Chryseobacterium joostei sp. nov., isolated from the dairy environment

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Among a large collection of South African dairy isolates, a novel *Chryseobacterium* taxon (DNA group 3) was previously delineated by a polyphasic taxonomic study (Hugo *et al.*, *Syst Appl Microbiol* **22**, 586–595, 1999). In the present paper, this taxon is further characterized using 16S rDNA sequencing, fatty acid methyl ester analysis and a comparative phenotypic analysis, resulting in the proposal of a novel species, *Chryseobacterium joostei* sp. nov. (type strain 1x 5a^T=LMG 18212^T=CCUG 46665^T).

INTRODUCTION

The taxonomy of the flavobacteria has changed drastically during the last 10 years, mainly as a result of rRNA similarity studies (Bernardet *et al.*, 1996; Segers *et al.*, 1993; Steyn *et al.*, 1998; Takeuchi & Yokota, 1992; Vancanneyt *et al.*, 1996; Vandamme *et al.*, 1994). The genus *Flavobacterium* was emended in 1996 (Bernardet *et al.*, 1996) and several former *Flavobacterium* species, as well as novel species, were classified in the new genera *Chryseobacterium*, *Empedobacter*, *Myroides*, *Sphingobacterium* and *Pedobacter* (Bernardet *et al.*, 1996; Steyn *et al.*, 1998; Takeuchi & Yokota, 1992; Vancanneyt *et al.*, 1996; Vandamme *et al.*, 1994; Yabuuchi *et al.*, 1983; Yamaguchi & Yokoe, 2000).

At the time of writing, the genus *Chryseobacterium* consists of six species, *Chryseobacterium balustinum*, *Chryseobacterium gleum* (type species), *Chryseobacterium indologenes*, *Chryseobacterium indoltheticum*, *Chryseobacterium meningosepticum* and *Chryseobacterium scophthalmum*, and the recently described protein-deaminating rice-field isolate '*Chryseobacterium proteolyticum*' (Yamaguchi & Yokoe, 2000). Except for *C. meningosepticum*, all these species belong to a multispecies taxonomic entity that also includes numerous unidentified clinical and environmental isolates that are often referred to as CDC group IIb (Holmes, 1992; Holmes *et al.*, 1984a, b; Jooste *et al.*, 1985, 1986a, b; King, 1959; Lijnen *et al.*, 2000; Owen & Snell, 1976; Ursing & Bruun, 1991). These organisms can cause a variety of defects in dairy products, such as surface taint and apple odour in

butter (Jooste, 1985; Jooste *et al.*, 1986a). The heat-stable metalloproteases of these organisms may also cause problems in the dairy industry (Venter, 1997).

In previous studies (Hugo & Jooste, 1997; Hugo *et al.*, 1999), 103 South African dairy isolates and a large number of reference strains, all identified as, or related to, *Chryseobacterium* species or CDC group IIb, were studied using a polyphasic approach. Forty of the 103 strains studied could be identified as *C. indologenes* and one as *C. gleum* (Hugo *et al.*, 1999). The other strains could not be classified in any of the existing species. Among these unidentified strains, two homogeneous taxa, one large and one small, were delineated. According to DNA–DNA hybridization values (Hugo *et al.*, 1999) and the generally accepted standards for describing novel species (Vandamme *et al.*, 1996; Wayne *et al.*, 1987), only the large taxon (DNA group 3), containing 11 strains, was a candidate for a novel *Chryseobacterium* species. This taxon was studied extensively in the present work. On the basis of fatty acid methyl ester analysis, 16S rDNA sequence analysis and phenotypic characterizations [biochemical tests, API ZYM (bioMérieux) profiles, antibiotic-susceptibility patterns and scanning and transmission electron microscopy], we propose the name *Chryseobacterium joostei* sp. nov. for this taxon.

METHODS

Bacterial strains. Strains used in this study are listed in Table 1. *C. joostei* strains were isolated from raw milk samples from various regions of South Africa (Jooste, 1985; Jooste *et al.*, 1985, 1986a, b; Welthagen & Jooste, 1992). The reference strains were received from culture collections or other research institutes (Mudarris *et al.*, 1994; Ursing & Bruun, 1991). A comparison with '*C. proteolyticum*' was made using data from the literature (Yamaguchi & Yokoe, 2000), as the proposed type strain was not available at the time of this study. *Bergeyella zoohelcum* and *Empedobacter brevis* were included for

The EMBL accession numbers for the 16S rDNA sequences of *C. joostei* sp. nov. LMG 18212^T and *C. scophthalmum* LMG 13028^T are AJ271010 and AJ271009.

