Chryseobacterium vrystaatense sp. nov., isolated from raw chicken in a chicken-processing plant

Hanli de Beer,¹ Celia J. Hugo,² Piet J. Jooste,³ Anne Willems,⁴ Marc Vancanneyt,⁵ Tom Coenye⁴ and Peter A. R. Vandamme⁴

Correspondence

Hanli de Beer
hdebeer@cut.ac.za

¹School for Agriculture and Environmental Science, Central University of Technology: Free State, P/bag x20539, 1 Pres. Brandt Street, Bloemfontein 9300, South Africa

²Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, Bloemfontein, South Africa

³Department of Biotechnology and Food Technology, Tshwane University of Technology, Pretoria, South Africa

^{4,5}Laboratorium of Microbiology⁴ and BCCM/LMG Bacteria Collection⁵, Ghent University, K.L. Ledeganckstraat 35, Ghent B-9000, Belgium

Yellow-pigmented, Gram-negative organisms isolated from raw chicken were investigated by means of a polyphasic taxonomic approach and were shown to represent a novel species in the genus *Chryseobacterium*, for which the name *Chryseobacterium vrystaatense* sp. nov. is proposed. Its nearest phylogenetic neighbours were *Chryseobacterium joostei*, *Chryseobacterium indologenes* and *Chryseobacterium gleum*, which showed 16S rRNA gene sequence similarity levels of 96.9, 97.1 and 96.1 %, respectively. Levels of DNA–DNA hybridization between strains of *C. vrystaatense* and *Chryseobacterium* reference species were below 46 %. Strain LMG 22846^T (=CCUG 50970^T) was chosen as the type strain and has a DNA G+C content of 37.1 mol%.

Flavobacteria and pseudomonads are traditionally known to cause spoilage in food and food products (Forsythe, 2000, pp. 96–98 and 101–103). In the literature on meat spoilage, 'flavobacteria' is used as a generic name for yellow-pigmented rods (Hendrie *et al.*, 1969). Usually, pathogens such as *Salmonella* and *Campylobacter* are associated with poultry, but large numbers of other bacteria often associated with spoilage are found on poultry carcasses. These include many so-called flavobacteria that may originate from the poultry itself or from the abattoir environment and which are responsible for spoilage (Hang'ombe *et al.*, 1999). Mai & Conner (2001) found that the incidence of members of the genus *Pseudomonas* and flavobacteria on chicken carcasses was 17 and 16 %, respectively. The incidence of flavobacteria

on poultry is much higher than on other fresh meat (Nychas & Drosinos, 1999).

Over the past decade, many changes have taken place in the taxonomy of the family *Flavobacteriaceae*, and the revised genus *Flavobacterium* comprises mainly aquatic bacteria that are not known in food microbiology (Bernardet *et al.*, 1996). Several former *Flavobacterium* species were transferred to the novel genus *Chryseobacterium* (Vandamme *et al.*, 1994) and novel species have been described, including *Chryseobacterium joostei* (Hugo *et al.*, 2003), *Chryseobacterium defluvii* (Kämpfer *et al.*, 2003), *Chryseobacterium miricola* (Li *et al.*, 2003) and, most recently, *Chryseobacterium formosense* (Young *et al.*, 2005), *Chryseobacterium daecheongense* (Kim *et al.*, 2005a) and *Chryseobacterium taichungense* (Shen *et al.*, 2005). '*Chryseobacterium proteolyticum*' was described by Yamaguchi & Yokoe (2000), but its name has not been validly published. In addition, two *Chryseobacterium* species, *Chryseobacterium meningosepticum* and *Chryseobacterium miricola*, have been reclassified into the novel genus *Elizabethkingia* (Kim *et al.*, 2005b). In general, these *Chryseobacterium* species are widely distributed in water, soil and the clinical environment, but they are also found in food specimens, such as milk, meat, poultry and fish (Jooste & Hugo, 1999).

Published online ahead of print on 3 June 2005 as DOI 10.1099/ijs.0.63746-0.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Chryseobacterium vrystaatense* LMG 22846^T and LMG 22954 are AJ871397 and AJ871398, respectively.

