics among the novel type strains and their closely-related streptococcal and lactococcal relatives are presented in Table 1. The DNA G+C contents for members of the family *Streptococcaceae* ranged from 33 to 46 mol% [1]. The DNA G+C contents for DF1^T and HibF3^T were 33.0 and 33.1 %, respectively, which were at the lower end of the range within this family. Major differences in phenotypic characteristic which differentiated DF1^T and HibF3^T from their relatives included growth at 40 °C and at pH 5.0, production of gas from glucose and production of leucine arylamidase and acid phosphatase. Both DF1^T and HibF3^T produced gas from glucose (for HibF3^T, gas production was weak). Even though gas production from glucose was not determined in those studies cited, generally speaking, streptococci and lactococci are characteristically unable to produce gas from glucose and [1, 23]. Also, production of leucine arylamidase was observed in all streptococcal and lactococcal relatives, but not in the novel isolates. Both DF1^T and HibF3^T were able to grow at 40 °C and pH 5.0 (Table S2), while growth was not observed for their lactococcal relatives [24–27]. The fatty acid profile of DF1^T was similar to those of its lactococcal relatives except for *Lactococcus piscium* DSM 6634^T (Table S3). The saturated fatty acid

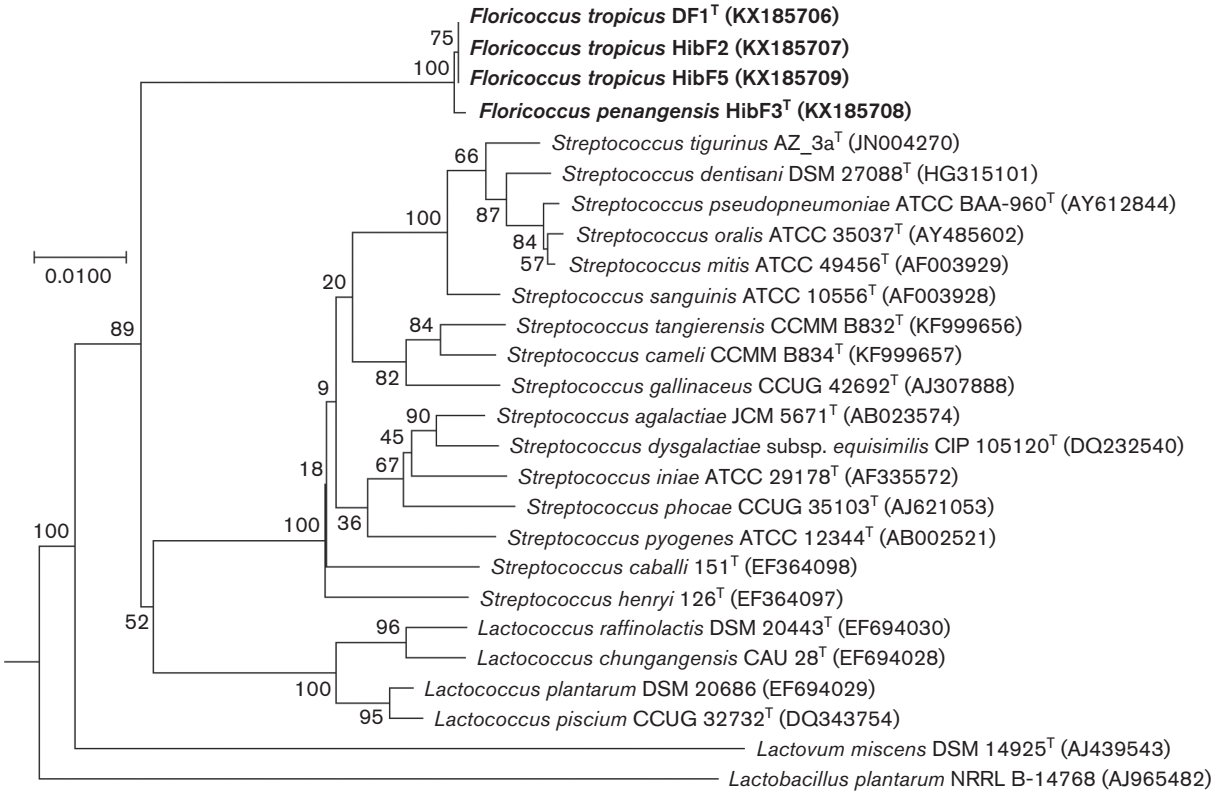

Fig. 1. Phylogenetic tree showing the relationships of strain DF1^T, HibF2, HibF5 and HibF3^T and closely-related species. 16S rRNA gene sequences of DF1^T, HibF2, HibF3 and HibF5^T have been deposited in GenBank under the accession numbers KX185706, KX185707, KX185708 and KX185709, respectively. *Lactobacillus plantarum* NRRL B-14768 was used as an outgroup. Bootstrap percentages (based on 100 replications) are shown at nodes.

