Chryseobacterium hominis sp. nov., to accommodate clinical isolates biochemically similar to CDC groups II-h and II-c

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A collection of eight clinical strains from Belgian hospitals and three clinical strains of the CCUG collection were characterized biochemically as being similar to CDC groups II-h and II-c; the latter differs from group II-h only by positivity for sucrose acidification. These 11 strains were found to cluster according to 16S rRNA gene sequence similarity at a level of $\geq 99.5\%$, and on the basis of their tDNA-PCR profile. Based on 16S rRNA gene sequence analysis, this collection of strains was related most closely to *Chryseobacterium hispanicum* (97.2%), but they differed from the type strain of this species by the following phenotypic characteristics: growth at 37 °C, negativity for xylose acidification, positivity for acetate assimilation–alkalinization on Simmons' agar base and absence of flexirubin pigments, and by their tDNA-PCR profile. Strain NF802^T showed only 57.8% DNA–DNA relatedness to the type strain of *C. hispanicum*. Fatty acid composition did not enable differentiation from *C. hispanicum*. The DNA G+C content of strain NF802^T is 36.5 mol%. The name *Chryseobacterium hominis* sp. nov. is proposed for this taxon, with type strain NF802^T (=CCUG 52711^T=CIP 109415^T).

The family *Flavobacteriaceae*, emended by Bernardet *et al.* (2002), currently comprises more than 50 genera, including *Bergeyella*, *Chryseobacterium*, *Elizabethkingia*, *Kaistella*, *Riemerella*, *Sejongia* and the recently described *Wautersiella* (Kämpfer *et al.*, 2006).

At present, the genus *Chryseobacterium* comprises 20 species, including some of clinical importance (Quan *et al.*, 2007). In addition, Schreckenberger *et al.* (2003) mentioned a number of phenotypically related strains called 'CDC groups II-c, II-e, II-g, II-h and II-i', which are recovered rarely from clinical material, according to the authors. CDC group II-h and group II-c strains are distinguished from each other by acid production from sucrose (CDC group II-c), from group II-e by aesculin hydrolysis, from group II-i by the absence of xylose acidification and from group II-g because the latter is non-saccharolytic. In this study, we characterized seven CDC II-h and four CDC II-c strains, of which the clinical and geographical origin and the year of isolation are listed in

Table 1. On the basis of this study, we propose that the 11 strains represent a novel species in the genus *Chryseobacterium*, for which the name *Chryseobacterium hominis* sp. nov. is proposed.

Biochemical and morphological tests were performed as described previously (Laffineur *et al.*, 2002; Schreckenberger *et al.*, 2003); the morphological characteristics of the novel species are given in the species description. Inhibition zones were determined on tryptic soy agar around discs of tetracycline (30 µg), ciprofloxacin (5 µg), trimethoprim/cotrimoxazole (23.75 µg/1.25 µg), ampicillin (10 µg), temocillin (30 µg), cephalothin (30 µg), cefotaxime (30 µg), erythromycin (15 µg), gentamicin (10 µg) and colistin (10 µg).

Acid production from carbohydrates was tested on oxidation–fermentation medium and low-peptone phenol red agar as described for ethylene glycol (Wauters *et al.*, 1998). Assimilation–alkalinization of organic compounds was detected on Simmons' citrate agar base, replacing citrate by 0.2% (w/v) of various organic substrates, according to Martin *et al.* (1981). Enzymic reactions were carried out by using diagnostic tablets from Rosco. The KOH test was used to detect flexirubin pigments (Bernardet *et al.*, 2002). Susceptibility to antibiotics was

Abbreviation: tDNA-PCR, tRNA intergenic length polymorphism analysis.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *C. hominis* are AM261868 and AM423079–AM423088.

