

# *Chryseobacterium pennae* sp. nov., isolated from poultry feather waste

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## Abstract

A Gram-stain-negative, rod-shaped, non-motile, non-spore-forming, aerobic, yellow-pigmented bacterium was isolated from chicken feather waste collected from an abattoir in Bloemfontein, South Africa. A polyphasic taxonomy study was used to describe and name the bacterial isolate, strain 1\_F178<sup>T</sup>. The 16S rRNA gene sequence analysis and sequence comparison data indicated that strain 1\_F178<sup>T</sup> was a member of the genus *Chryseobacterium* and was closely related to *Chryseobacterium jejuense* (99.1%) and *Chryseobacterium nakagawai* (98.7%). Overall genome similarity metrics (average nucleotide identity, digital DNA–DNA hybridization and average amino acid identity) revealed greatest similarity to the *C. jejuense* and *C. nakagawai* type strains but were below the threshold for species delineation. Genome sequencing revealed a genome size of 6.18 Mbp and a G+C content of 35.6 mol%. The major respiratory quinone and most abundant polar lipid of strain 1\_F178<sup>T</sup> were menaquinone-6 and phosphatidylethanolamine, respectively. Strain 1\_F178<sup>T</sup> had a typical fatty acid composition for *Chryseobacterium* species. On the basis of physiological, genotypic, phylogenetic and chemotaxonomic data, strain 1\_F178<sup>T</sup> constitutes a novel species of *Chryseobacterium*, for which the name *Chryseobacterium* pennae sp. nov. is proposed. The type strain is 1\_F178<sup>T</sup> (=LMG 30779<sup>T</sup>=KCTC 62759<sup>T</sup>).

The genus Chryseobacterium initially belonged to the family Flavobacteriaceae and was first described by Vandamme et al. [1]. The genus Chryseobacterium has undergone several changes since its description and currently consists of 132 species [2]. Chryseobacterium has recently been reclassified into the family Weeksellaceae [3]. Another study suggested that nine Chryseobacterium species (Chryseobacterium arachidiradicis, Chryseobacterium bovis, Chryseobacterium caeni, Chryseobacterium hispanicum, Chryseobacterium hominis, Chryseobacterium hungaricum, Chryseobacterium molle, Chryseobacterium pallidum, Chryseobacterium zeae) should be transferred to the genus Epilithonimonas, 11 species (Chryseobacterium anthropi, Chryseobacterium antarcticum, Chryseobacterium carnis, Chryseobacterium chaponense, Chryseobacterium haifense, Chryseobacterium jeonii, Chryseobacterium montanum, Chryseobacterium palustre, *Chryseobacterium solincola, Chryseobacterium treverense, Chryseobacterium yonginense*) to the genus *Kaistella* and three (*Chryseobacterium frigidisoli, Chryseobacterium humi, Chryseobacterium marina*) to the genus *Halpernia* [4].

Chryseobacterial cells are Gram-stain-negative rods, aerobic, non-sporulating and non-motile. Colonies are circular, yellow-pigmented due to the presence of flexirubin, oxidase-positive and catalase-positive. Their predominant branched chain fatty acids are iso- $C_{15:0}$ , iso- $C_{17:0}$  3-OH and iso- $C_{17:1}$   $\omega$ 9c, with menaquinone-6 (MK-6) as the major respiratory quinone and phosphatidylethanolamine (PE) as the major polar lipid [5].

*Chryseobacterium* species have been isolated from a variety of food sources including fish, meat, poultry and dairy products [6–11]. Some species cause spoilage in milk due to lipolytic and

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Keywords: Chryseobacterium; pennae; taxonomy.