Full details of the antimicrobial sensitivity of strains of the novel species and reference strains are available as supplementary material in IJSEM Online.

Table 1. Strains investigated and their sources of isolation

Abbreviations: ATCC, American Type Culture Collection, Manassas, VA, USA; CCUG, Culture Collection of the University of Göteborg, Sweden; GIFU, Department of Medical Microbiology, Gifu University School of Medicine, Japan; LMG, BCCM/LMG Bacteria Collection Laboratorium voor Microbiologie, Universiteit Gent, Belgium; NCTC, National Collection of Type Cultures, London, UK. Other strain designations were used by Jooste (1985), Welthagen & Jooste (1992), Hugo & Jooste (1997) and Hugo *et al.* (1999). RSA, Republic of South Africa.

Strain	Isolation
<i>Chryseobacterium joostei</i> sp. nov.	
LMG 18207 (=Eh 9=32)	Raw tanker milk, Eikenhoff, Gauteng, RSA, 1981
LMG 18208 (=Hb 7a=37)	Raw tanker milk, Heidelberg I, Gauteng, RSA, 1981
LMG 18209 (=Hb 7b=38)	Raw tanker milk, Heidelberg I, Gauteng, RSA, 1981
LMG 18210 (=Hbg 12b=52)	Raw tanker milk, Heidelberg II, Gauteng, RSA, 1981
LMG 18211 (=Ip 8b=55)	Raw tanker milk, Ixopo I, Kwazulu-Natal, RSA, 1981
LMG 18212 ^T (=CCUG 46665 ^T =Ix 5a ^T =56 ^T)	Raw tanker milk, Ixopo II, Kwazulu-Natal, RSA, 1981
LMG 18213 (=Ix 11=63)	Raw tanker milk, Ixopo II, Kwazulu-Natal, RSA, 1981
LMG 18214 (=Sp 6a=81)	Raw tanker milk, Steenkoolspruit, Gauteng, RSA, 1981
LMG 18215 (=J 68=105)	Raw tanker milk, Dewetsdorp, Free State, RSA, 1989
LMG 18216 (=J 75=106)	Raw tanker milk, Jagersfontein, Free State, RSA, 1989
LMG 18217(=J 79=107)	Raw tanker milk, Bloemfontein-South, Free State, RSA, 1989
<i>C. gleum</i>	
NCTC 11432 ^T (=95 ^T)	High vaginal swab, London, UK
NCTC 10795 (=92)	Sputum, South Carolina, USA
<i>C. indologenes</i>	
NCTC 10796 ^T (=93 ^T)	Trachea at autopsy, USA
GIFU 1392 (=119)	Pharynx, Japan
Bv 3b (=8)	Raw tanker milk, RSA
Ct 12a (=17)	Raw tanker milk, Cape Town, Western Cape, RSA
Hb 12 (=40)	Raw tanker milk, Heidelberg I, Gauteng, RSA, 1981
Hbg 4a (=44)	Raw tanker milk, Heidelberg I, Gauteng, RSA, 1981
Ix 9a (=61)	Raw tanker milk, Ixopo II, Kwazulu-Natal, RSA, 1981
<i>C. balustinum</i> LMG 8329 ^T	Blood of freshwater fish, France
<i>C. indoltheticum</i> ATCC 27950 ^T (=126 ^T)	Marine mud
<i>C. scopthalmum</i>	
LMG 13028 ^T (=129 ^T)	Gills of diseased turbot, Scotland, UK
LMG 13032 (=130)	Sea water, Scotland, UK
<i>C. meningosepticum</i> NCTC 10016 ^T (=127 ^T)	Spinal fluid, Massachusetts, USA
<i>Bergeyella zoohelcum</i> LMG 8351 ^T	Sputum, Nebraska, USA
<i>Empedobacter brevis</i> NCTC 11099 ^T (=131 ^T)	Human bronchial secretion, Switzerland

comparative purposes. All the isolates and reference strains were reactivated in nutrient broth (CM67; Oxoid) and checked for purity on nutrient agar [1.5% (w/v) agar] at 25 °C for 24–48 h. The strains were maintained in a freeze-dried state on filter-paper discs and stored in screw-capped tubes at –20 °C, and for longer times at –80 °C or by lyophilization.