Table 1. Data regarding clinical and geographical origin and phenotypic and genotypic data of the *C. hominis* strains studied

Strain designation	CDC group	Origin and year of isolation	Sequence similarity*
NF802 ^T	II-h	Blood, La Louvière, Belgium, 1998	NA
NF1335	II-h	Dialysis fluid, Brussels, Belgium, 1982	99.8
NF675	II-c	Blood, La Louvière, Belgium, 1995	99.9
NF690	II-c	Blood, La Louvière, Belgium, 1995	99.9
NF974	II-c	Blood, La Louvière, Belgium, 2001	99.8
NF696	II-h	Pus, Brussels, Belgium, 1995	100.0
NF1141	II-h	Blood, Brussels, Belgium, 2003	99.8
NF1146	II-h	Blood, Yvoir, Belgium, 2003	99.5
CCUG 13649, CDC A6180	II-h	Infraorbital drain, CA, USA, 1966	98.9
CCUG 15261, CDC F4158	II-h	Eye, MD, USA, 1984	99.9
CCUG 36748, CDC 9064	II-c	Aortic valve, CO, USA, 1963	99.9

*16S rRNA gene sequence similarity to NF802^T (1483 bp); NA, not applicable.

performed by the agar diffusion method on Mueller–Hinton agar and interpreted according to the guidelines of CLSI (2005).

Table 2 summarizes the biochemical data obtained. All strains of *C. hominis* were positive for oxidase and catalase activities, growth at 30 and 37 °C (with optimal growth at 30 °C), aerobic growth, acid production from glucose, maltose and ethylene glycol, indole production, hydrolysis of aesculin, starch and gelatin, alkaline phosphatase, trypsin (benzylarginine arylamidase) and pyrrolidonyl-aminopeptidase (except for CCUG 36749) activities, and resistance to desferrioxamine. Acid production from sucrose, reduction of nitrate and nitrite, and hydrolysis of tyrosine were variable. Schreckenberger *et al.* (2003) reported positive nitrate reduction (90 %) in group II-c and a negative result in group II-h. However, in our series, four strains out of seven with a II-h profile also reduced nitrate. All strains were negative for production of flexirubin pigments, growth at 42 °C, urease activity, H₂S production on Kligler agar, Tween 80 hydrolysis, ornithine and lysine decarboxylases, arginine dihydrolase, alkalization of citrate on Simmons' agar and L-phenylalanine deaminase.

Phenotypically, *C. hominis* can be differentiated from *Chryseobacterium hispanicum* (Gallego *et al.*, 2006) by growth at 37 °C, lack of acid production from xylose and arabinose and positive alkalization of acetate on Simmons' agar base, and from *Chryseobacterium caeni* by lack of acid production from arabinose, trehalose and xylose and absence of β -galactosidase (ONPG) activity. The absence of flexirubin pigments was a unique feature differentiating *C. hominis* from all other *Chryseobacterium* species. It should be noted that some of our results obtained with *C. caeni* were different from those of the original description (Quan *et al.*, 2007). For *C. caeni* N4^T, which was reported originally as being asaccharolytic, we detected acid production from several sugars by using oxidation–fermentation medium and phenol red agar with low peptone content (Wauters *et al.*, 1998). The type strain

of *C. caeni* produced indole on urea–indole broth and was urease-negative on Christensen's urea broth and urea–indole broth.

The 16S rRNA gene sequences were determined for all isolates as described previously (Wauters *et al.*, 2003), and phylogenetic tree construction based on 16S rRNA gene sequences was done as described by Nemec *et al.* (2001). Cluster analysis was performed by using GeneBase (Applied Maths) and was based on the neighbour-joining method. The four CDC II-c strains were found to cluster together with the seven CDC II-h strains according to 16S rRNA gene sequence similarity (Table 1; Fig. 1). This should not be surprising, as CDC II-c strains differ phenotypically from CDC II-h strains only by positive acidification of sucrose. The similarity of the type strain (*C. hominis* NF802^T) to the other 10 strains ranged between 99.5 and 100 %. A strain submitted to GenBank as '*Chryseobacterium aquaticum*' (accession no. AM398648) and isolated from the drinking-water distribution system of Sevilla, Spain, was found to have a sequence identical to those of four *C. hominis* strains (NF802^T, NF696, CCUG 36748 and CCUG 15261). Based on 16S rRNA gene sequence, this collection of strains was related most closely to *C. hispanicum* (97.2 %). A maximum-parsimony tree showed essentially the same topology (data not shown).

For DNA–DNA hybridization, a microplate method was used, modified after Lind & Ursing (1986) and described previously (Ziemke *et al.*, 1998). *C. hominis* NF802^T showed >90 % DNA–DNA similarity to strains CCUG 36748, CCUG 15261 and CCUG 13649, but only 57.8 % DNA–DNA similarity to the type strain of *C. hispanicum*.