Abbreviations: AAI, average amino acid identity; ANI, average nucleotide identity; BPW, buffered peptone water; dDDH, digital DNA–DNA hybridization; FAME, fatty acid methyl ester; MK-6, menaquinone-6; NB, nutrient broth; OGRI, overall genomic relatedness index; PE, phosphatidylethanolamine. The GenBank/EMBL/DDBJ accession for the 16S rRNA gene sequence of *Chryseobacterium pennae* strain 1\_F178<sup>T</sup> is MH059518.1. The DDBJ/ENA/ Genbank accession for the whole genome shotgun sequence is QNVT00000000. The version described in this paper is version QNVT01000000. One supplementary table and five supplementary figures are available with the online version of this article.

proteolytic activities [12]. *Chryseobacterium/Flavobacterium* were also reported to cause spoilage in butter [13, 14] and creamed rice [15]. In contrast, *Chryseobacterium* species can also play a protective role, as evidenced by the presence of four *Chryseobacterium balustinum* strains on potatoes where they play an antagonistic role against plant-pathogenic fungi and a plant-parasitic nematode [16].

In this study, a putative novel species of the genus *Chryseobacterium* was isolated from chicken feather waste. A polyphasic taxonomy approach was followed to characterize strain  $1_F178^{T}$ .

## **ISOLATION AND ECOLOGY**

Strain 1\_F178<sup>T</sup> was isolated from chicken feather waste. The samples were obtained from a chicken abattoir in Bloemfontein, Free State, South Africa (approx. 29.108405° S 26.183772° E). Ten grams of feather waste were added to 90 ml buffered peptone water (BPW; Merck 63725) and mixed vigorously. Serial dilutions were made in BPW and pour-plated in duplicate using nutrient agar (Oxoid CM3). The plates were incubated aerobically for 48 h at 25 °C. Yellow-pigmented colonies were enumerated and streaked on nutrient agar to obtain a pure culture of strain 1\_F178<sup>T</sup>. The isolates were maintained as freeze-dried cultures on filter paper discs and stored in sealed Petri dishes at -20 °C. For short-term maintenance, they were cultured on nutrient agar slants, stored at 4 °C and re-streaked every 4–6 weeks.

# **16S rRNA GENE PHYLOGENY**

The DNA of strain 1\_F178<sup>T</sup> was extracted with the NucleoSpin Microbial DNA kit (Macherey-Nagel), according to the manufacturer's instructions. The extracted genomic DNA quantity and quality was assessed using the Nanodrop ND-1000 pectrophotometer (version 3.3.0, Thermo Scientific).

The DNA was subjected to PCR amplification of the 16S rRNA gene according to the manufacturer's instructions (Applied Biosystems). The forward primer used was 27F (5'-AGAGTTTGATCCTGGCTCAG-3', Integrated DNA Technologies) and the reverse primer was 1492R (5'-GGTTACCTTGTTACGACTT-3', Integrated DNA Technologies). Thermal cycling was conducted using a 2720 Thermocycler (Applied Biosystems).

The PCR products were visualized on a 1% w/v agarose gel (Seakem LE Agarose, Lonza). PCR amplicons were viewed using a Gel Doc EZ Imager (Bio-Rad) and photographed using ImageLab software (version 5.0, Bio-Rad).

Amplicon lengths of approximately 1500 bp corresponding to the expected amplicon bands were excised and purified with the Wizard SV Gel and PCR Clean-Up system (Promega) as per manufacturer's instructions. Sequencing was done with the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems). The forward primer used was 27F (5'-AGAGTTTGATCCTGGCTCAG-3', Integrated DNA Technologies) and the reverse primer was 1492R (5'-GGTTACCTTGTTACGACTT-3', Integrated DNA Technologies).

Sequence data was analysed and aligned using Geneious Pro R9 software (www.geneious.com) [17] and compared with sequences in the EzBioCloud (www.ezbiocloud.net) database [18] and in GenBank (www.ncbi.nlm.nih.gov) to identify closely related validly published species. The 16S rRNA gene sequence analysis clearly showed that strain 1\_F178<sup>T</sup> was associated with members of the genus *Chryseobacterium*. *Chryseobacterium jejuense* (99.10%) and *Chryseobacterium nakagawai* (98.75%) showed the highest 16S rRNA sequence similarity values, which were above the threshold value (98.7%) for species delineation, hence further genomic analysis was conducted to determine whether strain 1\_F178<sup>T</sup> was a novel species [19, 20].