16S rDNA sequencing. Two strains, *C. joostei* LMG 18212^T and *C. scopthalmum* LMG 13028^T, were studied. High-molecular-mass DNA was prepared according to Vandamme *et al.* (1992). Part of the rDNA operon, comprising the nearly complete 16S rDNA, was amplified using a PCR. The sequence of the forward primer was 5'-AGAGTTTGATCCTGGCTCAG-3' and that of the reverse primer was 5'-AAGGAGGTGATCCAGCCGCA-3' (respectively corresponding to positions 8–27 and 1541–1522 of the *Escherichia coli* 16S rRNA numbering system). PCR-amplified 16S rDNAs were purified using the QIAquick PCR purification kit (Qiagen). Sequence analysis was performed using an Applied Biosystems 377 DNA sequencer and the protocols of the manufacturer with the ABI PRISM BigDye

Terminator cycle sequencing ready reaction kit (with AmpliTaq DNA Polymerase, FS). The sequencing primers were those described by Coenye *et al.* (1999). Sequence assembly was performed by using the program AutoAssembler (Applied Biosystems) and phylogenetic analysis was performed by using the BIONUMERICS software (Applied Maths). The consensus sequence and sequences of strains belonging to the same phylogenetic group (retrieved from the EMBL database) were aligned and a phylogenetic tree was constructed on the basis of the neighbour-joining method.

Fatty acid methyl ester analysis. Reactivated cultures were streaked on trypticase soy agar [BBL; solidified with 1.5% (w/v) Difco Bacto agar] and incubated at 28 °C for exactly 24 h. From a loopful of harvested cells, fatty acid methyl esters were prepared, separated by GLC and identified by the Microbial Identification System software package (MIS version 3.9; Microbial ID), as described previously (Vandamme *et al.*, 1992). Mean percentages and standard deviations were calculated for each taxon.

Phenotypic characterization of the isolates. A battery of tests was selected to differentiate among Gram-negative, yellow-pigmented bacteria (Holmes *et al.*, 1984a). Tests were performed at 25 °C with different incubation times, according to Cowan (1974), MacFaddin (1980) or Gerhardt *et al.* (1981), unless indicated otherwise.

Colony morphology was observed on nutrient agar and the presence of flexirubin pigments was investigated by flooding the plates with 20% (w/v) potassium hydroxide (Fautz & Reichenbach, 1980). Pigmentation was also examined on tyrosine agar and fluorescence was assessed using King's medium B (Gerhardt *et al.*, 1981). Gram staining was done using the modified protocol of Lillie, as described by Cowan (1974). Motility was examined by phase-contrast microscopy from cells grown in nutrient broth at 22 and 37 °C. Gliding motility was determined on CY agar, as described by Jooste *et al.* (1985). Growth was investigated at different concentrations of sodium chloride (0–5%, w/v), at different temperatures (5, 22, 37 and 42 °C) and on cetrimide agar (Brown & Lowbury, 1965), β -hydroxybutyrate agar, MacConkey agar and Simmons' citrate agar. Haemolytic activity was determined using sheep-blood (5%, v/v) agar plates.

Additional biochemical tests were performed: oxidative or fermentative metabolism of glucose; oxidase, catalase, methyl red and Voges-Proskauer reactions; gluconate oxidation; reduction of selenite (0.4%, w/v), nitrate and nitrite (Cleve's acid was used instead of α -naphthylamine); alkaline reaction on Christensen's citrate (Holmes *et al.*, 1975); production of hydrogen sulphide (lead acetate paper and triple-sugar-iron methods), indole (Ehrlich's reagent and Kovács' indole reagent), 3-ketolactose; ammonia from arginine; activity of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, phosphatase, DNase (Oxoid CM321; supplemented

Fig. 1. Neighbour-joining tree of *Chryseobacterium* species and related taxa of the *Flavobacteriaceae*, showing the position of *C. joostei* sp. nov. LMG 18212^T. Accession numbers of 16S rDNA sequences are indicated in parentheses. Bootstrap probability values are indicated at branch-points (100 trees resampled).

with 0.01% toluidine blue), β -galactosidase (ONPG), lecithinase (opalescence on lecithovitellin agar), phenylalanine deaminase, urease on Christensen's urea agar; hydrolysis of aesculin, casein, gelatin (tube and plate method), starch, Tweens 20 and 80 and tyrosine; potassium cyanide tolerance; malonate utilization; and acid production from 10% (w/v) glucose and lactose, from 1% (w/v) adonitol, L-arabinose, cellobiose, dulcitol, ethanol, D-fructose, D-glucose, glycerol, inositol, lactose, maltose, D-mannitol, raffinose, rhamnose, sorbitol, sucrose, trehalose and D-xylose and from 0.5% (w/v) salicin.