A dendrogram derived from the unweighted pair group mean linkage of correlation coefficients between the whole-cell protein profiles of *C. vrystaatense* and reference strains of related taxa and a table detailing the strains investigated and their origin are available as supplementary material in IJSEM Online.

During a study investigating the incidence of yellow-pigmented strains isolated from meat, 36 yellow-pigmented isolates were obtained from raw chicken, at different stages of processing, from a local chicken-processing plant in the Vrystaat province (Free State, South Africa). Information regarding the investigated isolates, their origin and number is given in the Supplementary Table (available in IJSEM Online). The spoilage ability of this group of strains has not yet been determined. *Chryseobacterium* reference strains were available from the BCCM/LMG Bacteria Collection. After growth for 24 h on trypticase soy agar (BBL), cells were harvested and whole-cell protein extracts were prepared and subjected to whole-cell protein electrophoresis as described by Pot *et al.* (1994). A densitometric analysis, normalization and interpolation of the protein profiles, and a numerical analysis were performed by using the GelCompar software package (Applied Maths). The 36 isolates formed three subgroups comprising 19, four and 13 isolates, respectively. Isolates belonging to the three subgroups differed mainly in the density of some protein bands (see Supplementary Figure in IJSEM Online).

High-molecular-mass DNA was prepared according to the methods of Pitcher *et al.* (1989) from a representative isolate of each of these three subgroups, i.e. isolates LMG 22846^T, LMG 22848 and LMG 22847, and from *Chryseobacterium* reference strains (see below). DNA–DNA hybridizations were performed with photobiotin-labelled probes in microplate wells as described by Ezaki *et al.* (1989), using an HTS7000 Bio Assay Reader (Perkin Elmer) for the fluorescence measurements. The hybridization temperature was 33 °C. Hybridization values obtained among the three

isolates were >92 %, demonstrating that the three subgroups formed a homogeneous genospecies.

In order to determine the phylogenetic position of this taxon, the 16S rRNA gene sequences of strains LMG 22846^T and LMG 22954 were determined as described by Willems *et al.* (2003). The 16S rRNA gene sequences of strains LMG 22846^T and LMG 22954 showed 99.9 % similarity to each other and 96.9, 97.1 and 97.1 % similarity to those of *C. joostei*, *Chryseobacterium indologenes* and *Chryseobacterium gleum*, respectively, demonstrating that the taxon represented by these two strains belonged to the genus *Chryseobacterium*. Values towards the other type strains investigated were <96 %. Fig. 1 illustrates the phylogenetic position of this taxon within the genus *Chryseobacterium*. Subsequent DNA–DNA hybridizations between the type strains of the latter three species yielded a value of 46 % between strain LMG 22846^T and *C. joostei* LMG 18212^T; the other values were between 27 and 32 %.

In order to determine its G + C content, DNA was degraded enzymically into nucleosides as described by Mesbah *et al.* (1989). The nucleoside mixture obtained was then separated by HPLC using a Waters SymmetryShield C8 column regulated at 37 °C. The solvent was 0.02 M NH₄H₂PO₄, pH 4.0, with 1.5 % acetonitrile. Non-methylated lambda phage DNA (Sigma) was used as the calibration reference. The detector used was a Waters model 484 UV-VIS absorbance detector set at 270 nm. The DNA G + C contents of strains LMG 22846^T, LMG 22847 and LMG 22848 were 37.1, 37.0 and 37.6 mol%, respectively, within the range of 34–38 mol% reported for the genus *Chryseobacterium* (Vandamme *et al.*, 1994).

Fig. 1. Neighbour-joining phylogenetic tree of *Chryseobacterium vrystaatense* sp. nov. and related bacteria based on 16S rRNA gene sequence comparisons. Bootstrap values above 60 % obtained from 1000 repetitions are indicated. The 16S rRNA gene sequence of *Ornithobacterium rhinotracheale* LMG 9086^T was included as outgroup.