Phylogenetic and molecular evolutionary analyses were conducted with MEGA software version 7 [21] using the neighbour-joining method [22] with Kimura's two-parameter distance model to determine the relationship of the unidentified isolate to the most similar validly named Chryseobacte*rium* type strains (www.bacterio.net/chryseobacterium.html) (Fig. 1). Confidence values were estimated from bootstrap analysis of 1000 replicates [23]. Phylogenetic trees were also reconstructed with the maximum-likelihood method (Fig. S1, available in the online version of this article) [24]. The topological structure of both phylogenetic trees clearly indicated that strain 1 F178<sup>T</sup> clustered with C. jejuense and C. nakagawai, although with poor bootstrap support. These strains, together with Chryseobacterium gleum NCTC 11432<sup>T</sup> (type species of the genus) were chosen as the reference organisms for phenotypic and genomic comparisons. C. jejuense DSM 19299<sup>T</sup> was obtained from the Deutsche Sammlung fur Microorganism, C. nakagawai CCUG 60563<sup>T</sup> was obtained from the Culture Collection of the University of Göteburg and *C. gleum* NCTC 11432<sup>T</sup> was obtained from the National Collection of Type Cultures.

## **GENOME FEATURES**

The genome of strain 1\_F178<sup>T</sup> was sequenced and assembled as described by Oosthuizen et al. [11]. Assembly of reads resulted in 88 contigs with a total sequence length of 6.18 Mbp and a G+C content of 36.6 mol%. Assessment of the assembly quality with CheckM [25] as implemented in KBase [26] revealed a completeness score of 100% and 'contamination' score of 1.5%. The whole-genome shotgun project was deposited in DDBJ/ENA/GenBank under the accession QNVT00000000. The version described in this paper is QNVT01000000. The assembled 1\_F178<sup>T</sup> genome was identified by the Genome Taxonomy Database (GTDB) [27, 28] as a representative of a new species. The bacterial phylogenomic tree reconstructed from alignment of 120 concatenated protein sequences from more than 45000 species level genomes was downloaded from the GTDB website (https://gtdb.ecogenomic.org/) and opened with NCBI Genome WorkBench version 3.5 [29]. The strain 1 F178<sup>T</sup> assembly accession (GCF 003385515)



**Fig. 1.** Neighbour-joining tree showing phylogenetic relationships between strain  $1_F178^{T}$  and representatives of some related taxa. The tree was computed with MEGA 7 and rooted by using *Elizabethkingia meningoseptica* ATCC  $13253^{T}$  as the outgroup. The GenBank accession numbers for the 16S rRNA genes are shown in parentheses. Numbers at nodes represent bootstrap values (based on 1000 resamplings). Only values  $\geq$ 70% are shown. Bar, 0.005 nucleotide substitutions per position.

was used to search the tree to identify the appropriate branch (Fig. S2). As with the 16S rRNA gene tree, *C. nakagawai* was confirmed as the closest relative, with *C. jejuense* present on the adjacent clade.

The genome was uploaded to the RAST server [30] for annotation and analysed using the SEED Viewer [31]. To identify closely related strains based on genes other than the 16S rRNA, the *rpoB* gene was retrieved from the genome and BLAST was used to identify similar sequences in the RefSeq database, which were downloaded, aligned by ClustalW and used to reconstruct a neighbour-joining tree with MEGA 7. The search identified a very similar sequence in a genome (RKHU01) labelled as *Chryseobacterium nakagawai* BIGb0215 derived from a strain isolated from the nematode *Caenorhabditis*  *elegans*. These genome sequences also clustered together in the genome BLAST distance phylogeny tree reconstructed by the Type Strain Genome Server [32].