Downloaded from www.microbiologyresearch.org by
IP: 163.152.24.7
On: Tue, 31 Oct 2017 01:01:53

Table 1. Differential phenotypic characteristic of DF1^T, HibF3^T and type strains of closely-related species from the family *Streptococcaceae*

Taxa: 1, *Floricoccus tropicus* gen. nov., sp. nov. DF1^T (data from this study); 2, *Floricoccus penangensis* gen. nov., sp. nov. HibF3^T (data from this study); 3, *Streptococcus tigurinus* DSM 24864^T [26]; 4, *Streptococcus cameli* LMG 27685^T [27]; 5, *Streptococcus caballi* DSM 19004^T [28]; 6, *Streptococcus henryi* DSM 19005^T [28]; 7, *Streptococcus hongkongensis* DSM 26014^T [29]; 8, *Lactococcus chungangensis* KCTC 13185^T [30]; 9, *Lactococcus piscium* DSM 6634^T [31, 32]; 10, *Lactococcus plantarum* DSM 20686^T [33]. +, Positive; −, negative; NA, not available; W, weak.

Characteristic	1	2	3	4	5	6	7	8	9	10
DNA G+C content (mol%)	33.0	33.1	40.0	40.8	46.8	38.7	35.6	NA	38.5	36.9–38.1
Gas production from glucose	+	W	−*	−*	−*	−*	−*	−†	−†	−†
Hydrolysis of aesculin	+	W	−	+	+	+	+	+	+	+
Hydrolysis of arginine	+	+	−	NA	−	−	+	NA	−	−
Hydrolysis of hippuric acid	−	−	−	−	−	−	−	+	NA	−
Voges–Proskauer test	+	+	−	NA	+	−	−	+	NA	+
Acid production from:										
L-Arabinose	−	−	−	−	−	−	−	−	+	−
Ribose	−	−	−	−	−	−	+	−	+	−
Galactose	−	−	+	−	NA	NA	+	−	+	−
Mannitol	+	+	−	−	−	+	+	+	+	+
Lactose	−	−	+	+	−	+	+	−	+	−
Melibiose	−	−	−	−	+	+	NA	−	+	−
Melezitose	+	+	−	−	−	−	NA	−	+	+
Glycogen	+	+	−	−	+	+	+	−	−	−
Enzyme production:										
β-glucosidase	−	−	−	NA	+	+	+	−	NA	NA
Leucine arylamidase/aminopeptidase	−	−	+	+	+	+	+	+	+	+
β-glucuronidase	−	−	−	−	−	−	NA	−	W	+
Acid phosphatase	−	−	NA	NA	NA	NA	NA	+	+	+

*Streptococci are characteristically unable to produce gas from glucose [1].

†Lactococci are characteristically unable to produce gas from glucose [23].

