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TAXONOMIC DESCRIPTION

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Chryseobacterium pennipullorum sp. nov., isolated from poultry feather waste

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Abstract

Strain 7_F195^T was previously isolated from chicken feather waste collected from an abattoir in Bloemfontein, South Africa. A polyphasic approach was followed to determine if strain 7_F195^T belongs to the genus *Chryseobacterium* and if the organism can be classified as a new species. The nearest neighbours, based on 16S rRNA gene sequence similarity values (indicated in parentheses), were *Chryseobacterium flavum* KCTC 12877^T (98.42 %), *Chryseobacterium indologenes*LMG 8337^T (98.24 %) and *Chryseobacterium gleum* ATCC 35910^T (97.71 %). Genome sequencing revealed a genome size of 4 796 535 bp and a DNA G+C content of 38.6 mol%. The ANI values of strain 7_F195^T compared to *C. flavum, C. indologenes* and *C. gleum* were 81.45, 81.86 and 82.38 %, respectively. The digital DNA–DNA hybridization values for strain 7_F195^T with *C. flavum*, *C. indologenes* and *C. gleum* were 23.7, 23.7 and 24.9 %, respectively. Notable phenotypic differences include the presence of urease activity in *C. indologenes* LMG 8337^T and *C. gleum* NCTC 11432^T, but not in strain 7_F195^T or *C. flavum* KCTC 12877^T. The predominant fatty acids of strain 7_F195^T were iso-$C_{15:0}$, iso-$C_{17:1}\omega9c$ and iso-$C_{17:0}$ 3-OH and the most abundant polar lipid was phosphatidylethanolamine. Menaquinone-6 was the only respiratory quinone. Based on the data generated from this polyphasic study, strain 7_F195^T represents a novel *Chryseobacterium* species for which the name *Chryseobacterium pennipullorum* sp. nov. is proposed. The type strain is 7_F195^T (=LMG 30781^T=KCTC 62760^T).

Chryseobacterium, a genus of the family *Flavobacteriaceae*, was proposed in 1994 and six species previously included in the genus *Flavobacterium* were reclassified as members of *Chryseobacterium* [1]. The six species were *Chryseobacterium scophthalmum*, *Chryseobacterium indologenes*, *Chryseobacterium gleum*, *Chryseobacterium balustinum*, *Chryseobacterium indoltheticum* and *Chryseobacterium meningosepticum*. *Chryseobacterium gleum* was identified as the type species of the genus [2]. *Chryseobacterium* species are known to be Gram-stain-negative straight rods that are yellow-pigmented, non-motile, non-spore-forming, oxidase-positive, catalase-positive and aerobic [3]. In 2013, the genus *Chryseobacterium* consisted of only 61 validly named species [4]. At the time of this study the genus consists of 112 validly published *Chryseobacterium* species [5].

Classification methods that were used in the past relied more on morphological and physiological characteristics, but the use of genetic information of an organism has become more common [6]. DNA–DNA hybridization (DDH) is still known as the 'gold standard' for delineating species of prokaryotes [7], but pairwise genome-sequence derived similarity has been proposed to replace this method [8]. Overall genome related indexes (OGRIs) are analogous to DDH values and can be used to determine if a strain belongs to a known species. Average nucleotide identity (ANI), amino acid identity (AAI) and digital DDH (dDDH) are examples of frequently used metrics [8].

Members of the genus *Chryseobacterium* have been isolated from environments such as terrestrial, aquatic, diseased animals, humans and food [3, 9] and some species may play a significant role in food spoilage [10–13]. Food sources

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Abbreviations: AAI, amino acid identity; ANI, average nucleotide identity; BPW, buffered peptone water; CDS, coding sequences; dDDH, digital DNA–DNA hybridization; DDH, DNA–DNA hybridization; FAME, fatty acid methyl ester; GGDC, Genome-to-Genome Distance Calculator; NA, nutrient agar; NB, nutrient broth; NJ, neighbour-joining; OGRI, overall genome related index; TSBA, trypticase soy broth agar.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 7_F195^T is MH051720. The DDBJ/ENA/Genbank accession for the whole genome shotgun sequence is QNVV00000000. The version described in this paper is version QNVV01000000.

One supplementary table and four supplementary figures are available with the online version of this article.

where *Chryseobacterium* species were found include poultry, red meat, milk, milk products and fish [3, 4, 14–16].