Overall genomic relatedness indices (OGRIs) [30] of strain 1\_ F178<sup>T</sup> were calculated relative to the genome of closely related *Chryseobacterium* strains including the type species (Table S1). Average nucleotide identity (ANI) values were calculated using the Kostas Lab ANI calculator (http://enveomics.ce. gatech.edu/ani/). Digital DNA–DNA hybridization (dDDH) values were calculated using the Genome-t0-Genome Distance calculator [32]. Average amino acid identity (AAI) values were determined with a custom calculator (http:// lycofs01.lycoming.edu/~newman/AAI.html). **Table 1.** Differential characteristics of strain  $1_F178^{T}$  and the type strains of closely related species of the genus *Chryseobacterium* 

Strains: 1, 1 F178<sup>T</sup>; 2, C. jejuense DSM 19299<sup>T</sup>; 3, C. nakagawai CCUG 60563<sup>T</sup>: 4. C. aleum NCTC 11432<sup>T</sup>. All data were obtained under the same cultivation conditions. -, Negative; +, positive; w, weak reaction. All the strains were positive for: oxidase, catalase, rod-shaped cell morphology, fruity odour and flexirubin-type pigment production; growth on MacConkey, nutrient, tryptic soy, brilliant green, brain heart infusion and  $\beta$ -hydroxybutyrate agars; growth at 25, 32 °C; growth at pH 4-6: hydrolysis of gelatine, aesculin, tyrosine and Tween 80: production of DNase, urease, caseinase, tryptophanase, acid and alkaline phosphatase. All strains were negative for the production of lecithinase,  $\beta$ -galactosidase (ONPG); arginine-, lysine- and ornithinedecarboxylase, phenylalanine deaminase; acid production from 10% lactose and ethanol; acid production from 10% glucose, fructose, glucose, mannitol, glycerol, lactose, maltose, malonate, mannitol, trehalose and xylose; ammonia production from arginine; 3-ketolactose production; methyl red and Vogues-Proskauer reactions; growth on 0.4% selenite; gluconate oxidation and H<sub>2</sub>S production; fluorescence on King's medium; growing anaerobically in a Gas Pak, and aerobically at 42 °C

Characteristic	1	2	3	4
Growth at:				
4 °C	+	-	-	-
20 °C	+	-	_	-
35 °C	W	+	+	+
37 °C	-	w	+	+
Acid production from:				
Glucose	-	-	+	-
Mannitol	w	-	-	-
Glycerol	w	-	-	-
Maltose	w	-	w	-
Trehalose	-	+	+	+
Simmon's citrate	+	-	+	+
Reduction of:				
Nitrate	_	+	+	-
Nitrite	-	-	-	+
Hydrolysis of:				
Tween 20	-	+	-	-
Tyrosine	+	+	-	+
Production of:				
Amylase	-	+	-	-
Growth on:				
Mannitol salt agar	-	w	+	+
Cetrimide	-	+	+	+

Organisms that are members of the same species have dDDH values of 70 % or greater [33], ANI values greater than 95 % [34] and AAI values above 95 % [35]. Comparison of the genome sequences of strain  $1\_F178^T$  and '*Chryseobacterium nakagawai*' BIGb0215 (RKHU01) with each other revealed values (Table S1) above the species thresholds, indicating that they are members of the same species. Comparison to the genomes of validly published species revealed values below the species thresholds, indicating that these two strains represent a single novel species. Most notably, despite the assignment of strain BIGb0215 to *C. nakagawai* in GenBank, the ANI value of this strain relative to the type strain was only 87.1%, clearly indicating that these are different species.