The DNA G+C content of the type strain, determined by HPLC at the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany, according to Mesbah *et al.* (1989), was 36.5 mol%.

Fatty acid analysis was carried out on cells grown for 48 h on tryptone soy agar at 28 °C as described previously

Table 2. Phenotypic characteristics useful to differentiate *C. hominis* from related bacteria

Taxa: 1, *C. hominis*; 2, *Chryseobacterium balustinum*; 3, *C. caeni*; 4, *Chryseobacterium defluvii*; 5, *Chryseobacterium formosense*; 6, *Chryseobacterium gleum*; 7, *C. hispanicum*; 8, *Chryseobacterium indologenes*; 9, *Chryseobacterium indoltheticum*; 10, *Chryseobacterium joosteii*; 11, '*Chryseobacterium proteolyticum*'; 12, *Chryseobacterium scophthalmum*; 13, *Chryseobacterium taichungense*; 14, *Elizabethkingia meningoseptica*; 15, *Elizabethkingia miricola*. Data for *C. hominis*, *C. hispanicum* and *C. caeni* are from this study. Data for reference species were taken from de Beer *et al.* (2005), Kämpfer *et al.* (2003), Kim *et al.* (2005a, b), Li *et al.* (2003), Hugo *et al.* (2003), Park *et al.* (2006), Quan *et al.* (2007), Shen *et al.* (2005), Shimomura *et al.* (2005), Tai *et al.* (2006), Weon *et al.* (2006), Yamaguchi & Yokoe (2000) and Young *et al.* (2005). +, All strains tested positive; (+), weakly positive; –, all strains tested negative; v, variable; NA, not available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Number of strains	11	1	1	1	1	12	1	13	1	11	2	7	1	49	1
Acid production from:															
Glucose	+	+	+	+	(+)	+	+	+	+	+	+	–	(+)	+	+
Sucrose	4*	–	+	–	–	–	+	–	–	–	+	–	–	–	–
Mannitol	–	–	–	–	–	–	–	4*	–	+	+	–	–	31*	+
Arabinose	–	–	(+)	–	–	10*	+	–	–	–	+	–	–	1*	–
Cellobiose	1*	–	(+)	(+)	–	–	(+)	–	–	–	–	–	–	4*	–
Lactose	–	–	–	NA	NA	–	–	–	–	–	–	–	–	27*	+
Maltose	+	–	+	+	–	+	+	+	+	+	+	–	+	46*	+
Trehalose	–	–	+	+	+	+	–	+	–	+	+	–	+	42*	+
Xylose	–	–	(+)	–	(+)	4*	+	–	–	–	NA	–	+	3*	–
Growth at 36–37 °C	+	–	+	+	–	+	–	+	+	–	+	–	+	+	+
Growth on MacConkey agar	–	+	–	–	–	+	–	v	+	+	–	–	–	+	(+)
Nitrate reduction	8*	+	–	–	–	7*	+	v	–	–	–	–	–	–	–
Urease activity	–	–	–	–	–	7*	–	–	–	+	–	+	–	18*	+
Indole production	+	+	+	+	+	+	+	+	+	+	+	–	(+)	24*	+
Acetate alkalization	+	NA	+	NA	NA	NA	–	NA	NA	NA	NA	NA	NA	NA	NA
Production of flexirubin pigments	–	+	+	+	+	+	+	+	+	+	+	+	+	–	–
Hydrolysis of:															
Aesculin	+	–	+	+	+	+	+	+	+	+	+	–	+	47*	+
Tween 80	–	+	–	NA	–	+	–	+	+	+	+	+	NA	–	+
Starch	+	–	+	+	NA	+	+	+	–	+	+	–	NA	–	–
Tyrosine	4*	–	+	NA	NA	+	+	–	+	+	+	+	NA	(+)	–
DNase	+/(+)	+	+	NA	NA	+	(+)	+	+	+	+	+	NA	+	+
ONPG	–	–	+	–	–	–	(+)	–	–	–	+	–	(+)	+	+

*Number of positive strains.

