Transfer of *Pseudomonas flectens* Johnson 1956 to *Phaseolibacter* gen. nov., in the family *Enterobacteriaceae*, as *Phaseolibacter flectens* gen. nov., comb. nov.

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*Pseudomonas flectens* Johnson 1956, a plant-pathogenic bacterium on the pods of the French bean, is no longer considered to be a member of the genus *Pseudomonas sensu stricto*. A polyphasic approach that included examination of phenotypic properties and phylogenetic analyses based on 16S rRNA, *rpoB* and *atpD* gene sequences supported the transfer of *Pseudomonas flectens* Johnson 1956 to a new genus in the family *Enterobacteriaceae* as *Phaseolibacter flectens* gen. nov., comb. nov. Two strains of *Phaseolibacter flectens* were studied (ATCC 12775T and LMG 2186); the strains shared 99.8 % sequence similarity in their 16S rRNA genes and the housekeeping gene sequences were identical. Strains of *Phaseolibacter flectens* shared 96.6 % or less 16S rRNA gene sequence similarity with members of different genera in the family *Enterobacteriaceae* and only 84.7 % sequence similarity with *Pseudomonas aeruginosa* LMG 1242T, demonstrating that they are not related to the genus *Pseudomonas*. As *Phaseolibacter flectens* formed an independent phyletic lineage in all of the phylogenetic analyses, it could not be affiliated to any of the recognized genera within the family *Enterobacteriaceae* and therefore was assigned to a new genus. Cells were Gram-negative, straight rods, motile by means of one or two polar flagella, fermentative, facultative anaerobes, oxidase-negative and catalase-positive. Growth occurred in the presence of 0–60 % sucrose. The DNA G+C content of the type strain was 44.3 mol%. On the basis of phenotypic properties and phylogenetic distinctiveness, *Pseudomonas flectens* Johnson 1956 is transferred to the novel genus *Phaseolibacter* gen. nov. as *Phaseolibacter flectens* gen. nov., comb. nov. The type strain of *Phaseolibacter flectens* is ATCC 12775T = CFBP 3281T = ICMP 745T = LMG 2187T = NCPPB 539T.

*Pseudomonas flectens* Johnson 1956 was first isolated from *Phaseolus vulgaris* and identified in Australia. It was described as a plant-pathogenic bacterium on pods of French bean (Johnson, 1956). The type strain ATCC 12775T was isolated in 1956 (Johnson, 1956) and another strain, LMG 2186, was isolated in 1957 (De Vos *et al.*, 1985).

*Pseudomonas flectens* is no longer considered to be a member of the genus *Pseudomonas sensu stricto* (Kersters *et al.*, 1996; Young *et al.*, 1978; Euzéby, 1997). De Vos *et al.* (1985) studied the rRNA cistron sequence similarities of phytopathogenic *Pseudomonas* species; *Pseudomonas flectens* exhibited no relationship with any of the rRNA branches, indicating its uncertain taxonomic status. De Vos *et al.* (1985) concluded that *Pseudomonas flectens* should be removed from the genus *Pseudomonas*. Anzai *et al.* (2000) demonstrated that *Pseudomonas flectens* is included in the cluster of the family *Enterobacteriaceae*. Here, we reclassify *Pseudomonas flectens* Johnson 1956 within a novel genus *Phaseolibacter* in the family *Enterobacteriaceae* as *Phaseolibacter flectens* gen. nov., comb. nov.

The family *Enterobacteriaceae* is a large, heterogeneous group of Gram-negative, facultatively anaerobic, rod-shaped
bacteria, most of which are catalase-positive and oxidase-negative (Brenner & Farmer, 2005). Members of this family play critical roles not only in enteric disease of humans and animals but also as phytopathogens, as insect pathogens and in industrial processes (Janda, 2006).

A polyphasic approach that included the study of phenotypic properties and phylogenetic analysis was used to re-examine the phylogenetic position of two *Pseudomonas flectens* strains (ATCC 12775\textsuperscript{T} and LMG 2186). Sequences of the 16S rRNA gene as well as the housekeeping genes *rpoB* and *atpD* were determined to analyse the phylogenetic position of these strains.