$C_{12:0}$ was one of the major components present in HibF3^T but not in its lactococcal relatives [24–27]. The phenotypic analysis further supported the hypothesis that DF1^T, HibF2, HibF5, and HibF3^T were clearly separated from other type strains of species of the genera *Lactococcus* and *Streptococcus*. Additionally, DF1^T and HibF3^T could be differentiated phenotypically from members of the genus *Lactovum*, another member of the family *Streptococcaceae*, on the basis of cell shape, production of gas from glucose, ranges of temperature and pH permitting growth and growth on galactose. Cells of species of the genus *Lactovum* are ovoid while cells of the novel isolates are cocci. Members of the genus *Lactovum* produced insignificant amount of gas from glucose, were able to grow in galactose and able to grow at 0–7 °C but not at 40 °C and pH 8.1 [28]. DF1^T and HibF3^T produced gas from glucose, were unable to utilise galactose and able to grow at 40 °C and pH 9 but not at 7 °C (Table S2). These strains are proposed to represent a novel genus, for which the name *Floricoccus* gen. nov. is proposed.

To determine if the four strains represented the same species, similarities between strains DF1^T, HibF2, HibF5 and HibF3^T based on 16S rRNA, *rpoA* and *pheS* genes were calculated by using the pairwise nucleotide sequence alignments. Results obtained in this study indicated that DF1^T, HibF2, HibF5 and HibF3^T shared 99–100, 97.7–100 and 97.7–100 % similarities based on the 16S rRNA, *rpoA* and *pheS* gene sequences, respectively (Table S1). Stackebrandt and Ebers [29] reported a cut-off value of 98.7–99 % similarity for species delineation based on 16S rRNA gene sequences. Naser *et al.* [21] reported on the intraspecies similarities of 98 and 97 % for the *rpoA* and *pheS* gene sequences, respectively. Determination of genetic relatedness between strains is compulsory for species delineation in cases where multiple strains are present in the novel taxon and those strains share ≥97 % 16S rRNA, 98 % *rpoA* and 97 % *pheS* gene sequences similarity. Comparison of the ANI indices (as an alternative to DDH in species delineation) was performed, for which 94–95 % ANI is recognised as the cut-off value [14, 15]. Results of comparison of the 16S rRNA, *rpoA* and *pheS* gene sequences indicated that HibF3^T is most likely to represent a different species from DF1^T, HibF2 and HibF5. ANI values between genomes of DF1^T and HibF3^T were 93 %, thus strongly supporting the classification of these isolates into two novel species (Fig. S2). Phenotypic and chemotaxonomic characterisation were also performed (Tables S2 and S3). Similar phenotypic characteristics were observed among strains DF1^T, HibF2 and HibF5. Differences between HibF3^T and the other three strains were observed in gas production from glucose, bile aesculin

Downloaded from www.microbiologyresearch.org by
IP: 163.152.24.7
On: Tue, 31 Oct 2017 01:01:53

hydrolysis, carbohydrate utilisation (Table S2) and fatty acid methyl ester (FAME) profiles (Table S3). DF1^T, HibF2 and HibF5 produced abundant amounts of gas from glucose and hydrolysed aesculin while HibF3^T produced gas and hydrolysed aesculin weakly. Variable results were observed in carbohydrate utilisation profiles as DF1^T, HibF2 and HibF5, but not HibF3^T, were able to utilise amygdalin, β-gentiobiose and turanose and HibF3^T could only utilize salicin and cellobiose weakly. Fatty acid profiles indicated that DF1^T, HibF2, HibF5 and HibF3^T produced $C_{18:1}\omega7c$ and $C_{16:0}$ but HibF3^T could be distinguished from the other strains as it also produced large amounts of $C_{12:0}$ and $C_{14:0}$ (Table S3). ANI values below the cut-off value and distinctive chemotaxonomic and phenotypic characteristics supported the hypothesis that these strains represent two novel species, for which the names *Floricoccus* gen. nov. *tropicus* sp. nov (DF1^T, HibF2 and HibF5) and *Floricoccus* gen. nov. *penangensis* sp. nov (HibF3^T) are proposed. *Floricoccus tropicus* is proposed as the type species.