Susceptibility to 26 antimicrobial agents was determined according to

Table 2. Long-chain fatty acid composition of *Chryseobacterium* species and related bacteria

Taxa are listed as: 1, *C. joostei* sp. nov. ($n=11$); 2, *C. gleum* ($n=5$); 3, *C. indologenes* ($n=45$); 4, *C. balustinum* ($n=1$); 5, *C. indoltheticum* ($n=1$); 6, *C. scophthalmum* ($n=2$); 7, *C. meningosepticum* ($n=1$); 8, *B. zoohelcum* ($n=1$); 9, *Empedobacter brevis* ($n=6$). All strains identified as *C. gleum*, *C. indologenes* and *Empedobacter brevis* in an earlier study (Hugo *et al.*, 1999) were included. Fatty acid percentages amounting to less than 1% of the total fatty acids in all strains were not included. Means $\pm$ SD are given. tr, Trace (less than 1.0%); ND, not detected.

Fatty acid	1	2	3	4	5	6	7	8	9
13:0 iso	tr	tr	tr	tr	ND	tr	1.4	1.5	tr
Unknown 13:566*	1.1 $\pm$ 0.2	1.2 $\pm$ 0.4	2.1 $\pm$ 0.7	1.6	1.7	2.9 $\pm$ 0.2	1.5	1.8	1.9 $\pm$ 1.4
15:0 iso	34.6 $\pm$ 2.0	35.4 $\pm$ 2.9	34.3 $\pm$ 4.9	32.3	29.4	35.0 $\pm$ 0.7	41.4	47.8	23.6 $\pm$ 2.2
15:0 iso 3-OH	2.9 $\pm$ 0.3	2.5 $\pm$ 0.1	2.6 $\pm$ 0.2	2.7	2.3	2.7 $\pm$ 0.1	3.5	3.95	4.8 $\pm$ 0.7
15:0 anteiso	tr	tr	tr	tr	5.9	tr	2.3	ND	tr
16:0	tr	1.3 $\pm$ 0.4	tr	1.6	1.0	1.2 $\pm$ 0.2	tr	tr	3.8 $\pm$ 0.4
16:0 3-OH	1.2 $\pm$ 0.2	1.1 $\pm$ 0.1	1.0 $\pm$ 0.2	1.4	tr	1.0 $\pm$ 0.1	2.2	ND	3.6 $\pm$ 0.7
16:0 iso 3-OH	ND	ND	ND	tr	1.3	ND	tr	ND	tr
Unknown 16:580*	1.6 $\pm$ 0.1	1.7 $\pm$ 0.1	1.7 $\pm$ 0.2	1.3	1.3	1.5 $\pm$ 0.1	1.7	1.4	1.4 $\pm$ 0.1
17:0 2-OH	ND	ND	ND	tr	3.0	tr	tr	ND	tr
17:0 iso	tr	1.6 $\pm$ 0.6	tr	1.0	tr	tr	tr	ND	tr
17:0 iso 3-OH	20.1 $\pm$ 1.2	21.8 $\pm$ 0.3	19.2 $\pm$ 1.8	16.8	14.0	16.3 $\pm$ 0.1	16.3	13.5	17.1 $\pm$ 2.1
17:1 iso ω 9c	22.9 $\pm$ 1.9	20.2 $\pm$ 3.9	24.2 $\pm$ 3.1	27.1	25.6	24.8 $\pm$ 0.4	7.0	17.5	3.9 $\pm$ 0.5
18:1 ω 5c	tr	tr	tr	tr	tr	tr	tr	1.5	ND
Summed feature 4†	12.1 $\pm$ 1.3	11.8 $\pm$ 0.8	11.1 $\pm$ 1.3	9.2	11.2	11.5 $\pm$ 0.3	18.1	9.0	19.4 $\pm$ 1.6
Summed feature 5†	tr	tr	tr	tr	ND	tr	tr	1.5	6.7 $\pm$ 0.8

*Unknown fatty acid; numbers indicate equivalent chain length.