Table 1. Long-chain fatty acid composition of *Chryseobacterium* species and related taxa

Taxa: 1, *C. vrystaatense* sp. nov. ($n=7$); 2, *C. indoltheticum* ($n=1$); 3, *C. balustinum* ($n=1$); 4, *C. gleum* ($n=5$); 5, *C. indologenes* ($n=45$); 6, *C. scophthalmum* ($n=2$); 7, *C. joostei* ($n=11$); 8, *C. defluvii* ($n=1$); 9, *C. formosense* ($n=1$); 10, *C. daecheongense* ($n=1$); 11, *C. taichungense* ($n=1$); 12, *Elizabethkingia meningoseptica* ($n=1$); 13, *Elizabethkingia miricola* ($n=1$). Fatty acid percentages amounting to $<1.0\%$ of the total fatty acids in all strains were not included. tr, Trace (for values $<1\%$); NA, not available; ND, not detected; ECL, equivalent chain length (i.e. the identity of the fatty acids is unknown). Means $\pm$ SD are given for *C. vrystaatense* only. Data are taken from Hugo *et al.* (2003) (2–7, 12), Li *et al.* (2003) (13), Young *et al.* (2005) (9), Kim *et al.* (2005a) (10) and Shen *et al.* (2005) (11).

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13
Iso-C _{13:0}	1.1 $\pm$ 0.4	ND	tr	tr	tr	tr	tr	2.8	3.6	1.6	tr	1.4	tr
ECL 13:566	1.4 $\pm$ 0.3	1.7	1.6	1.2	2.1	2.9	1.1	tr	tr	1.5	6.7	1.5	tr
Anteiso-C _{15:0}	1.7 $\pm$ 0.7	5.9	tr	tr	tr	tr	tr	3.2	2.1	1.0	tr	2.3	tr
Iso-C _{15:0}	41.8 $\pm$ 1.4	29.4	32.3	35.4	34.3	35.0	34.6	58.5	52.2	51.2	35.4	41.4	33.0
Iso-C _{15:0} 3-OH	2.7 $\pm$ 0.3	2.3	2.7	2.5	2.6	2.7	2.9	2.6	1.8	2.0	4.3	3.5	3.0
C _{16:0}	1.1 $\pm$ 0.3	1.0	1.6	1.3	tr	1.2	tr	1.3	1.5	1.8	1.3	tr	2.0
C _{16:0} 3-OH	1.3 $\pm$ 0.3	tr	1.4	1.1	1.0	1.0	1.2	tr	tr	tr	2.6	2.2	tr
Iso-C _{16:0} 3-OH	tr	1.3	tr	ND	ND	ND	ND	tr	1.1	tr	1.4	tr	8.0
ECL 16:580	1.2 $\pm$ 0.2	1.3	1.3	1.7	1.7	1.5	1.6	tr	1.0	1.0	1.7	1.7	tr
Iso-C _{17:0}	tr	tr	1.0	1.6	tr	tr	tr	2.0	2.3	3.0	tr	tr	1.0
Iso-C _{17:0} 3-OH	15.4 $\pm$ 1.8	14.0	16.8	21.8	19.2	16.3	20.1	14.1	10.9	15.7	22.4	16.3	40.0
Iso-C _{17:1} ω 9c	19.7 $\pm$ 2.3	25.6	27.1	20.2	24.2	24.8	22.9	4.8	4.3	7.6	8.9	7.0	tr
C _{18:1} ω 5c	tr	tr	tr	tr	tr	tr	tr	NA	ND	ND	ND	tr	NA
Summed feature 3*	9.1 $\pm$ 0.9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Summed feature 4*	tr	11.2	9.2	11.8	11.1	11.5	12.1	8.4	6.5	8.4	13.8	18.1	12
Summed feature 5*	NA	ND	tr	tr	tr	ND	tr	NA	ND	ND	ND	tr	NA

*Summed feature 3 comprises C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH; summed feature 4 comprises iso-C_{15:0} 2-OH and C_{16:1} ω 7c; summed feature 5 comprises iso-C_{17:1} I and/or anteiso-C_{17:1} B.

The whole-cell fatty acid components of seven strains representing the three subgroups and of *Chryseobacterium* reference strains were prepared from a loopful of well-grown cells incubated for 24 h at 28 °C. Separation and identification of esters were performed with the Sherlock Microbial Identification system (MIDI version 3.0) as described by Vandamme *et al.* (1992). Mean percentages and SD were calculated and are shown in Table 1. As for other *Chryseobacterium* species, the predominant fatty acids were iso-C_{15:0} (40–43 %), iso-C_{17:1} ω 9c (16–21 %) and iso-C_{17:0} 3-OH (15–18 %).