DESCRIPTION OF *FLORICOCCUS* GEN. NOV.

Floricoccus (Flo.ri.coc′cus. L. masc. n. *flos*, *floris* flower; N.L. masc. n. *coccus* grain; N.L. masc. n. *Floricoccus* a coccus from a flower).

Cells are Gram-staining-positive, catalase-negative, oxidase-negative, facultatively anaerobic, heterofermentative and non-spore forming cocci. They produce gas from glucose and lack leucine arylamidase and acid phosphatase. The major fatty acids consistently produced by all members of the genus *Floricoccus* are $C_{18:1}\omega7c$ and $C_{16:0}$.

Floricoccus tropicus is proposed as the type species.

DESCRIPTION OF *FLORICOCCUS TROPICUS* SP. NOV.

Floricoccus tropicus [tro′pi.cus. L. masc. adj. *tropicus*, tropical, of or pertaining to the tropic(s), relating to its isolation from tropical flowers].

Cells are Gram-staining-positive, non-endospore-forming and non-motile cocci. Cells in broth culture are mostly in chains or occur in pairs or singly. Colonies are about 1–3 mm in diameter, smooth, circular with even margins and convex elevation, cream–white and opaque, after growth on APT media supplemented with 1 % (w/v) sodium pyruvate, at 30 °C for 24–48 h. Major cellular fatty acids are unsaturated $C_{18:1}\omega7c$ and saturated $C_{16:0}$. Cells are able to grow on bile aesculin agar but not on MRS and M17 lactose agar, and weakly on BHI agar. Weak α-haemolysis was observed when grown aerobically on sheep blood agar for 24 h. Cells are catalase- and oxidase-negative, and do not hydrolyse hippuric acid or utilise citrate. Positive reactions are observed in Voges–Proskauer and methyl red tests and for hydrolysis of arginine and gelatin. They produce gas and L-lactic acid from glucose, suggesting heterolactic fermentation. Growth was observed at pH 5, 6, 7.5 and 9 but not pH 3. Cells are able to grow at a temperature range of 15–40 °C, with optimum growth observed at 30–37 °C. Growth at 7 and 45 °C was not observed. All the tested strains were not tolerant to 4, 5 and 6.5 % NaCl. Acid was produced from D-glucose, D-fructose, D-mannose, mannitol, *N*-acetyl-glucosamine, arbutin, aesculin, maltose, sucrose, trehalose, melezitose, starch, glycogen, amygdalin, salicin, cellobiose, β-gentiobiose and turanose, but not from glycerol, erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, methyl β-xyloside, galactose, L-sorbose, rhamnose, dulcitol, inositol, sorbitol, methyl α-D-mannoside, methyl α-D-gulucoside, lactose, melibiose, inulin, raffinose, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate, 5-ketogluconate and starch. Acidifying activity in milk was not observed. In API ZYM and API 20 Strep tests, cells produce naphthol-AS-BI-phosphohydrolase and α-galactosidase, but production of valine arylamidase was weak. They do not produce alkaline phosphatase, esterase (C 4), esterase lipase (C 8), lipase (C 14), leucine arylamidase/aminopeptidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, pyrrolidonyl arylamidase and arginine dihydrolase.

The proposed type strain, DF1^T (=LMG 29833^T=JCM 31733^T) was isolated from fresh flowers of durian (*Durio zibethinus*) in an orchard in Teluk Bahang, Penang, Malaysia on 16th December 2014. The DNA G+C content of the type strain is 33.0 mol%.

DESCRIPTION OF *FLORICOCCUS PENANGENSIS* SP. NOV.

Floricoccus penangensis (pe.nang.en′sis N.L. masc. adj. *penangensis* of Penang, named after the northern state in Malaysia, from where the species was isolated).