In this study, strain 7_F195^T isolated from chicken feather waste collected from an abattoir in Bloemfontein, Free State, South Africa is described. The strain belongs to the genus *Chryseobacterium* and the species name, *Chryseobacterium pennipullorum* sp. nov. is proposed.

ISOLATION AND ECOLOGY

Strain 7_F195^T was isolated in a previous study in 2010 [13] from chicken feather waste collected from a small-scale chicken abattoir in Bloemfontein, Free State, South Africa with coordinates approximately 29.108405 S 26.183772 E. Ten grams of feather waste was 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 (NA; Oxoid). Incubation was aerobic for 48 h at 25 °C. Yellow-pigmented colonies were streaked on NA until pure cultures were obtained. One of the yellow colonies was designated strain 7_F195^T. The strain was freeze-dried until further investigation. For short-term maintenance the culture was freeze-dried on AA Whatman filter paper discs and stored at −20 °C. The culture was also stored on NA slants at 4 °C and re-streaked every 4–6 weeks. Long-term maintenance was by freeze-drying. Routine cultivation was in nutrient broth (NB; Oxoid) and on NA at 25 °C for 24–48 h.

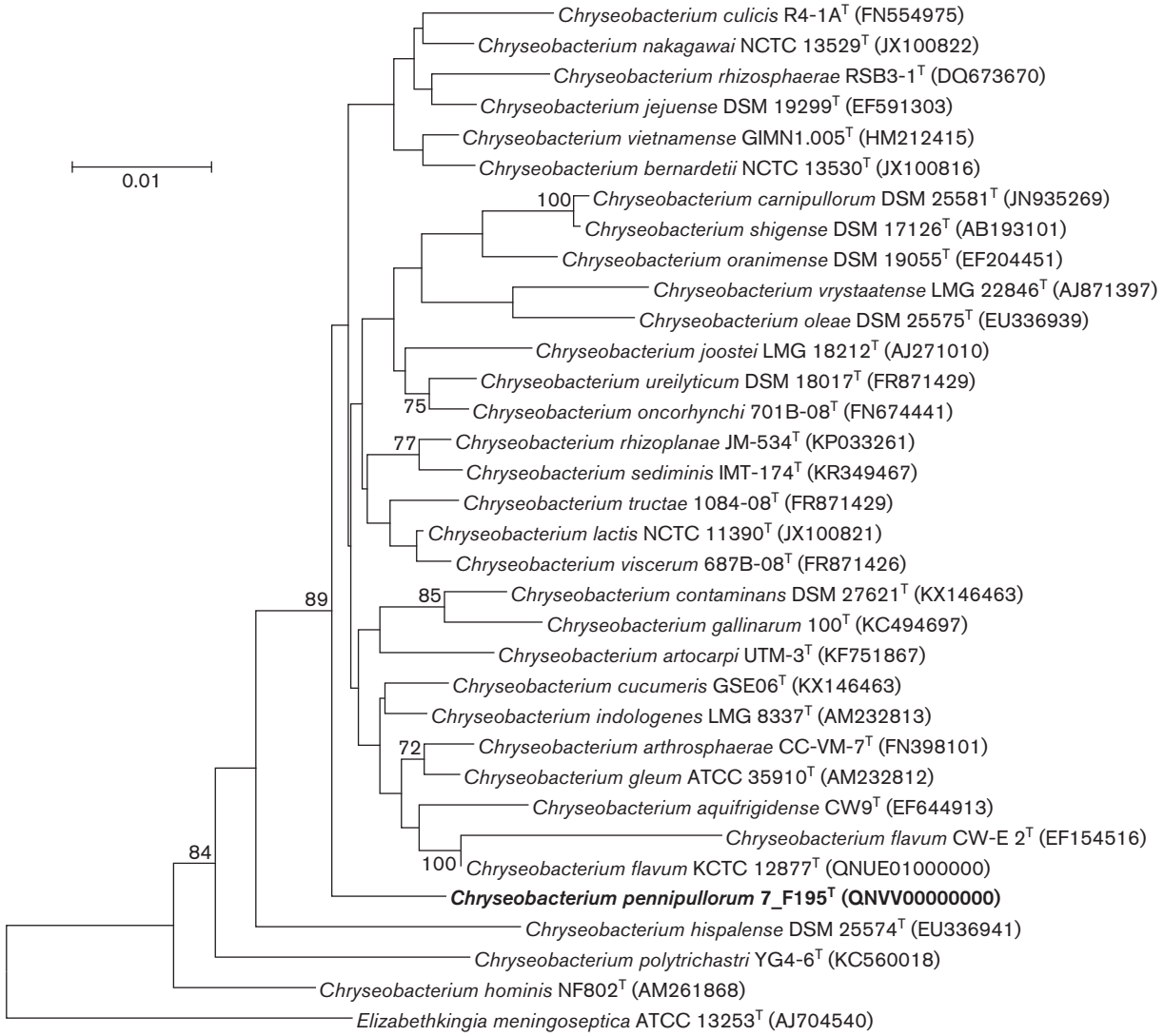

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences (accession numbers given in parentheses) showing the phylogenetic relationships between strain 7_F195^T and related taxa. Bootstrap values above 70 % are shown at the nodes as percentages of 1000 replicates. *Elizabethkingia meningoseptica* ATCC 13253^T was used as an outgroup. Bar, 0.01 changes per nucleotide position.

16S RNA GENE PHYLOGENY

The genomic DNA from strain 7_F195^T was extracted using the NucleoSpin kit (Machery Nagel) according to the manufacturer's instructions. PCR-amplification of the 16S rRNA gene region was performed using an Applied Biosystems 2720 Thermal Cycler and forward primer 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse primer 1492R (5′-GGTTACCTTGTTACGACTT-3′) [17]. The PCR product was visualized on a 1 % agarose gel. The amplicons were purified using the Wizard SV Gel and PCR Clean-Up System (Promega) according to the manufacturer's instructions. Sanger sequencing was performed using the BigDye Terminator Cycle Sequencing Kit version 3.1 on the Applied Biosystems 3130xl sequencer. The forward primer was 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and the reverse primer was 1492R (5′-GGTTACCTTGTTACGACTT-3′). Sequence data was analysed and aligned using Geneious Pro R9 software (www.geneious.com) and compared with sequences on the EzBioCloud (www.ezbiocloud.net/) database [18] and on GenBank (www.ncbi.nlm.nih.gov/) to identify closely related validly published species. The 16S rRNA gene sequencing results confirmed that strain 7_F195^T (1422 nt) showed the highest similarity to the genus *Chryseobacterium*.

Phylogenetic trees were reconstructed as described by Bernardet and co-workers [9] by using MEGA software version 7 [19]. The neighbour-joining (NJ) method [20] with the parameter distance measure Kimura two (KP2) was used. The reliability of the branching was validated by using the bootstrap analysis with 1000 replicates [9]. Although bootstrap values were very low, the NJ tree (Fig. 1) showed that strain 7_F195^T formed a separate lineage from the other *Chryseobacterium* species in the tree. These results suggested that strain 7_F195^T belonged to the genus *Chryseobacterium* and the separate lineage indicated that this strain could be regarded as a new species in the genus. This phylogeny was also obtained using the maximum-likelihood method (Fig. S1, available in the online version of this article) [21]. The nearest neighbours of strain 7_F195^T based on 16S rRNA gene sequence similarity values (indicated in parentheses) were *Chryseobacterium flavum* KCTC 12877^T (98.42 %), *Chryseobacterium indologenes* LMG 8337^T (98.24 %), *Chryseobacterium tructae* 1084-08^T (98.22 %), *Chryseobacterium lactis* NCTC 11390^T (98.19 %), *Chryseobacterium vietnamense* GIMN1.005^T (97.99 %), *Chryseobacterium viscerum* 687B-08^T (97.90 %), *Chryseobacterium gleum* ATCC 35910^T (97.71 %), *Chryseobacterium cucumeris* GSE06^T (97.70 %), *Chryseobacterium rhizoplanae* JM-534^T (97.69 %) and *Chryseobacterium arthrosphaerae* CC-VM-7^T (97.65 %). Strains that have 16S rRNA gene similarity values of 98.7 % or less are regarded as different species [8].

Based on this data and the separate lineage in the phylogenetic trees, *C. flavum* KCTC 12877^T, *C. indologenes* LMG 8337^T (nearest neighbours) and *C. gleum* NCTC 11432^T (type species of *Chryseobacterium*) were chosen as related type strains used in this study. The related type strains were obtained from the Korean Collection of Type Cultures (KCTC), the Laboratorium voor Microbiologie (LMG) and the National Collection of Type Cultures (NCTC).