(Kämpfer & Kroppenstedt, 1996; Kämpfer *et al.*, 2003). Table 3 compares the data obtained during this study for four *C. hominis* strains with those reported in the literature for related *Chryseobacterium* species.

tRNA-intergenic length polymorphism analysis (tDNA-PCR) was carried out as described previously (Baele *et al.*, 2000). The tDNA-PCR patterns of 10 *C. hominis* strains were compared with the pattern of the *C. hispanicum* type strain. All 11 strains had tRNA-intergenic spacers in common, with lengths of 57.6, 90.2, 91.3 and 92.3 bp. *C. hominis* had spacers of 81.1 bp (compared with 82.9 bp for *C. hispanicum*) and of 86.7, 89.3 and 110.6 bp that were absent in the type strain of *C. hispanicum*, whereas the latter had spacers of 94.5, 138.8, 232.0 and 236.1 bp, not observed in any of the 10 *C. hominis* strains. On the basis of the data presented, the 11 strains should be assigned to the genus *Chryseobacterium*

as representing a novel species, for which the name *Chryseobacterium hominis* sp. nov. is proposed.

Description of *Chryseobacterium hominis* sp. nov.

Chryseobacterium hominis (ho'mi.nis. L. gen. n. *hominis* of a man, of a human being, named as such because most of the known isolates at the time of description are of human origin, in opposition to most other *Chryseobacterium* species).

Non-motile, Gram-negative rods, 1–3 µm in length and 1.0–1.5 µm in width, growing aerobically at 20, 30 and 37 °C on standard media such as tryptic soy agar and blood agar, with optimal growth at 30 °C. No growth on MacConkey agar, cetrimide agar or 3 % NaCl agar. Colonies are circular and mucoid; some are also sticky.

characteristics are listed in Tables 2 and 3. The major cellular fatty acids are 15:0 iso, 17:0 iso 3-OH, 17:0 iso ω 9c and 15:0 anteiso. Strains are susceptible to tetracycline, ciprofloxacin and trimethoprim/cotrimoxazole and resistant to ampicillin and temocillin. Susceptibility is variable to cephalothin, cefotaxime, erythromycin, gentamicin and colistin.

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Table 3. Long-chain fatty acid composition of *C. hominis* strains and related bacteria

Taxa: 1, *C. hominis* NF802^T; 2, *C. hominis* CCUG 15261; 3, *C. hominis* CCUG 13649; 4, *C. hominis* CCUG 36748; 5, *C. balustinum*; 6, *C. caeni* (*n*=1); 7, *C. defluvii* (*n*=1); 8, *C. formosense* (*n*=1); 9, *C. gleum* (*n*=5); 10, *C. hispanicum* (*n*=1); 11, *C. indologenes* (*n*=45); 12, *C. indoltheticum* (*n*=1); 13, *C. joostei* (*n*=11); 14, *C. scophthalmum* (*n*=2); 15, *C. taichungense* (*n*=1); 16, *E. meningoseptica* (*n*=5); 17, *E. miricola* (*n*=2). '*C. proteolyticum*' is not listed, as no data are available. Values are percentages of total fatty acids. Fatty acids amounting to <1 % of the total fatty acids in all strains are not listed. Some of the strains/species were not cultivated under the same conditions. Where applicable, means $\pm$ SD are given. Tr, Trace (<1.0 %); ND, not detected. Data are from de Beer *et al.* (2005), Hugo *et al.* (2003), Kämpfer *et al.* (2003), Kim *et al.* (2005a, b), Li *et al.* (2003), Park *et al.* (2006), Quan *et al.*, (2007), Shen *et al.* (2005), Shimomura *et al.* (2005), Tai *et al.* (2006), Weon *et al.* (2006), Yamaguchi & Yokoe (2000) and Young *et al.* (2005).