The sequence of the 16S rRNA gene of the type strain (ATCC 12775\textsuperscript{T}) has already been deposited by Anzai et al. (2000) under the accession number AB021400. We confirmed this sequence and determined the 16S rRNA gene sequence of strain LMG 2186. The universal bacterial primers 8f and 1512r (Felske et al., 1997) were used to amplify internal fragments of the 16S rRNA gene. Purified PCR products were sequenced with primers 8f, 534r, 968f and 1512r as described in detail by Raats & Halpern (2007), resulting in sequences of approximately 1500 bp. Identification of phylogenetic neighbours was carried out initially by using the BLAST (Altschul et al., 2007), resulting in sequences of approximately 1500 bp. Sequence alignment was performed using the CLUSTAL W program, and a phylogenetic tree (Fig. 1) was generated using the neighbour-joining method in the MEGA 4.1 version of the EzTaxon server (http://www.eztaxon.org/; Chun et al., 2007). Sequence alignment was performed using the CLUSTAL W program, and a phylogenetic tree (Fig. 1) was generated using the neighbour-joining and maximum-parsimony methods in the MEGA 4.1 version software (Tamura et al., 2007) (Fig. 1). As high congruence was noted between the tree-drawing methods, only the neighbour-joining trees are shown.

*Pseudomonas flectens* ATCC 12775\textsuperscript{T} and strain LMG 2186 shared 99.8 % sequence similarity in their 16S rRNA gene sequences. *Pseudomonas flectens* ATCC 12775\textsuperscript{T} shared the highest sequence similarity with the type strains of with *Pantoea anthophila* (96.6 %), *Pantoea vagans* (96.5 %) and *Tatumella morbirosei* (96.5 %), and shared less than 96.4 % similarity with type strains of species from other genera in the family Enterobacteriaceae (Fig. 1). Only 84.7 % sequence similarity was found between the 16S rRNA gene sequences of *Pseudomonas flectens* ATCC 12775\textsuperscript{T} and *P. aeruginosa* LMG 1242\textsuperscript{T}. This demonstrates that *Pseudomonas flectens* is not related to the genus *Pseudomonas* (Fig. 1).

Species of different genera of the family Enterobacteriaceae share 97.2 % or less 16S rRNA gene sequence similarity. For example, the type strain of *Mangrovibacter plantisponsor* (Rameshkumar et al., 2010) shares 97.2 % 16S rRNA gene sequence similarity with *Cronobacter mcytjensei* E603\textsuperscript{T} and 97.1 % similarity with *Enterobacter cloacae* subsp. dissolvens LMG 2683\textsuperscript{T} and *Enterobacter radicicinats* D5/23\textsuperscript{T}. Moreover, 98 % 16S rRNA gene sequence similarity and 94 % *rpoB* sequence similarity appeared reasonable cut-off values to describe *Klebsiella* and *Raoultella* as different genera (Drancourt et al., 2001). Thus, *Pseudomonas flectens* ATCC 12775\textsuperscript{T} should be reclassified within a novel genus of the family Enterobacteriaceae.

To support the phylogenetic position of strains ATCC 12775\textsuperscript{T} and LMG 2186 further as members of a novel genus, their phylogenetic position was analysed based on partial *rpoB* and *atpD* gene sequences (Figs S1 and S2, available in IJSEM Online). Amplification of these genes was performed according to Brady et al. (2008) and Dahllöf et al. (2000).

For *rpoB*, it has been shown that species from different genera share 91.0 % or less gene sequence similarity (Rameshkumar et al., 2010). Sequence similarity with the type strain of *Phaseolibacter flectens* (LMG 22050\textsuperscript{T}) was used as an outgroup. Bootstrap values (>50 %) resulting from 1000 replicates are indicated at branching nodes. Asterisks indicate branches of the tree that were also formed by using the maximum-parsimony method. Bar, 0.005 substitutions per nucleotide position.

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**Fig. 1.** Neighbour-joining tree based on 16S rRNA gene sequences, showing the nearest neighbours of *Phaseolibacter flectens* gen. nov., comb. nov. The tree shows a clear separation of *Phaseolibacter flectens* from other genera of the family Enterobacteriaceae. *Pseudomonas aeruginosa* LMG 1242\textsuperscript{T} was used as an outgroup. Bootstrap values (>50 %) resulting from 1000 replicates are indicated at branching nodes. Asterisks indicate branches of the tree that were also formed by using the maximum-parsimony method. Bar, 0.005 substitutions per nucleotide position.
Strains ATCC 12775<sup>T</sup> and LMG 2186 shared 100% rpoB gene sequence similarity, and they shared 82.6% or less rpoB gene sequence similarity with members of different genera in the family Enterobacteriaceae, demonstrating that they belong to a novel genus (Fig. S1).