GENOME FEATURES

Whole genome sequencing of strain 7_F195^T was performed at the Next-Generation Sequencing Unit, Department of Medical Virology, Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa. The genomic DNA was prepared for sequencing according to the manufacturer's instructions using the Nextera XT DNA Library Prep Reference Guide. The genome was sequenced using an Illumina MiSeq sequencer (2×300 b) and the assembly performed on PATRIC (www.patricbrc.org/) using SPAdes 3.10.0.

The genome of strain 7_F195^T was uploaded to the Rapid Annotation with Subsystems Technology (RAST) website (http://rast.nmpdr.org) [22] for annotation. The Whole Genome Shotgun project has been deposited at DDBJ/ENA/Genbank (www.ncbi.nlm.nih.gov/) as accession QNVV00000000. The version described in this paper is version QNVV01000000. The information from the genome was as follows: genome size, 4 796 535 bp; number of contigs, 61; coding sequences (CDS), 4348; N50 value, 208 445; coverage, 33.0x and G+C value, 38.6 mol%. *Chryseobacterium* species generally have G+C values ranging from 29 to 39 mol% [3].

OGRIs [23] were compared to *Chryseobacterium* genomes available in NCBI and the most similar genomes are shown in Table S1. Average nucleotide identity (ANI) values were calculated using the Kostas Lab ANI calculator (http://enveomics.ce.gatech.edu/ani/). dDDH values were calculated using the Genome-Genome Distance calculator (GGDC) [24]. Average Amino Acid Identity (AAI) values were determined with a custom calculator (http://lycofs01.lycoming.edu/~newman/AAI.html).

Strains that have DNA–DNA similarity values of 70 % or more are classified as part of the same species [7, 9]. The digital DNA–DNA reassociation values from whole genome sequencing for strain 7_F195^T with *C. flavum*, *C. indologenes* and *C. gleum* were 23.7, 23.7 and 24.9 %, respectively.

Strains that have ANI values of 95~96 % or higher are classified as part of the same species [25]. The ANI values of strain 7_F195^T compared to *C. flavum, C. indologenes* and *C. gleum* were 81.45, 81.86 and 82.38 %, respectively.

Strains that have AAI values of 95 % or higher are classified as part of the same species [26]. The AAI values of strain 7_F195^T compared to *C. flavum, C. indologenes* and *C. gleum* were 84.94, 85.39 and 85.14 %, respectively.

Values for dDDH, ANI and AAI support the conclusion that strain 7_F195^T does not belong to *C. flavum*, *C. indologenes* or *C. gleum*.

Table 1. Differential characteristics of strain 7_F195^T and the type strains of closely related species of the genus *Chryseobacterium*

Strains: 1, 7_F195^T; 2, *C. flavum* KCTC 12877^T; 3, *C. indologenes* LMG 8337^T; 4, *C. gleum* NCTC 11432^T. 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 agar; growth at 25, 32 °C; growth at pH 6; hydrolysis of gelatine, aesculin, starch, and Tween 80; production of DNase, lecithinase; acid production from D-glucose. All the strains were negative for: Gram-reaction and motility; anaerobic conditions at 25 °C; growth at 5 and 42 °C; growth at pH 5; growth in the presence of 4–5% NaCl; production of H_2S, phenylalanine deaminase; acid from adonitol, L-arabinose, cellobiose, dulcitol, ethanol, glycerol, D-xylose, lactose, maltose, D-mannitol, raffinose, rhamnose, salicin, sorbitol and sucrose.

Characteristics	1	2	3	4
Growth on/at:				
Cetrimide agar	−	−	+	−
37 °C	−	+	+	+
%NaCl w/v (optimum)	0–2.5 (0)	0–2.5 (0)	0–3 (0)	0–2.5 (0)
Enzyme activities:				
Urease production	−	−	+	+
Nitrate reduction	−	−	−	+
Tyrosine hydrolysis	−	+	−	+
Tween 20 hydrolysis	−	−	+	−
β-Galactosidase (ONPG) production	−	+	−	+
Acid production from:				
D-Fructose	−	−	−	+
Trehalose	−	−	−	+
Oxidation of (Biolog Gen III):				
D-Fructose-6-PO_4	−	−	+	+
Maltose	−	+	−	+
D-Mannitol	+	−	−	−
D-Salicin	−	+	−	+
Glycerol	−	+	−	+
Formic acid	+	−	w	−
G+C content (mol%)*	38.6	36.7	37.2	36.8

*The DNA G+C contents were calculated based on their genomes in this study.