Cells are Gram-staining-positive, non-endospores forming and non-motile cocci. Cells in broth culture are mostly in chains or occur in pairs or singly. Colonies are about 1–2 mm in diameter, smooth, circular with even margins and convex elevation, cream–white and opaque, after growth on APT media supplemented with 1 % (w/v) sodium pyruvate, at 30 °C for 24–48 h. Major cellular fatty acids are the saturated $C_{16:0}$, unsaturated $C_{18:1}\omega7c$, saturated $C_{12:0}$ and saturated $C_{14:0}$. Cells grow weakly on bile aesculin agar and BHI agar but not on MRS and M17 lactose agar. Weak α-haemolysis was observed when grown aerobically on sheep blood agar for 24 h. Cells are catalase- and oxidase-negative, do not hydrolyse hippuric acid or utilise citrate. Positive reactions were observed in Voges–Proskauer and methyl red tests and for hydrolysis of arginine and gelatin. They produce gas weakly and L-lactic acid from glucose, suggesting heterolactic fermentation. Growth was observed at pH 5, 6, 7.5 and 9 but not pH 3. Cells are able to grow at a temperature range of 15–40 °C, optimally at 30–37 °C. Growth at 7 or 45 °C was not observed. The strain is not tolerant to 4, 5 and 6.5 % NaCl. Acid was produced from D-glucose, D-fructose, D-mannose, mannitol, *N*-acetyl-glucosamine,

Downloaded from www.microbiologyresearch.org by
IP: 163.52.24.7
On: Tue, 31 Oct 2017 01:01:53

arbutin, aesculin, maltose, sucrose, trehalose, melezitose, starch and glycogen but not from glycerol, erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, amygdalin, methyl β-xyloside, galactose, L-sorbose, rhamnose, dulcitol, inositol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, lactose, melibiose, inulin, raffinose, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, β-gentiobiose, turanose, 2-ketogluconate, 5-ketogluconate and starch. Acid was produced weakly from salicin and cellobiose. Acidifying activity in milk was not observed. In API ZYM and API 20 Strep tests, cells produce naphthol-AS-BI-phosphohydrolase and α-galactosidase and valine arylamidase were produced weakly, but alkaline phosphatase, esterase (C 4), esterase lipase (C 8), lipase (C 14), leucine arylamidase/aminopeptidase, cystine arylamidase, trypsin, acid phosphatase, α-chymotrypsin, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, pyrrolidonyl arylamidase and arginine dihydrolase were not produced.

The proposed type strain, HibF3^T (=LMG 29831^T=DSM 31735^T) was isolated from fresh flowers of Hibiscus (*Hibiscus rosa-sinensis* L.) in the campus ground of Universiti Sains Malaysia, in Penang, Malaysia on 16th December 2014. The DNA G+C content of the type strain is 33.1 mol%.

Funding information
This work, including the efforts of Li-Oon Chuah, was funded by the School of Industrial Technology, Universiti Sains Malaysia (Graduate Assistant Scheme) and the Ministry of Higher Education, Malaysia (MOHE) (MyBrain15). This work, including the efforts of Gulam Rusul, was funded by the MOHE [Fundamental Research Grant Scheme (203/PTEKIND/6711238)]. This work was also funded by the University of Malaya (UM.C/625/1/HIR/MOHE/CHAN/02).

Acknowledgements
We would like to thank Proffessor Paul A. Lawson for advice on genus name and Latin usage.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
Ethical approval was not necessary.