Strains ATCC 12775<sup>T</sup> and LMG 2186 shared 100% atpD gene sequence similarity, and showed less than 85.1% similarity to members of other genera in the family Enterobacteriaceae, again demonstrating that they belong to a novel genus (Fig. S2).

The neighbour-joining and maximum-parsimony trees based on 16S rRNA, rpoB and atpD gene sequences (Figs 1, S1 and S2) showed that strains ATCC 12775<sup>T</sup> and LMG 2186 clustered together but not with any species of other genera in the family Enterobacteriaceae. As strains ATCC 12775<sup>T</sup> and LMG 2186 shared very high sequence similarities (99.8–100%) in their 16S rRNA, rpoB and atpD genes (Figs 1, S1 and S2) and showed almost identical biochemical characteristics (see below; Table 1), they clearly belong to the same species.

For electron microscopy, bacteria from R2A (Himedia) agar plates and from R2A agar plates supplemented with 10% sucrose were resuspended in saline. The samples were fixed to a carbon-coated grid and stained with 2% uranyl acetate and photographed under a JEM-1200EX electron microscope (JEOL).

For cellular fatty acid analysis, cells were cultured on tryptic soy agar (Difco) for 24 h at 28 °C (exponential phase) and the fatty acids were extracted and methylated (Ben-Ze’ev et al., 2005). Fatty acid methyl esters were analysed by gas chromatography using the MIDI/Hewlett Packard Microbial Identification System (Analytical Services Inc.) and the results were analysed using the Sherlock database, version 3.10.

For determination of DNA G+C content, genomic DNA of strains ATCC 12775<sup>T</sup> and LMG 2186 was extracted according to a modified version of the procedure of Wilson (1987). The G+C content of the DNA sample was determined in three independent analyses using the HPLC technique (Mesbah et al., 1989) by the BCCM/LMG Bacteria Collection Identification Service (Laboratory of Microbiology, Ghent University, Ghent, Belgium).

For phenotypic characterization, LB agar (Himedia) was used as the basal growth medium. Growth at 4, 10, 18, 20, 28, 30, 35, 37, 40, 44 and 50 °C was measured using LB agar supplemented with 10% sucrose. The ability to grow in the presence of sucrose was measured on LB supplemented with 0–60% sucrose. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on LB agar supplemented with 10% sucrose or with 0.5% glucose. Biochemical tests were performed by using the API 20E and 50CH identification systems (bioMérieux), according to the manufacturer’s instructions except that the tests were incubated at 28 °C for 2 days. Catalase activity was shown by bubble production in a 3% (v/v) hydrogen peroxide solution. Oxidase activity was determined using 1% N,N,N’,N’-tetramethyl p-phenylenediamine dihydrochloride (Sigma-Aldrich). Growth was tested on MacConkey agar.

Phenotypic traits of the two isolates and representative members of other genera from the family Enterobacteriaceae are given in the species description and in Table 1.

Being Gram-negative, facultatively anaerobic, oxidase-negative, catalase-positive, glucose-fermenting, chemoheterotrophic rods, the strains share characteristics typical of the family Enterobacteriaceae. Phylogenetic trees based on 16S rRNA, rpoB and atpD gene sequences also support the fact that strains ATCC 12775<sup>T</sup> and LMG 2186 are members of the family Enterobacteriaceae (Figs 1, S1 and S2) and not of the genus Pseudomonas [as was already suggested by Anzai et al. (2000)]. They can be distinguished from species of the genera Tatumella and Pantoaea, which are their closest relatives phylogenetically, by their colony colour (Phaseolibacter colonies are greyish white, while those of Tatumella are yellow and those of Pantoaea are yellow, beige or non-pigmented), motility, the presence of gelatinase, acid production from mannitol, their ability to grow at 41 °C and to reduce nitrate to nitrogen and their major fatty acids (Table 1). The results of this study support the transfer of Pseudomonas flectens to a new genus, Phaseolibacter gen. nov., in the family Enterobacteriaceae, as Phaseolibacter flectens gen. nov., comb. nov.

**Description of Phaseolibacter gen. nov.**

*Phaseolibacter* [Pha.se.o’li.bac’ter. L. n. *phaseolus* a kind of bean with an edible pod and also a botanical genus name; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Phaseolibacter* a rod isolated from French bean (*Phaseolus vulgaris* L.).]