The protein-coding gene content of strain 7_F195^T was compared to those of the related type strains. Coding sequences with bidirectional best hits were used to generate a Venn diagram (Fig. S2) by using a calculator available at http://lycofs01.lycoming.edu/~newman/CurrentResearch.html. Strain 7_F195^T has a total of 540 unique genes and there are 2786 genes shared by all the strains. Among the genes not found in the related type strain genomes were several that were present in other poultry associated bacteria. A gene encoding a unique acyl-coA synthetase (WP_115928641) was most similar to an ortholog in *Chryseobacterium vrystaatense*, isolated from a chicken portion, and *Chryseobacterium arachidis*. An ortholog of this gene is present on plasmids in *Chryseobacterium shandongense* and *Chryseobacterium balustinum*, but this seems unlikely to be the case in strain 7_F195^T because there are several ribosomal proteins encoded on the same contig. There is a large cluster of genes encoding proteins such as WP_115928909, likely to be involved in nonribosomal peptide synthesis, in which the product appears to be a siderophore. A similar cluster is present in *Chryseobacterium vrystaatense*, *Chryseobacterium nakagawai*, *Chryseobacterium aurantiacum* and *Chryseobacterium ureilyticum*. There is a large cluster of 26 mostly unique genes involved in the synthesis of lipopolysaccharide, exopolysaccharide and/or capsular polysaccharide. A specific unique gene of interest in this cluster encodes a polysaccharide pyruvyl transferase (WP_115926819) that is also found in *Flavobacterium marinum*, *Dyadobacter koreensis*, and the poultry pathogen *Gallibacterium anatis*.

A C-terminal processing S41 type peptidase (WP_115927967) and an adjacent AraC type transcriptional regulator (WP_115927968) are most similar to proteins encoded by *Chryseobacterium* sp. 1_F178 (isolated from chicken feather waste), *Chryseobacterium jejuense*, *Chryseobacterium nakagawai*, *Chryseobacterium hungaricum*, *Chryseobacterium zeae* and many *Elizabethkingia* species. The next open reading frame is predicted to encode a S58 type D-aminopeptidase (WP_115927969) that is also present in *Chryseobacterium piperi* and *C. lactis*. An anticodon nuclease (WP_115928350) linked to a DNA restriction-modification system is also predicted in *Chryseobacterium culicis*, *Chryseobacterium taklimakanense*, *Elizabethkingia*

Table 2. Cellular fatty acid profiles (%) of strain 7_F195^T and the type strains of closely related species of the genus *Chryseobacterium*

Strains: 1, 7_F195^T; 2, *C. flavum* KCTC 12877^T; 3, *C. indologenes* LMG 8337^T; 4, *C. gleum* NCTC 11432^T. All data are from this study except *C. indologenes* [37]. Values are percentages of the total fatty acids. Fatty acids that are <1.0 % in all strains are not shown. ECL, equivalent chain length; TR, trace (<1.0 %). The major fatty acids (>10 %) are indicated in bold.

Fatty acid	1	2	3	4
Saturated:				
$C_{16:0}$	1.0	TR	1.1	1.1
$C_{16:0}$ 3-OH	TR	1.1	TR	TR
Unsaturated:				
iso-$C_{17:1}\omega 9c$	**25.3**	**22.8**	**25.5**	**22.2**
Branched:				
iso-$C_{15:0}$	**38.1**	**33.2**	**34.5**	**35.0**
iso-$C_{15:0}$ 3-OH	2.8	2.7	2.3	2.9
iso-$C_{17:0}$	1.0	1.2	1.0	1.6
iso-$C_{17:0}$ 3-OH	**13.1**	**16.0**	**12.7**	**15.8**
Unknown:				
ECL 13.565	4.6	4.8	5.0	4.2
ECL 16.582	1.4	1.5	7.0	1.5
Summed features:*				
3	8.2	**12.5**	**11.3**	**12.1**
4	TR	TR	1.0	TR

*Summed features are groups of two or three fatty acids that are treated together for the purposes of evaluation in the MIDI system and include both peaks with discrete ECLs as well as those where the ECLs are not reported separately. Summed feature 3 listed as iso-$C_{15:0}$ 2-OH/$C_{16:1}$7*c*; summed feature 4 listed as anteiso-$C_{17:1}$ B and/or iso-$C_{17:1}$.

anopheles and *Elizabethkingia meningoseptica*. Proteins WP_115929211 and WP_115929212 are predicted to be insecticidal toxin/sugar binding proteins encoded by strain 7_F195^T, with orthologs in *Chryseobacterium nakagawai* and *Chryseobacterium bernardetii*.