†Fatty acids that could not be separated by GC using the Microbial Identification System (Microbial ID) software were considered summed features. Summed feature 4 contains 15:0 iso 2-OH and/or 16:1 ω 7c and/or 16:1 ω 7t. Summed feature 5 contains 17:1 iso I and/or 17:1 anteiso B.

the standard methods of the NCCLS (1983). Strains were grown overnight on Mueller–Hinton agar (Oxoid) at 25 °C and the diameters of inhibition zones were measured.

Hydrolysis of 19 substrates was investigated using the API ZYM system according to the recommendations of the manufacturer (bioMérieux). The intensity of the developed colour was measured on a scale from 0 to 5 and interpreted as being negative when values ranged between 0 and 1 and positive for values between 2 and 5 (Mudarris *et al.*, 1994).

Electron microscopy. Dense suspensions of *C. joostei* LMG 18212^T were cultivated in nutrient broth at 25 °C for 72 h. Cell preparation was done according to Spurr (1969) and Reynolds (1963). For transmission electron microscopy, a Philips CEM 300 electron microscope was used; a JEOL 6400 WINSEM electron microscope was used for scanning electron microscopy.

RESULTS AND DISCUSSION

Phylogenetic allocation and strain characterization

16S rDNA sequence analysis and subsequent construction of a neighbour-joining tree (Fig. 1) confirmed that *C. joostei* LMG 18212^T and *C. scopthalmum* LMG 13028^T, a species for which no sequence was previously available, are authentic members of the genus (Mudarris *et al.*, 1994). Respective sequence similarities of 97·7 and 97·6 % between *C. joostei* LMG 18212^T and the type strains of *C. gleum* and *C. indologenes* did not exclude a possible relationship at the species level with one of these taxa (Vandamme *et al.*, 1996). Similarities in the range 95·8–87·3 % between *C. joostei*

Table 3. Differential phenotypic characteristics of *Chryseobacterium* species and related bacteria

Species are listed as: 1, *C. joostei* sp. nov. (*n*=11); 2, *C. gleum* (*n*=2); 3, *C. indologenes* (*n*=7); 4, *C. balustinum* (*n*=1); 5, *C. indoltheticum* (*n*=1); 6, *C. scopthalmum* (*n*=2); 7, *C. meningosepticum* (*n*=1); 8, *B. zoohelcum* (*n*=1); 9, *Empedobacter brevis* (*n*=1); 10, '*C. proteolyticum*' (*n*=2; data from Yamaguchi & Yokoe, 2000). +, Positive; –, negative; V, variable; D, delayed; NA, not available; W, weakly positive.

Characteristic	1*	2	3	4	5	6	7	8	9	10
Growth on/at:										
Cetrimide agar	+	–	+	–	–	–	+	–	–	NA
MacConkey agar	+	+	+	+	+	–	+	–	+	–
5 °C	+	–	–	D	+	D	–	–	–	NA
37 °C	–	+	–	+	–	–	+	+	–	+
42 °C	–	–	–	–	–	–	–	+	–	–
Enzyme activities:										
DNase	+	+	+	+	+	+	+	–	+	+
Urease	V ^a	–	–	–	–	+	D	–	+	–
Lecithinase	+	+	+	+	+	+	–	–	–	NA
Nitrate reduction	–	+	–	+	–	–	–	–	–	–
Nitrite reduction	–	+	–	–	–	–	+	–	–	–
Aesculin hydrolysis	+	+	+	+	+	+	+	–	–	+
Starch hydrolysis	+	+	+	–	–	–	–	–	+	+
Tween 80 hydrolysis	+	+	+	+	+	+	–	–	–	+
Tyrosine hydrolysis	+	+	–	–	+	+	D	–	–	+
Production of:										
Hydrogen sulphide	–	–	–	–	+	–	–	–	–	W
Indole (Kovács' reagent)	+	+	+	+	+	–	D	+	+	+
Acid production from:										
L-Arabinose	–	+	–	–	–	–	–	–	–	+
Ethanol	–	–	–	+	–	+	+	–	–	–
D-Fructose	+	+	D	+	–	–	+	–	–	NA
Glycerol	V ^b	+	D	–	–	–	+	–	–	W
Lactose	–	–	–	–	–	–	+	–	–	–
Maltose	+	+	+	–	+	–	+	–	+	+
D-Mannitol	V ^b	–	–	–	–	–	+	–	–	W
Trehalose	+	+	+	–	–	+	+	–	–	+
D-Xylose	–	+	–	–	–	–	–	–	–	NA
Alkaline reaction on Christensen's citrate	V ^c	+	–	–	+	+	D	–	–	NA
Mean DNA G+C content (mol%)	36·8	38·0	38·5	33·1	33·8	34·2	37·0	36·0	32·0	37·1