The protein-coding gene content of strain 1\_F178<sup>T</sup> was compared to those of the related strains. Coding sequences identified as bidirectional best hits by the SEED Viewer sequence-based comparison tool [30] were used to generate a Venn diagram (Fig. S3) using a calculator available at http://lycofs01lycomingedu/~newman/CurrentResearchhtml. Strain 1 F178<sup>T</sup> had 743 unique genes, of which 219 encoded proteins with assigned functions. These included Tra genes associated with a two separate conjugative transposons, several phage genes (e.g. WP\_115971329, WP\_115971998) and many genes annotated as 'mobile element proteins' (e.g. WP\_115971419). Given that strain 1\_F178<sup>T</sup> was isolated from feather waste, proteases would be expected to play an important role in this niche. Unique genes present were predicted to encode enzymes such as cysteine proteases (e.g. WP\_115971399), serine proteases (e.g. WP\_115973573, WP\_115973076) and zinc metalloproteases (WP\_115973574, WP\_115973075). Interestingly, the only other Chryseobacterium genome currently in RefSeq encoding orthologs of WP\_115973076 and WP\_115973075, is derived from *Chryseobacterium arthrosphaerae* CC-VM-7<sup>T</sup>. One unique gene found in strain 1\_F178<sup>T</sup> that is not seen in any other Weeksellaceae genomes in RefSeq, encodes an aquaporin (WP\_115970958). Orthologs of this gene are seen in other Bacteroidota, and the gene is close to genes encoding an integrase (WP\_115970810) and a tetracycline resistance element mobilization regulatory protein (WP\_115970813), suggesting that it was acquired by horizontal gene transfer.

There were 496 genes shared by strains 1\_F178<sup>T</sup> and BIGb0215 but absent in the other three strains and there were 3059 genes shared by all five strains.

## PHYSIOLOGY AND CHEMOTAXONOMY

For phenotypic comparison, reference strains *C. jejuense* and *C. nakagawai*, together with the type species, *C. gleum* were used. Gram-staining and the production of oxidase and catalase were determined [36]. The cultures produced a fruity odour, similar to that produced by closely related species. Motility was determined by phase-contrast examination of wet mounts from nutrient broth (NB; CM67; Oxoid). Gliding motility was determined according to the protocol of Jooste [37]. The presence of flexirubin-type pigments was assessed by flooding the colonies with 20% (w/v) KOH solution [23].

Table 2. Cellular fatty acid profiles (%) of strain  $1\_F178^{\mathsf{T}}$  and reference strains

Strains: 1, 1\_F178<sup>T</sup>; 2, Chryseobacterium jejuense DSM 19299<sup>T</sup>; 3, Chryseobacterium nakagawai CCUG 60563<sup>T</sup>; 4, Chryseobacterium gleum NCTC 11432<sup>T</sup>. All results are from this study. Values correspond to the percentage of total fatty acids. Summed features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total (summed feature 3 listed as iso-C<sub>15:0</sub> 2-OH/C<sub>16:</sub>  $\omega$ 17c). TR, Trace (<1.0%). ECL, Equivalent chain length. ND, not determined. Major fatty acids (>10%) are indicated in bold.

Fatty acid	1	2	3	4
Saturated:				
C <sub>16:0</sub>	2.2	3.7	3.6	1.3
Unsaturated:				
iso-C <sub>17:1</sub> ω9c	24.6	15.6	30.3	23.6
Branched:				
iso-C <sub>15:0</sub>	37.9	34.1	38.9	35.7
iso-C <sub>15:0</sub> 3-OH	2.2	3.0	2.9	2.8
iso-C <sub>16:0</sub> 3-OH	TR	1.2	1.6	ND
iso-C <sub>17:0</sub> 3-OH	15.1	12.3	19.9	15.1
Summed feature 3	9.31	10.7	10.4	ND
Unknown ECL 16.587	1.2	TR	1.9	1.7
Unknown ECL 13.591	4.5	1.39	4.5	2.8

Cell morphology was determined using scanning (JSM-7800F Extreme-resolution Analytical Field Emission SEM) and transmission (Philips CM100 Transmission Electron Microscope, FEI) microscopy (Fig. S4). These were performed by the Centre of Microscopy at the University of the Free State [11].

Phenotypic and biochemical tests were carried out as reported by Oosthuizen *et al.* [11]. Strains were cultivated in NB at 25 °C for 48 h. The cells were centrifuged at 3000 *g* for 10 min (Eppendorf). The supernatant was discarded and the cell pellets were washed twice with phosphate buffer (0.1 M, pH 7). The cell pellets were re-suspended in 10 ml fresh phosphate buffer and standardized in comparison with a McFarland number 2 density ( $6 \times 10^8$  c.f.u. ml<sup>-1</sup>) standard (Difco 0691326). A multi-inoculation device was used to perform the inoculations.