References

1. **Whiley RA, Hardie JM.** Family VI. *Streptococcaceae* Deibel and Seeley 1974, 490^{AL}. Genus I. *Streptococcus* Rosenbach 1884, 22AL. In: De Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W *et al.* (editors). *Bergeys Manual of Systematic Bacteriology*, 2nd ed. New York: Springer; 1974. pp. 655–711.
2. **Euzéby JP.** List of bacterial names with standing in nomenclature: a folder available on the Internet. *Int J Syst Bacteriol* 1997;47:590–592.
3. **Parte AC.** LPSN-list of prokaryotic names with standing in nomenclature. *Nucleic Acids Res* 2014;42:D613–D616.
4. **Choi WY, Lee HY.** Complete nucleotide sequence of the 16S rRNA from *Lactobacillus paracasei* HS-05 isolated from women's hands. *AMB Express* 2015;5:78–83.
5. **Leisner JJ, Vancanneyt M, van der Meulen R, Lefebvre K, Engelbeen K *et al.*** *Leuconostoc durionis* sp. nov., a heterofermenter with no detectable gas production from glucose. *Int J Syst Evol Microbiol* 2005;55:1267–1270.
6. **Yan Yang S, Zheng Y, Huang Z, Min Wang X, Yang H *et al.*** *Lactococcus nasutitermitis* sp. nov. isolated from a termite gut. *Int J Syst Evol Microbiol* 2016;66:518–522.
7. **Pisano MB, Fadda ME, Melis R, Ciusa ML, Viale S *et al.*** Molecular identification of bacteriocins produced by *Lactococcus lactis* dairy strains and their technological and genotypic characterization. *Food Control* 2015;51:1–8.
8. **Endo A, Okada S.** *Lactobacillus satsumensis* sp. nov., isolated from mashes of shochu, a traditional Japanese distilled spirit made from fermented rice and other starchy materials. *Int J Syst Evol Microbiol* 2005;55:83–85.
9. **Naser SM, Thompson FL, Hoste B, Gevers D, Dawyndt P *et al.*** Application of multilocus sequence analysis (MLSA) for rapid identification of *Enterococcus* species based on *rpoA* and *pheS* genes. *Microbiology* 2005;151:2141–2150.
10. **Hall TA.** BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 1999;41:95–98.
11. **Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM *et al.*** Ribosomal database project: data and tools for high throughput rRNA analysis. *Nucleic Acids Res* 2014;42:D633–D642.
12. **Wang Q, Garrity GM, Tiedje JM, Cole JR.** Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 2007;73:5261–5267.
13. **Chuah LO, Yap KP, Thong KL, Liong MT, Ahmad R *et al.*** Genome sequence of *"Anthococcus,"* a novel genus of the family *Streptococcaceae* isolated from flowers. *Genome Announc* 2016;4:e01410-16.
14. **Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P *et al.*** DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 2007;57:81–91.
15. **Konstantinidis KT, Tiedje JM.** Genomic insights that advance the species definition for prokaryotes. *Proc Natl Acad Sci USA* 2005;102:2567–2572.
16. **Adékambi T, Shinnick TM, Raoult D, Drancourt M.** Complete *rpoB* gene sequencing as a suitable supplement to DNA-DNA hybridization for bacterial species and genus delineation. *Int J Syst Evol Microbiol* 2008;58:1807–1814.
17. **Collins MD, Lawson PA, Willems A, Cordoba JJ, Fernandez-Garayzabal J *et al.*** The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. *Int J Syst Bacteriol* 1994;44:812–826.
18. **Qin QL, Xie BB, Zhang XY, Chen XL, Zhou BC *et al.*** A proposed genus boundary for the prokaryotes based on genomic insights. *J Bacteriol* 2014;196:2210–2215.
19. **Whiteson KL, Hernandez D, Lazarevic V, Gaia N, Farinelli L *et al.*** A genomic perspective on a new bacterial genus and species from the *Alcaligenaceae* family, *Basilea psittacipulmonis*. *BMC Genomics* 2014;15:169–14.
20. **Yarza P, Yilmaz P, Pruesse E, Glöckner FO, Ludwig W *et al.*** Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nat Rev Microbiol* 2014;12:635–645.
21. **Naser SM, Dawyndt P, Hoste B, Gevers D, Vandemeulebroecke K *et al.*** Identification of lactobacilli by *pheS* and *rpoA* gene sequence analyses. *Int J Syst Evol Microbiol* 2007;57:2777–2789.
22. **Park HK, Yoon JW, Shin JW, Kim JY, Kim W.** *rpoA* is a useful gene for identification and classification of *Streptococcus pneumoniae* from the closely related viridans group streptococci. *FEMS Microbiol Lett* 2010;305:58–64.
23. **Teuber M.** Family VI. *Streptococcaceae* Deibel and Seeley 1974, 490^{AL}. Genus II. *Lactococcus* Schleifer, Kraus, Dvorak, Kilpper-Bälz, Collins and Fischer 1986, 354 VP. In: De Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W *et al.* (editors). *Bergeys Manual of Systematic Bacteriology*. New York: Springer; 2009. pp. 712–722.
24. **Cho SL, Nam SW, Yoon JH, Lee JS, Sukhoom A *et al.*** *Lactococcus chungangensis* sp. nov., a lactic acid bacterium isolated from activated sludge foam. *Int J Syst Evol Microbiol* 2008;58:1844–1849.