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
13:0 iso	3.6	4.8	1.9	Tr	Tr	1.0	2.8	3.6	Tr	1.2	Tr	ND	Tr	Tr	Tr	1.3 $\pm$ 0.3	2.0 $\pm$ 0.5
Unknown 13.566*	6.3	ND	6.9	ND	1.6	2.6	Tr	Tr	1.2 $\pm$ 0.4	7.6	2.1 $\pm$ 0.7	1.7	1.1 $\pm$ 0.2	2.9 $\pm$ 0.2	6.7	1.9 $\pm$ 0.3	1.5 $\pm$ 0.2
15:0 iso	31.5	32.8	28.2	22.4	32.3	26.4	58.5	52.2	35.4 $\pm$ 2.9	22.6	34.3 $\pm$ 4.9	29.4	34.6 $\pm$ 2.0	35.0 $\pm$ 0.7	35.4	43.9 $\pm$ 2.0	46.4 $\pm$ 2.2
15:0 iso 3-OH	6.0	5.5	5.7	4.3	2.7	3.2	2.6	1.8	2.5 $\pm$ 0.1	4.5	2.6 $\pm$ 0.2	2.3	2.9 $\pm$ 0.3	2.7 $\pm$ 0.1	4.3	2.8 $\pm$ 0.3	3.0 $\pm$ 0.6
15:0 anteiso	8.8	8.1	8.3	9.6	Tr	4.3	3.2	2.1	Tr	3.0	Tr	5.9	Tr	Tr	0.7	1.1 $\pm$ 0.8	1.0 $\pm$ 0.6
16:0	ND	ND	ND	3.0	1.6	13.5	1.3	1.5	1.3 $\pm$ 0.4	3.5	Tr	1.0	Tr	1.2 $\pm$ 0.2	1.3	Tr	1.2 $\pm$ 0.1
16:0 3-OH	ND	4.8	2.0	ND	1.4	6.7	Tr	Tr	1.1 $\pm$ 0.1	5.7	1.0 $\pm$ 0.2	Tr	1.2 $\pm$ 0.2	1.0 $\pm$ 0.1	2.6	2.6 $\pm$ 0.4	3.0 $\pm$ 0.6
16:0 iso 3-OH	ND	ND	2.5	2.7	Tr	ND	Tr	1.1	ND	ND	ND	1.3	ND	ND	1.4	Tr	Tr
16:1 ω 5c	ND	ND	0.6	3.0	ND	ND	ND	ND	ND	4.5	ND	ND	ND	ND	ND	ND	ND
Unknown 16.580*	ND	ND	ND	ND	1.3	ND	ND	1.0	1.7 $\pm$ 0.1	ND	1.7 $\pm$ 0.2	1.3	1.6 $\pm$ 0.1	1.5 $\pm$ 0.1	1.7	1.6 $\pm$ 0.1	1.3 $\pm$ 0.6
17:0 2-OH	ND	ND	ND	ND	Tr	ND	ND	ND	ND	ND	ND	3.0	ND	Tr	ND	ND	ND
17:0 iso	ND	ND	ND	ND	1.0	ND	2.0	2.3	1.6 $\pm$ 0.6	ND	Tr	Tr	Tr	Tr	0.8	Tr	Tr
17:0 iso 3-OH	18.1	16.3	19.9	19.6	16.8	9.8	14.1	10.9	21.8 $\pm$ 0.3	17.5	19.2 $\pm$ 1.8	14.0	20.1 $\pm$ 1.2	16.3 $\pm$ 0.1	22.4	14.6 $\pm$ 1.0	15.3 $\pm$ 0.2
17:1 iso ω 9c	15.6	10.3	13.7	17.5	27.1	ND	4.8	4.3	20.2 $\pm$ 3.9	1.5	24.2 $\pm$ 3.1	25.6	22.9 $\pm$ 1.9	24.8 $\pm$ 0.4	8.9	7.8 $\pm$ 1.3	6.6 $\pm$ 0.2
18:1 ω 5c	ND	ND	ND	ND	Tr	ND	ND	ND	Tr	ND	Tr	Tr	Tr	Tr	ND	Tr	Tr
Summed feature 4†	7.5	17.3	13.2	17.8	9.2	32.4	8.4	6.5	11.8 $\pm$ 0.8	26.7	11.1 $\pm$ 1.3	11.2	12.1 $\pm$ 0.3	11.5 $\pm$ 0.3	13.8	19.6 $\pm$ 1.0	17.0 $\pm$ 1.3
Summed feature 5†	2.4	ND	ND	ND	Tr	ND	ND	ND	Tr	ND	Tr	ND	Tr	Tr	ND	Tr	ND

*Unknown fatty acid; number indicates equivalent chain length.

†Fatty acids that could not be separated by GC using the Microbial Identification (MIDI; Microbial ID) system: summed feature 4, 15:0 iso 2-OH and/or 16:1 ω 7t; summed feature 5, 17:1 iso I and/or 17:1 anteiso B.

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