*Variable reactions are scored as: *a*, positive for LMG 18212^T and 18213; *b*, positive for LMG 18207–18211, 18212^T, 18213, 18214 and 18217; *c*, positive for LMG 18208–18211, 18212^T, 18215 and 18216.

LMG 18212^T and reference strains of *C. balustinum*, *C. indoltheticum*, *C. meningosepticum*, '*C. proteolyticum*', *C. scophthalmum*, *Riemerella anatipestifer*, *B. zoohelcum*, *Empedobacter brevis* and *Weeksella virosa* were below the level indicating relatedness at the species level (Stackebrandt & Goebel, 1994).

The mean long-chain fatty acid contents of all *C. joostei* strains and related taxa (except '*C. proteolyticum*') are presented in Table 2. Fatty acid methyl ester analysis revealed minor differences between *C. joostei* and its nearest phylogenetic neighbours *C. gleum*, *C. indologenes*, *C. indoltheticum*, *C. balustinum* and *C. scophthalmum*. Dominant fatty acids for all strains investigated (mean levels above 5 %) were 15:0 iso, 17:0 iso 3-OH, 17:1 iso ω 9c and summed feature 4 (15:0 iso 2-OH and/or 16:1 ω 7c and/or 16:1 ω 7t; Table 2). This fatty acid composition is characteristic of all of the above-mentioned taxa, including CDC group IIb strains (Hugo *et al.*, 1999). Significant differences were observed between the latter organisms and *C. meningosepticum*, *B. zoohelcum* and *Empedobacter brevis* (Table 2).

Evidence for the proposal of a novel species

DNA–DNA hybridizations between *C. joostei* (DNA group 3; Hugo *et al.*, 1999) and the two species that showed significant 16S rDNA similarity for possible relatedness at the species level, *C. gleum* and *C. indologenes*, showed low DNA binding values (19–23 %; Hugo *et al.*, 1999). A separate position for the *C. joostei* strains with respect to the other *Chryseobacterium* species was also shown previously by SDS-PAGE of whole-cell proteins (SDS-PAGE group 5; Hugo *et al.*, 1999).

In view of the separate position determined genomically and chemotaxonically, an extensive comparative phenotypic study was performed. Results from this polyphasic approach confirm that *C. joostei* constitutes a novel species of the genus *Chryseobacterium* (Mudarris *et al.*, 1994; Vandamme *et al.*, 1994). Phenotypic features that distinguish the novel species from its nearest neighbours are summarized in Table 3.

Description of *Chryseobacterium joostei* sp. nov.

Chryseobacterium joostei (joos'te.i. N.L. gen. n. *joostei* of Jooste, in honour of P. J. Jooste, who isolated the first strains of this species from South African raw milk).

Cells are Gram-negative, non-spore-forming rods, approximately $1.0 \times 0.5 \mu\text{m}$ with parallel sides and rounded ends (Fig. 2). The cell wall is approximately 50 nm thick (Fig. 2). Cells do not show gliding motility on CY agar and are non-motile after incubation in nutrient broth at 22 and 37 °C (phase-contrast microscopy). Growth is aerobic. Colonies on nutrient agar are smooth, shiny, circular with entire edges and butyrous in consistency, becoming mucoid after incubation for 5 days. A bright-yellow pigment (flexirubin)

Fig. 2. Transmission electron micrograph of a cross-section of cells of *C. joostei* sp. nov. LMG 18212^T. Bar, 500 nm.