A detailed phenotypic characterization of the 36 isolates and of *Chryseobacterium* reference species was subsequently performed. A 24 h culture, incubated at 25 °C, was suspended in a quarter-strength Ringer solution to give a McFarland Barium Sulphate Standard 2 suspension. A battery of tests was selected to differentiate species in the genus at 25 °C according to the methods described by Cowan (1974), MacFaddin (1980), Gerhardt *et al.* (1981) and Hugo *et al.* (2003). Twenty-two isolates were subjected to Biolog GN2 MicroPlate (Biolog Inc.) tests according to the manufacturer's protocol. Test results are given below in the species description and in Table 2, which includes the biochemical tests useful for the differentiation of the novel species from recognized *Chryseobacterium* species.

The results of the present study demonstrate that the collection of 36 yellow-pigmented isolates from raw chicken represent a novel species in the genus *Chryseobacterium*, for which we propose the name *Chryseobacterium vrystaatense* sp. nov.

Description of *Chryseobacterium vrystaatense* sp. nov.

Chryseobacterium vrystaatense (vry.staa.ten'se. N.L. neut. adj. *vrystaatense* named after Vrystaat (Free State), the South African province where these bacteria were isolated).

Colonies are shiny, orange–yellow and translucent with entire edges. Cells are Gram-negative, catalase- and oxidase-positive and produce flexirubin-type pigments; they are non-motile and neither oxidative nor fermentative. Colonies are seen after 24 h growth at 4, 15, 25 and 32 °C on nutrient agar, but no growth occurs at 37 °C or above. Growth on MacConkey agar no. 3 (Oxoid CM115) is observed after 48 h at 15, 25 and 32 °C for all but one strain that grows only on MacConkey agar after 48 h at 32 °C. All strains grow in nutrient broth containing 1 and 2 % NaCl (w/v); growth in 3 % NaCl broth is strain-dependent (14 strains, excluding the type strain, test positive for growth). Although several strains initially show growth at different salt concentrations, only five show growth in 4 % NaCl

Table 2. Phenotypic properties that differentiate *C. vrystaatense* sp. nov. from other *Chryseobacterium* species and related taxa

Taxa: 1, *C. vrystaatense* (n=36); 2, *C. indoltheticum* (n=1); 3, *C. balustinum* (n=1); 4, *C. gleum* (n=1); 5, *C. indologenes* (n=1); 6, *C. scopthalmum* (n=1); 7, *C. joostei* (n=1); 8, *C. defluvii* (n=1); 9, *C. formosense* (n=1); 10, *C. daecheongense* (n=1); 11, *C. taichungense* (n=1); 12, *Elizabethkingia meningoseptica* (n=1); 13, *Elizabethkingia miricola* (n=1). Data are taken from Li *et al.* (2003) (13), Young *et al.* (2005) (9), Kim *et al.* (2005a) (10) and Shen *et al.* (2005) (11). +, Positive; w, weakly positive; –, negative; D, delayed; NA, not available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	
Growth on:														
Cetrimide agar	+	*	–	–	–	+	–	+	–	NA	NA	NA	+	+
MacConkey agar	–	†	+	+	+	+	–	+	–	–	–	–	+	+
Growth on nutrient agar at/in:														
5 °C	+	+	+	+	–	–	+	+	–	–	–	–	–	NA
37 °C	–	–	+	+	+	–	–	+	–	+	+	+	+	–
42 °C	–	–	–	–	+	–	–	+	–	–	–	–	+	NA
3 % NaCl	–	W	W	+	+	W	W	–	NA	NA	NA	W	NA	NA
Enzyme activity:														
Urease	+	–	–	–	–	+	+	–	–	–	–	–	D	+
Lecithinase	+	+	+	+	+	+	+	+	NA	NA	NA	–	+	–
Nitrate reduction	–	–	+	+	–	–	–	–	–	+	NA	–	–	–
Nitrite reduction	+	–	–	+	–	–	–	–	–	–	NA	+	–	–
Aesculin hydrolysis	+	+	+	+	+	+	+	+	+	+	NA	+	+	+
Starch hydrolysis	–	–	–	+	+	–	+	W	NA	+	NA	–	–	–
Tween 80 hydrolysis	+	+	+	+	+	+	+	NA	–	–	NA	–	+	+
Tyrosine hydrolysis	–	D	–	+	–	+	+	+	NA	NA	NA	D	–	–
Production of:														
Hydrogen sulfide	23‡	+	–	–	–	–	–	–	–	–	–	–	–	+
Indole	+	+	+	+	+	–	W	W	+	–	W	D	+	–