Cells are Gram-negative, straight rods. Motile by means of one or two polar flagella. Fermentative, facultative anaerobes. Oxidase-negative and catalase-positive. The following results are obtained for the type species from API 20E strips after 48 h of incubation at 28 °C: D-glucose, sucrose and melibiose are fermented, acetoin is produced, H₂S and indole are not produced, gelatin and urea are not hydrolysed and nitrate is reduced to nitrogen. The major fatty acids are C₁₆:₀ summed feature 2 (one or more of C₁₄:₀ 3-OH, iso-C₁₆:₁ 1 and unknown ECL 10.928) and summed feature 3 (C₁₆:₀10c and/or iso-C₁₅:₀ 2-OH). The genus-specific signature regions in the 16S rRNA gene are 5′-AGGCGTGAGTGTTAATATCATCTTGGC and 5′-ACC-GAGCAGATGCTTTGG (the positions of the 5′ nucleotides in the 16S rRNA gene of *Escherichia coli* are 451 and 1004, respectively). The genus belongs to the family Enterobacteriaceae and comprises one species of plant origin. The type species is *Phaseolibacter flectens* (formerly *Pseudomonas flectens*), originally identified from French bean by Johnson (1956).

**Description of Phaseolibacter flectens** *(Johnson 1956)* comb. nov.

Table 1. Biochemical differentiation of *Phaseolibacter flectens* gen. nov., comb. nov. and other members of the family *Enterobacteriaceae*

<table>
<thead>
<tr>
<th>Characteristic</th>
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Acid from:

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- Mannitol
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  - v(-)
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- Sorbitol
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  - -
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- Sucrose
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- Inositol
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Growth at 41 °C

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Motility

- +
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Major fatty acids:

- C16:0
  - +
  - +
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- C14:1^T
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- C17:0 cyclo
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- C18:1^T
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Summed feature 3

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Summed feature 7

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*At 25 °C but not at 36 °C (according to Hollis et al., 1981).
†Data from API 20E tests.
‡Data for the type species of *Phaseolibacter* and *Pantoea* (data from the present study and from Mergaert et al., 1993), the type strain of *Enterobacter radicicinans* (Kämpfer et al., 2005) and *Tatumella citrea* LMG 22049^T^ (data from the present study).
§Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI System. Summed feature 2 contains one or more of C14:0 3-OH, iso-C16:1 I and unknown ECL 10.928; summed feature 3 contains C16:1^T^ and/or iso-C15:0 2-OH; summed feature 7 contains one or more of C18:1ω7c, C18:1ω9t and C18:1ω7c.

Displays the following properties in addition to those described for the genus. Cells are 0.5–0.8 μm wide and 1.2–2.3 μm long. Motility is observed only when the cells are grown on media without sucrose. When grown on LB or R2A agar for 48 h, colonies are 1 mm in diameter; however, when grown on the same media supplemented with sucrose, colonies are 3–5 mm in diameter, smooth, foggy and greyish white in colour and produce huge amounts of mucus. Growth is observed under anaerobic conditions. Grows at 4–44 °C (optimum 28–30 °C) and with 0–60 % sucrose (optimum 10–25 %). Grows on MacConkey agar. The following results are obtained from API 20E strips after 48 h of incubation at 28 °C: L-arabinose fermentation is variable (negative for the type strain), D-mannitol, inositol, sorbitol, rhamnose and amygdalin are not fermented; acetoin is produced.
(Voges–Proskauer), gelatin and urea are not hydrolysed, citrate utilization is variable (positive for the type strain), H₂S and indole are not produced, tryptophan deaminase activity is present and β-galactosidase, arginine dihydrolase and lysine and ornithine decarboxylase activities are absent. In API 50CH strips incubated for 48 h at 28 °C, acid production occurs for glycerol, L-arabinose (weak), D-ribose (weak), D-xylene (weak), D-galactose (variable, negative for the type strain), D-glucose, D-fructose, aesculin, melibiose (variable, negative for the type strain), sucrose and D-fucose (weak). Acid production does not occur for erythritol, D-arabinose, L-xylose, D-adonitol, methyl β-D-xlyopyranoside, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, glycogen, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, L-fucose, D- and L-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate. Minor fatty acids are unknown ECL 13.957, C₁₇:0 cyclo, C₁₈:1ω7c, C₁₂:0 2-ОH and C₁₄:0 2-ОH.

The type strain is ATCC 12775ᵀ = CFBP 3281ᵀ = LMG 2187ᵀ = NCPPB 539ᵀ, isolated from French bean. Strain LMG 2186 (=ICMP 744 = NCPPB 538) is a reference strain. The DNA G+C contents of strains ATCC 12775ᵀ and LMG 2186 are 44.3 and 43.9 mol% respectively.

Acknowledgements

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References