Downloaded from www.microbiologyresearch.org by
IP: 163.152.24.7
On: Tue, 31 Oct 2017 01:01:53

25. Cai Y, Yang J, Pang H, Kitahara M. *Lactococcus fujiensis* sp. nov., a lactic acid bacterium isolated from vegetable matter. *Int J Syst Evol Microbiol* 2011;61:1590–1594.
26. Williams AM, Fryer JL, Collins MD. *Lactococcus piscium* sp. nov. a new *Lactococcus* species from salmonid fish. *FEMS Microbiol Lett* 1990;68:109–113.
27. Schleifer KH, Kraus J, Dvorak C, Kilpper-Bälz R, Collins MD *et al.* Transfer of *Streptococcus lactis* and related Streptococci to the genus *Lactococcus* gen. nov. *Syst Appl Microbiol* 1985;6:183–195.
28. Matthies C, Gössner A, Acker G, Schramm A, Drake HL. *Lactovum miscens gen. nov., sp. nov.*, an aerotolerant, psychrotolerant, mixed-fermentative anaerobe from acidic forest soil. *Res Microbiol* 2004;155:847–854.
29. Stackebrandt E, Ebers J. Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today* 2006;33:152–155.
30. Zbinden A, Mueller NJ, Tarr PE, Spröer C, Keller PM *et al.* *Streptococcus tigurinus sp. nov.*, isolated from blood of patients with endocarditis, meningitis and spondylodiscitis. *Int J Syst Evol Microbiol* 2012;62:2941–2945.
31. Kadri Z, Vandamme P, Ouadghiri M, Cnockaert M, Aerts M *et al.* *Streptococcus tangierensis* sp. nov. and *Streptococcus cameli* sp. nov., two novel *Streptococcus* species isolated from raw camel milk in Morocco. *Antonie van Leeuwenhoek* 2015;107:503–510.
32. Milinovich GJ, Burrell PC, Pollitt CC, Bouvet A, Trott DJ. *Streptococcus henryi sp. nov.* and *Streptococcus caballi* sp. nov., isolated from the hindgut of horses with oligofructose-induced laminitis. *Int J Syst Evol Microbiol* 2008;58:262–266.
33. Lau SK, Curreem SO, Lin CC, Fung AM, Yuen KY *et al.* *Streptococcus hongkongensis sp. nov.*, isolated from a patient with an infected puncture wound and from a marine flatfish. *Int J Syst Evol Microbiol* 2013;63:2570–2576.

Five reasons to publish your next article with a Microbiology Society journal

1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as 'excellent' or 'very good'.
5. Your article will be published on an interactive journal platform with advanced metrics.

Find out more and submit your article at microbiologyresearch.org.

Downloaded from www.microbiologyresearch.org by
IP: 163.152.24.7
On: Tue, 31 Oct 2017 01:01:53