PHYSIOLOGY AND CHEMOTAXONOMY

Conventional phenotypic tests were performed on strain 7_F195^T and the related type strains as described by Bernardet and co-workers [9]. The cultures were cultivated on NA for 24 h at 25 °C. Gram-staining, cell morphology, oxidase and catalase activities were determined [27]. The odour of the cultures was noted.

Gliding motility was determined by the hanging-drop technique [9]. Flexirubin-type pigment production was assessed by flooding a mass of cells smeared on a glass slide with 20 % (w/v) potassium hydroxide [10]. If the flexirubin-type pigment was present, the cell material turned from yellow to red, purple or brown. An acidic solution (1 N HCl) was used to remove the excess KOH and revert the cells to their initial colour [28].

Scanning (JSM-7800F Extreme-resolution Analytical Field Emission SEM) and transmission (Philips CM100 Transmission Electron Microscope, FEI) microscopy, were performed by the Centre of Microscopy at the University of the Free State. The micrographs are given in the Fig. S3. The cells ranged from 2 to 2.3 µm long and 0.4 µm wide. The cells had a slimy appearance, but its exact composition and purpose are unknown.

Phenotypic and biochemical tests were carried out by using cell suspensions that were grown under standard conditions of 25 °C in NB for 24 h. The cell suspensions were centrifuged at 3000 ***g*** for 10 min at 4 °C using a Beckman Coulter, Avanti J-26 XPI centrifuge and the supernatant decanted. The cells were washed with 0.1 M phosphate buffer (pH 7) and suspended in 0.1 M phosphate buffer (pH 7) to a McFarland number 1 (Difco 0691326) density standard to standardize the cell suspension. A multi-inoculation device was used to perform the inoculations.

The following phenotypic tests were performed according to Cowan [29] and MacFaddin [27] unless otherwise indicated: growth on MacConkey agar (Oxoid) and cetrimide agar (Oxoid); growth in anaerobic conditions at 25 °C for 14 days using a 2.5 l anaerobic jar and Anaerogen 2.5 l Thermo Scientific sachets; growth at different temperatures (5, 25, 32, 37 and 42 °C); growth in NB supplemented with sodium chloride (0–5 % w/v, in increments of 1 %); growth at pH 4–7 (adjusted with citrate–phosphate buffer) and pH 7.5–8.5 (adjusted with Tris–hydrochloride buffer), at intervals of 0.5; H_2S production on Kligler iron agar (Oxoid); hydrolysis of gelatin (liquefaction), aesculin [30], starch [31], Tween 80 [31], Tween 20, tyrosine [32]; production of DNase, urease (Oxoid), β-galactosidase (ONPG), lecithinase, phenylalanine deaminase; nitrate and nitrite reduction; acid production from adonitol, L-arabinose, cellobiose,

dulcitol, ethanol, D-fructose, D-glucose, glycerol, D-xylose, lactose, maltose, D-mannitol, raffinose, rhamnose, salicin, sorbitol, sucrose and trehalose.

In addition to the above-mentioned tests, strain 7_F195^T and related type strains were profiled in triplicate using the API 20NE and API ZYM systems (bioMérieux) and the Biolog Omnilog Gen III identification system according to the manufacturer's instructions.

Phenotypic characteristics of strain 7_F195^T are given in Table 1 and the species description. Notable differences include the presence of urease activity in *C. indologenes* LMG 8337^T and *C. gleum* NCTC 11432^T, but not strain 7_F195^T or *C. flavum* KCTC 12877^T. This can be correlated with the presence of a gene cluster encoding a urea transporter, three urease subunits, and several accessory proteins in the former two strains but neither of the latter strains. In the Biolog system, strain 7_F195^T could be differentiated from the related type strains in being the only strain that showed oxidation of D-mannitol and formic acid.