Variable reactions are scored as: *positive for all strains except R-23519, R-23533 and R-23598; †the majority of strains were negative, although some grew after 48 h at 25 °C. ‡Number of strains that tested positive, including the type strain.

broth after 5 days incubation (R-23493, R-23569, R-23575, R-23576 and R-23595). Strains show strong DNase activity and also urease and lecithinase activity, but no starch or tyrosine hydrolysis. Reactions on triple-sugar agar (Oxoid CM277) and 10 % lactose are alkaline. Ability to produce H₂S varies depending on incubation time on triple-sugar agar and SIM medium (Oxoid CM435). Both media contain sodium thiosulfate, but SIM medium also contains ferrous ammonium sulfate and >50 % of strains give a positive reaction. All strains tested (n=22) show positive reactions in the Biolog system for Tweens 40 and 80, gentobiose, α-D-glucose, D-mannose, D-trehalose, succinic acid monomethyl ester, acetic acid and L-asparagine. At least 19 of the strains (85 %) react for glycyl L-glutamic acid, glycerol, L-glutamic acid, L-serine and L-aspartic acid. Reactions in which more

than 50 % of the strains give a positive result are for dextrin (n=16), D-mannitol (n=18), α-ketovaleric acid (n=15), L-alanylglycine (n=12), L-threonine (n=16), inosine (n=13), uridine (n=15) and thymidine (n=12).

The type strain, LMG 22846^T (=CCUG 50970^T), was isolated from a raw chicken-portion sample obtained from a chicken-processing plant. The other (35) isolates were obtained from raw-chicken samples, collected at different processing stages (portion, whole bird, automatic line, manual line and the spiral freezing process) on different occasions. Three additional isolates have been deposited in the BCCM/LMG Bacteria Collection as LMG 22847, LMG 22848 and LMG 22954.

Acknowledgements

This project was funded by the National Research Foundation, South Africa. We are indebted to the Fund for Scientific Research – Flanders (Belgium) for positions as postdoctoral fellows (T.C. and A.W.) and research grants (P.A.R.V.).

References

Bernardet, J.-F., Segers, P., Vancanneyt, M., Berthe, F., Kersters, K. & Vandamme, P. (1996). Cutting a Gordian knot: emended classification and description of the genus *Flavobacterium*, emended description of the family *Flavobacteriaceae*, and proposal of *Flavobacterium hydatidis* nom. nov. (basonym, *Cytophaga aquatilis* Strohl and Tait 1978). *Int J Syst Bacteriol* **46**, 128–148.

Cowan, S. T. (1974). *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 2nd edn. Cambridge: Cambridge University Press.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

Forsythe, S. J. (2000). *The Microbiology of Safe Food*. London: Blackwell Science.

Gerhardt, P., Murray, R. G. E., Costilow, R. N., Nester, E. W., Wood, W. A., Krieg, N. R. & Phillips, G. B. (1981). *Manual of Methods for General Bacteriology*. Washington, DC: American Society for Microbiology.

Hang'ombe, B. M., Sharma, N. R., Skjerve, E. & Tuchili, L. M. (1999). Isolation of bacteria during processing of chicken carcasses for the market in Lusaka, Zambia. *Veterinarski Arhiv* **69**, 191–197.

Hendrie, M. S., Mitchell, T. G. & Shewan, J. M. (1969). The identification of yellow pigmented rods. In *Identification Methods for Microbiologists*, part B, pp. 67–78. Edited by B. M. Gibbs & D. A. Shapton. London: Academic Press.

Hugo, C. J., Segers, P., Hoste, B., Vancanneyt, M. & Kersters, K. (2003). *Chryseobacterium joostei* sp. nov., isolated from the dairy environment. *Int J Syst Evol Microbiol* **53**, 771–777.