The following range of phenotypic tests were done according to Cowan [38] and MacFaddin [36] unless otherwise stated: oxidative or fermentative metabolism of glucose; methyl red and Voges–Proskauer reactions; gluconate oxidation; potassium cyanide tolerance; malonate utilization; growth in 0–5% (w/v) sodium chloride; growth at 4, 20, 32, 35, 37 and 42 °C; growth on cetrimide agar (Merck 5284), MacConkey agar (Oxoid CM0007) and Simmon's citrate agar (Oxoid CM155); growth in 0–6% (w/v) sodium chloride; reduction of 0.4% selenite [39]; nitrate and nitrite reduction; production of acid from 10% (w/v) glucose and lactose; alkaline reaction on Christensen's citrate [40]; production of ammonia from arginine; lysine decarboxylase, ornithine decarboxylase, deoxyribonuclease (Oxoid CM321+ 0.01% toluidine blue),  $\beta$ -galactosidase (ONPG), hydrogen sulphide (TSI method), indole (Kovac's reagent), 3-ketolactose, phenylalanine deaminase, urease on Christensen's urea agar [39, 41]; hydrolysis of aesculin [42], casein, gelatin (plate method), starch [43], Tween 20, Tween 80 [38] and tyrosine [38]; and acid production in D-mannitol, L-arabinose, trehalose, ethanol and D-xylose. The sugars were encorporated at a final concentration of 1% (w/v).

Furthermore, physiological and biochemical features and enzymatic activities were assessed using API ZYM and API 20NE test strips (bioMérieux) and the Biolog Omnilog GEN III identification system in accordance with the manufacturer's instructions.

Strain  $1_{F178^{T}}$  could be distinguished from *C. jejuense* and *C. nakagawai* by its ability to grow at 4 and 20 °C (weakly) on nutrient agar, produce acid from glycerol and mannitol, inability to grow at 37 °C and not producing acid from trehalose, and ability to grow on mannitol salt agar but not on cetrimide agar (Table 1).

Analysis of fatty acids was done according to the method of Sasser [44]. For fatty acid methyl ester (FAME) analysis, strains were cultivated on trypticase soy broth agar at 25 °C. To ensure a standardized physiological age of the cells, a section of choice from a quadrant streak on the agar plate was taken. The cells were saponified, methylated and extracted according to the standard protocol of the Sherlock Microbial Identification System (MIS, version 6.1; MIDI). The FAMEs were analysed on an Agilent 6850 gas chromatograph using the method RTSBA6 of the MIS. The major fatty acids of strain 1\_F178<sup>T</sup> were iso-C<sub>15:0</sub>, iso-C<sub>17:1</sub>  $\omega$ 9*c* and iso-C<sub>17:0</sub> 3-OH, which were also predominant in the related species. The differences in cellular fatty acid content between strain 1\_F178<sup>T</sup> and related species of the genus *Chryseobacterium* are shown in Table 2.

Polar lipids were extracted from wet cells and analysed according to the methods of Nguyen et al. [45]. Extraction was done with chloroform-methanol-saline (6:10:3, v/v/v) and the lipids were separated using aluminium 20×20 cm silica gel 60 F254 plates (Merck Millipore), by two-dimensional chromatography using chloroform-methanol-water (65:25:4, v/v/v) for the first chromatography dimension, and chloroform-glacial acetic acid-methanol-water (40:7.5:6:2, v/v/v/v) in the second dimension. The plates were sprayed with the appropriate reagents to detect different lipids. Total lipid profiles were detected with phosphomolybdic acid, aminolipids and phospholipid groups were detected with ninhydrin reagent and glycolipids with a-naphtholsulphuric acid-methanol reagent. The major polar lipid of strain 1\_F178<sup>T</sup> was PE, along with several unidentified lipids (L1-5), glycolipids (G1-2) and other unidentified amino lipids (AL1-3) (Fig. S5).