Analysis of cellular fatty acid methyl esters (FAMEs), polar lipids and respiratory lipoquinones were carried out by the Identification Service and Dr Brian Tindall, DSMZ, Braunschweig, Germany. For FAME analysis, strains were cultivated on trypticase soy broth agar at 28 °C. To ensure a standardized physiological age of the cells, a sector 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; MIDI). The FAMEs were analysed by gas chromatography (model 6890 N, Agilent) using the standard protocol of the MIS. The MIS standard software (Microbial ID) version 6.1 was used to calculate the percentages and the names of the fatty acids. The peaks were automatically integrated. The cellular fatty acid profiles of strain 7_F195^T and its nearest neighbours are shown in Table 2. The predominant fatty acids were iso-$C_{15:0}$ (38.1 %), iso-$C_{17:1}\omega 9c$ (25.3 %) and iso-$C_{17:0}$ 3-OH (13.1 %). Strain 7_F195^T differed from the related type strains in having more than 35 % iso-$C_{15:0}$ and less than 10 % summed feature 3.

The two-stage method described by Tindall [33, 34] was used to extract the respiratory lipoquinones and the polar lipids. The quinones and polar lipids were extracted from 100 mg freeze-dried cell material. Respiratory lipoquinones were separated by thin-layer chromatography on silica gel (805 023, Macherey-Nagel), using hexane : tert-butylmethylether (9 : 1 v/v) as solvent. Quinones were analysed by using an LDC Analytical HPLC (Thermo Separation Products) fitted with a reverse phase column (Macherey-Nagel; 2 mm x 125 mm, 3 µm, RP18) using methanol:heptane 9 : 1 (v/v) as the eluant. Detection of respiratory lipoquinones was at 269 nm. Menaquinone-6 was the only respiratory quinone which is in accordance with members of the genus *Chryseobacterium* [1]. Polar lipids were separated by two-dimensional silica gel thin-layer chromatography (818 135, Macherey-Nagel). The first direction was developed in chloroform:methanol:water (65 : 25 : 4, v/v/v) and the second in chloroform:methanol:acetic acid:water (80 : 12 : 15 : 4, v/v/v/v). Molybdatophosphoric acid was used to detect the total lipid material and spray reagents, specific for defined functional groups, were used to detect specific functional groups [35]. The most abundant polar lipid was phosphatidylethanolamine (Fig. S4). Several unidentified lipids, unidentified aminolipids and a glycolipid were present, which is in accordance with members of the genus *Chryseobacterium* [36–38].

The results of the phylogenetic and chemotaxonomic analyses indicated that strain 7_F195^T is a member of the genus *Chryseobacterium*. The whole-genome sequencing data, especially the ANI, AAI and dDDH data, as well as the differential phenotypic characteristics, clearly indicated that strain 7_F195^T represents a novel species in the genus *Chryseobacterium* and the name *Chryseobacterium pennipullorum* sp. nov. is proposed.

DESCRIPTION OF *CHRYSEOBACTERIUM PENNIPULLORUM* SP. NOV.

Chryseobacterium pennipullorum (pen.ni.pul.lo'rum. L. fem. n. *penna* a feather; L. masc. n. *pullus* a chicken; N.L. gen. n. *pennipullorum* of chicken feathers).

Cells are Gram-stain-negative; non-motile; obligately aerobic and rod-shaped (2–2.3 µm long and 0.4 µm wide) without flagella. Cells produce a slime capsule. The colony shape is circular with a diameter of 1–3 mm after cultivation at 25 °C for 48 h on NA. The colonies produce a flexirubin-type pigment; have a fruity odour; they are translucent, flat with a smooth and entire surface and becomes mucoid on prolonged incubation. Positive for growth on nutrient and MacConkey agars but do not grow on cetrimide and β-hydroxybutyrate agars; growth at 25 abd 32 °C (optimal, 25 °C) and weak growth at 15 °C; no growth at 5, 37 and 42 °C; growth at pH 5.5–8 (optimal, pH 6–7) but not at pH less than 5 or more than 8; growth with 0–2.5% NaCl (optimal, 0 %) but not with 3–5% NaCl. Oxidase- and catalase-positive; positive for hydrolysis of gelatin, aesculin, starch and Tween 80; production of DNase and lecithinase; acid production from D-glucose. Negative for: hydrolysis of Tween 20 and tyrosine; production of H_2S, urease, β-galactosidase and phenylalanine deaminase; nitrate and nitrite reduction; acid production from adonitol, L-arabinose, cellobiose, dulcitol, ethanol, D-fructose, glycerol, D-xylose, lactose, maltose, D-mannitol, raffinose, rhamnose, salicin, sorbitol, sucrose and trehalose.