Jooste, P. J. & Hugo, C. J. (1999). The taxonomy, ecology and cultivation of bacterial genera belonging to the family *Flavobacteriaceae*. *Int J Food Microbiol* **53**, 81–94.

Kämpfer, P., Dreyer, U., Neef, A., Dott, W. & Busse, H.-J. (2003). *Chryseobacterium defluvii* sp. nov., isolated from wastewater. *Int J Syst Evol Microbiol* **53**, 93–97.

- Kim, K. K., Bae, H.-S., Schumann, P. & Lee, S.-T. (2005a). *Chryseobacterium daecheongense* sp. nov., isolated from freshwater lake sediment. *Int J Syst Evol Microbiol* **55**, 133–138.
- Kim, K. K., Kim, M.-K., Lim, J. H., Park, H. Y. & Lee, S.-T. (2005b). Transfer of *Chryseobacterium meningosepticum* and *Chryseobacterium miricola* to *Elizabethkingia* gen. nov. as *Elizabethkingia meningoseptica* comb. nov. and *Elizabethkingia miricola* comb. nov. *Int J Syst Evol Microbiol* **55**, 1287–1293.
- Li, Y., Kawamura, Y., Fujiwara, N., Naka, T., Liu, H., Huang, X., Kobayashi, K. & Ezaki, T. (2003). *Chryseobacterium miricola* sp. nov., a novel species isolated from condensation water of space station Mir. *Syst Appl Microbiol* **26**, 523–528.
- MacFaddin, J. F. (1980). *Biochemical Tests for Identification of Medical Bacteria*, 2nd edn. Baltimore: Williams & Wilkins.
- Mai, T. & Conner, D. (2001). Identification of bacteria found in broiler deboning operations. *J Dairy Sci* **84** (Suppl. 1), 297.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Nychas, G.-J. E. & Drosinos, E. H. (1999). Meat and poultry spoilage. In *Encyclopedia of Food Microbiology*, pp. 1253–1259. Edited by R. K. Robinson, C. A. Batt & P. D. Patel. San Diego: Academic Press.
- Pitcher, D. G., Saunders, N. A. & Owen, R. J. (1989). Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* **8**, 151–156.
- Pot, B., Vandamme, P. & Kersters, K. (1994). Analysis of electrophoretic whole-organism protein fingerprints. In *Chemical Methods in Prokaryotic Systematics*, pp. 494–521. Edited by M. Goodfellow & A. G. O'Donnell. Chichester: Wiley.
- Shen, F.-T., Kämpfer, P., Young, C.-C., Lai, W.-A. & Arun, A. B. (2005). *Chryseobacterium taichungense* sp. nov., isolated from contaminated soil. *Int J Syst Evol Microbiol* **55**, 1301–1304.
- Vandamme, P., Vancanneyt, M., Pot, B. & 10 other authors (1992). Polyphasic taxonomic study of the emended genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. *Int J Syst Bacteriol* **42**, 344–356.
- Vandamme, P., Bernardet, J.-F., Segers, P., Kersters, K. & Holmes, B. (1994). New perspectives in the classification of the flavobacteria: description of *Chryseobacterium* gen. nov., *Bergeyella* gen. nov., and *Empedobacter* nom. rev. *Int J Syst Bacteriol* **44**, 827–831.
- Willems, A., Fernández-López, M., Muñoz-Adelantado, E., Goris, J., De Vos, P., Martínez-Romero, E., Toro, N. & Gillis, M. (2003). Description of new *Ensifer* strains from nodules and proposal to transfer *Ensifer adhaerens* Casida 1982 to *Sinorhizobium* as *Sinorhizobium adhaerens* comb. nov. Request for an Opinion. *Int J Syst Evol Microbiol* **53**, 1207–1217.
- Yamaguchi, S. & Yokoe, M. (2000). A novel protein-deamidating enzyme from *Chryseobacterium proteolyticum* sp. nov., a newly isolated bacterium from soil. *Appl Environ Microbiol* **66**, 3337–3343.
- Young, C.-C., Kämpfer, P., Shen, F.-T., Lai, W.-A. & Arun, A. B. (2005). *Chryseobacterium formosense* sp. nov., isolated from the rhizosphere of *Lactuca sativa* L. (garden lettuce). *Int J Syst Evol Microbiol* **55**, 423–426.