Respiratory quinones were extracted from wet cells and analysed by the TLC method described by Komagata and Suzuki [46]. The only quinone was MK-6, which is the major menaquinone in the *Chryseobacterium* species [5].

Based on the results obtained from the taxonomic and morphological analyses, strain  $1\_F178^T$  shares characteristics (iso  $C_{15:0}$  as the major fatty acid, MK-6 as the major quinone and PE as major polar lipid) with other described species in the genus *Chryseobacterium*. Moreover, all the OGRI values of strain  $1\_F178^T$  were less than the cut-off values for species delineation, hence confirming the affiliation of strain  $1\_F178^T$  as representing a novel species in the genus *Chryseobacterium*, for which the name *Chryseobacterium pennae* sp. nov. is proposed.

## DESCRIPTION OF CHRYSEOBACTERIUM PENNAE SP. NOV.

*Chryseobacterium pennae* (pen'nae. L. gen. n. *pennae* of a feather).

Gram-stain-negative, rod-shaped (approximately 1-3 µm long devoid of flagella), non-gliding, non-motile and nonspore-forming cells with rounded ends. The colonies are yellowish and circular when cultivated at 25 °C for 48 h on nutrient agar, produce a fruity odour, are translucent, have a flat and smooth entire surface, and become mucoid on prolonged incubation. Cells are non-fluorescent, produce flexirubin-type pigment, are strictly aerobic and grow at 4 °C but not at 37 °C and 42 °C. Optimal growth is at 25-30 °C. Grows on MacConkey, tryptic soy agar, Simmons citrate, Christensen's citrate, nutrient agar and  $\beta$ -hydroxybutyrate, but not on cetrimide and mannitol salt agars. Grows in 0-3% NaCl (optimal at 0% NaCl), but not in 4-6% NaCl; growth at pH 5-8 (optimal at pH 6) but not at pH less than 5; produces acid weakly from mannitol, glycerol and maltose, but not from glucose, fructose, arabinose, lactose, trehalose, sucrose or xylose. Strain 1\_F178<sup>T</sup> produces caseinase, DNase, gelatinase, urease and tryptophanase but not amylase, lecithinase,  $\beta$ -galactosidase (ONPG) and phenylalanine deaminase. Negative for the production of 3-ketolactose and H<sub>2</sub>S, and unable to utilize malonate.

In the Biolog GEN III system, strain  $1\_F178^{T}$  is susceptible to troleandomycin, rifamycin SV, lincomycin, vancomycin (weakly), nalidixic acid (weakly) and aztreonam. The strain has a high reducing power to tetrazolium violet and tetrazolium blue. Strain  $1\_F178^{T}$  gave a positive response in wells containing the following amino acids: D-serine, glycyl-Lproline, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L-pyroglutamic acid and L-serine. On the API 20 NE test strip, positive for indole, urease and cytochrome oxidase production, aesculin and gelatin hydrolysis, assimilation of glucose, arabinose, mannose, mannitol, *N*-acetylglucosamine, maltose and potassium gluconate. On the API ZYM test strip, positive for alkaline phosphatase, esterase lipase, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase,  $\alpha$ -glucosidase and *N*-acetyl- $\beta$ -glucosaminidase. The predominant cellular fatty acids are iso- $C_{15:0}$ , iso- $C_{17:1}$   $\omega 9c$  and iso- $C_{17:0}$  3-OH. PE is the most abundant polar lipid with MK-6 as the only respiratory quinone.

The type strain is  $1_F178^{T}$  (=LMG  $30779^{T}$ =KCTC  $62759^{T}$ ), isolated from chicken feather waste collected from an abattoir in Bloemfontein, Free State, South Africa. The genome comparisons in this study indicated that the *C. 'nakagawai'* strain BIGb0215 (RKHU01) is a member of the same species, *C. pennae*.

General features of the genome assembly are as follows: genome size, approximately 6187872 bp; number of contigs, 88; coding sequences, 5682; N50 value, 162953; coverage, 25.0×. The DNA G+C content is 35.6 mol%.

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#### **Conflicts of interest**

The authors declare that there are no conflicts of interest.

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