is produced on nutrient agar: it is non-diffusible, non-fluorescent and turns reddish brown upon the addition of 20 % KOH. A water-soluble, non-diffusible brown pigment is produced on tyrosine agar. Strains grow at 5 °C and room temperature, but not at 37 or 42 °C. Growth is observed on cetrimide agar, MacConkey agar and β -hydroxybutyrate (without the production of β -hydroxybutyrate inclusion granules), but not on Simmons' citrate agar. All strains show catalase, oxidase, phosphatase, DNase and lecithinase (opalescence on lecithovitellin agar) activity, but not arginine, lysine or ornithine decarboxylase or phenylalanine deaminase activity; there is no gluconate oxidation. They produce indole when Kovács' reagent is used, but not when Ehrlich's reagent is used. Hydrogen sulphide and 3-ketolactose are not produced. Capable of hydrolysing aesculin, casein, gelatin, starch, Tweens 20 and 80 and tyrosine. Negative for the methyl red and Voges–Proskauer tests, does not tolerate KCN (0.0075 %), does not utilize malonate and cannot reduce nitrate, nitrite or 0.4 % selenite. Acid is produced from 1 % (w/v) D-fructose, D-glucose (not 10 %, w/v), maltose and trehalose, but not from L-arabinose, cellobiose, dulcitol, ethanol, inositol, lactose, raffinose, rhamnose, salicin, sorbitol, sucrose or D-xylose. Results from API ZYM tests are given in Table 4. Susceptible to the following antimicrobial agents: chloramphenicol (50 μg), nalidixic acid (30 μg), novobiocin (5 μg) and sulphamethoxazole (25 μg). Variable results for ampicillin (25 μg), carbenicillin (100 μg), cephaloridine (30 μg) and clindamycin (2 μg); full details are available as supplementary material in IJSEM Online. The cellular long-chain fatty acids characterizing the species are 15:0 iso, 15:0 iso 3-OH, 16:0 3-OH, 17:0 iso 3-OH, 17:1 iso ω 9c, summed feature 4 (15:0 iso 2-OH and/or 16:1 ω 7c and/or 16:1 ω 7t) and two unidentified fatty acids with equivalent chain lengths of 13.566 and 16.580 (Table 2). The G+C content of the DNA is about 36–37 mol% (36.7 mol% for the type strain).

Table 4. API ZYM profiles of *Chryseobacterium* species and related bacteria

Species are listed as: 1, *C. joostei* sp. nov.; 2, *C. gleum*; 3, *C. indologenes*; 4, *C. balustinum*; 5, *C. indoltheticum*; 6, *C. scopthalmum*; 7, *C. meningosepticum*; 8, *B. zoohelcum*; 9, *Empedobacter brevis*. Only type strains of each species were included. All the organisms gave positive reactions for 2-naphthyl phosphate, 2-naphthyl caprylate, L-leucyl 2-naphthylamide, L-valyl 2-naphthylamide, 2-naphthyl phosphate and naphthol-AS-BI phosphate and gave negative reactions for 2-naphthyl myristate, 2-naphthyl β -D-galactopyranoside, naphthol-AS-BI β -D-glucuronide and 6-Br-2-naphthyl α -D-mannopyranoside. API ZYM scores were interpreted as described in Methods.

Substrate	1	2	3	4	5	6	7	8	9
2-Naphthyl butyrate	–	–	–	–	–	+	–	–	+
L-Cystyl 2-naphthylamide	+	–	+	–	+	+	+	–	+
N-Benzoyl-DL-arginine 2-naphthylamide	+	–	–	–	–	+	+	–	+
N-Glutaryl-phenylalanine 2-naphthylamide	–	–	+	–	–	–	+	–	+
6-Br-2-naphthyl α -D-galactopyranoside	–	–	–	–	–	–	+	–	–
2-Naphthyl α -D-glucopyranoside	+	+	+	+	+	+	+	–	+
6-Br-2-naphthyl β -D-glucopyranoside	–	+	–	+	–	–	–	–	–
1-Naphthyl-N-acetyl β -D-glucosaminide	+	+	+	+	+	+	+	–	–
2-Naphthyl α -L-fucopyranoside	–	–	–	–	–	–	+	–	–

The type strain is LMG 18212^T (= Ix 5a^T = CCUG 46665^T), which was isolated in 1981 from raw milk in the Ixopo district of Kwazulu-Natal in South Africa. All *C. joostei* strains reported in this study were isolated from raw milk samples taken from different farms and from tankers arriving at dairy factories across South Africa.

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