On the API 20 NE test strip positive for: indole production; hydrolysis of aesculin and gelatin; assimilation of glucose, mannose, mannitol and maltose. Negative for: the reduction of nitrate to nitrite and nitrate to nitrogen; fermentation of glucose; production of urease, β-galactosidase and arginine dihydrolase; assimilation of arabinose, *N*-acetyl-glucosamine, capric acid, adipic acid, malate and phenylacetic

acid. Weak positive reaction for assimilation of potassium gluconate and trisodium citrate.

On the API ZYM test strip positive for: the production of esterase lipase, alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase. Negative for: the production of esterase, lipase, cystine arylamidase, trypsin, β-glucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase. Weak positive reaction for the production of α-chymotrypsin, α-glucosidase and *N*-acetyl-β-glucosaminidase.

With the Biolog Omnilog Gen III system, strain 7_F195^T shows a positive reaction against the following inhibitory substances: 1 % NaCl, 1 % sodium lactate, aztreonam, D-serine, guanidine HCl, potassium tellurite, lincomycin, pH 6, tetrazolium blue, tetrazolium violet and troleandomycin. Strain 7_F195^T shows sensitivity towards the following inhibitory substances: 4 % NaCl, 8 % NaCl, fusidic acid, LiCl, minocycline, sodium bromate, nalidixic acid, niaproof 4, pH 5, rifamycin SV and vancomycin.

Strain 7_F195^T gives a positive response with the following carbon sources in the Biolog Gen III system: gelatin, glycyl-L-proline, Tween-40, acetic acid, trehalose, dextrin, D-fructose, D-mannitol, D-mannose and formic acid. It shows weak response with the following carbon sources: acetoacetic acid, α-D-glucose, citric acid, D-galactose, D-glucuronic acid, gentiobiose, glucuronamide, L-arginine and L-glutamic acid. The strain gives a negative response the following carbon sources: 3-methyl glucose, lactose, α-hydroxy-butyric acid, α-keto-butyric acid, α-keto-glutaric acid, β-hydroxy-D,L-butyric acid, methyl β-D-glucoside, bromo-succinic acid, D-arabitol, D-aspartic acid, cellobiose, D-fructose-6-PO_4, D-fucose, D-galacturonic acid, D-gluconic acid, D-glucose-6-PO_4, D-lactic acid methyl ester, D-malic acid, maltose, melibiose, raffinose, D-saccharic acid, D-salicin, D-serine, D-sorbitol, turanose, γ-amino-butyric acid, glycerol, inosine, L-alanine, L-aspartic acid, L-fucose, L-galacturonic acid lactone, L-histidine, L-lactic acid, L-malic acid, L-pyroglutamic acid, L-rhamnose, L-serine, methyl pyruvate, mucic acid, myo-inositol, *N*-acetyl neuraminic acid, *N*-acetyl-β-D-mannosamine, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, pectin, p-hydroxy-phenylacetic acid, propionic acid, quinic acid, stachyose and sucrose.

The predominant fatty acids are iso-$C_{15:0}$, iso-$C_{17:1}\omega 9c$ and iso-$C_{17:0}$ 3-OH. The most abundant polar lipid is phosphatidylethanolamine. Contains menaquinone-6 as the only respiratory quinone.

The type strain is 7_F195^T (=LMG 30781^T=KCTC 62760^T), isolated from chicken feather waste collected from an abattoir in Bloemfontein, Free State, South Africa.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 7_F195^T is MH051720. General features of the genome assembly are as follows: genome size, 4 796 535 bp; number of contigs, 61; coding sequences, 4348; N50 value, 208445 and coverage, 33.0×; G+C value, 38.6 mol%. The Whole Genome Shotgun project has been deposited at DDBJ/ENA/Genbank (www.ncbi.nlm.nih.gov/) with accession number QNVV00000000. The version described in this paper is QNVV01000000.

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

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