Brevibacterium pityocampae sp. nov., isolated from caterpillars of *Thaumetopoea pityocampa* (Lepidoptera, Thaumetopoeidae)

Hatice Katı,[1] İkbal Agah İnce,[1,2]‡ İsmail Demir[2] and Zihni Demirbağ[2]

[1]Giresun University, Faculty of Arts and Sciences, Department of Biology, 28049 Giresun, Turkey

[2]Karadeniz Technical University, Faculty of Arts and Sciences, Department of Biology, 61080 Trabzon, Turkey

Correspondence
İkbal Agah İnce
agah.ince@wur.nl

This work deals with the taxonomic study of a bacterium, strain Tp12^T, isolated from caterpillars of the pine processionary moth (*Thaumetopoea pityocampa* Denis & Schiffermüller, 1775; Lepidoptera, Thaumetopoeidae). The isolate was assigned to the genus *Brevibacterium* on the basis of a polyphasic taxonomic study, including morphological and biochemical characteristics, 16S rRNA gene sequence analysis, fatty acid analysis and DNA G+C content. The highest 16S rRNA gene sequence similarity to this isolate was approximately 96 %, with the type strains of *Brevibacterium album* and *Brevibacterium samyangense*. Cellular fatty acids of the isolate are of the branched type, with the major components being anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$. The DNA G+C content was 69.8 mol%. Although the strain was related to *B. album* and *B. samyangense* according to 16S rRNA gene sequence analysis, it differed from any known species of *Brevibacterium*. Based on this evidence, the novel species *Brevibacterium pityocampae* sp. nov. is proposed, with strain Tp12^T (=DSM 21720^T =NCCB 100255^T) as the type strain.

The genus *Brevibacterium* was proposed by Breed (1957). While several Gram-positive, non-spore-forming, non-branching rods were classified as members of the genus *Brevibacterium*, further chemotaxonomic studies showed that not all of these species are members of the genus. Subsequently, these species have been classified in different coryneform genera (Collins, 1992) and the description of the genus was emended to include only those species that correspond to the type species, *Brevibacterium linens* (Collins *et al.*, 1980). In addition to *B. linens*, 40 species with validly published names were assigned to the genus *Brevibacterium* at the time of writing (http://www.bacterio.cict.fr/b/brevibacterium.html).

Brevibacteria have been isolated from diverse habitats such as milk products (Kollöffel *et al.*, 1999), clinical specimens (Wauters *et al.*, 2001), human body parts (McBride *et al.*, 1993; Wauters *et al.*, 2003, 2004), soil (Tang *et al.*, 2008), sediment (Gavrish *et al.*, 2004; Lee, 2006; Bhadra *et al.*, 2008), brown algae (Ivanova *et al.*, 2004), paintings (Heyrman *et al.*, 2004), poultry (Pascual & Collins, 1999) and marine environments (Lee, 2008). In the present study, strain Tp12^T, a novel member of the genus *Brevibacterium*, was isolated from larvae of the pine processionary moth (*Thaumetopoea pityocampa* Denis & Schiffermüller, 1775; Lepidoptera, Thaumetopoeidae). While many bacterial strains have been isolated from insects (Demir *et al.*, 2002; Sezen *et al.*, 2004, 2005, 2007; Yılmaz *et al.*, 2006; Bahar & Demirbağ, 2007), to our knowledge, the present report is the first time that a brevibacterium has been isolated from an insect.

T. pityocampa is one of the most harmful pests of pine species in Mediterranean countries including Turkey (İnce *et al.*, 2007). *T. pityocampa* nests were collected from Scots pine (*Pinus sylvestris* L.) from the Middle Black Sea region of Turkey while screening the bacterial flora of the pest (İnce *et al.*, 2008). Based on colony morphology and the colour of bacterial specimens removed from *T. pityocampa* larvae, 14 different isolates (Tp1–Tp14) were determined as members of the bacterial flora of the larvae (İnce *et al.*, 2008). Taxonomic identification of these isolates was finalized by a polyphasic taxonomic approach. One of the isolates obtained, strain Tp12^T, was clearly distinguished from the other isolates on the basis of some morphological, physiological and biochemical characteristics. Based on the current polyphasic study, strain Tp12^T represents a novel species of the genus *Brevibacterium*.

After macroscopic examination, dead, diseased and healthy larvae were distinguished and were sterilized in 70 %

‡Present address: Laboratory of Virology, Wageningen University and Research Center, 6700 AP Wageningen, The Netherlands.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Tp12^T is EU484189.

A comparison of the fatty acid profile of strain Tp12^T and related type strains is available as supplementary material with the online version of this paper.

ethanol for 1.5–2 min to remove external contaminants and then washed twice with sterile distilled water (Lipa & Wiland, 1972; Poinar & Thomas, 1978; Osborn *et al.*, 2002). Using standard aseptic techniques, the contents of surface-sterilized insect larvae were collected with a sterile injector and suspended in 5 ml PBS (pH 7.4). Serial dilutions of the suspension were spread on nutrient agar (Case & Johnson, 1992) and the plates were incubated at 30 °C for 48–72 h. At the end of the incubation period, discrete bacterial colonies were removed aseptically by using an inoculation loop, restreaked on nutrient agar and incubated aerobically for 24–48 h. According to the colour and morphology of the colonies, isolates were separated and pure cultures were prepared from colonies. The isolates were identified by morphological, physiological, biochemical and molecular methods according to Palleroni (1986) and Kandler & Weiss (1986). Finally, a selected colony of isolate Tp12^T was purified by subculturing on plates and the bacterium was identified by various conventional and molecular tests.

Tryptic soy agar (TSA) was used for determination of phenotypic and physiological characteristics of strain Tp12^T. Colony and cell morphology were determined by direct and stereomicroscopic observations of single colonies grown for 24, 48, 72 and 120 h. Gram staining was performed according to the procedure described by Claus (1992) and confirmed by the KOH string test (Murray *et al.*, 1999). The KOH test result was compared with the positive control *Pseudomonas aeruginosa* ATCC 27853 and the negative control *Staphylococcus aureus* ATCC 25923. Cell motility was examined by the presence of turbidity throughout tubes of semi-solid medium (Leifson, 1960) and by light microscopy (Olympus model CX21).

Growth of strain Tp12^T was tested at 25, 30, 40, 45 and 50 °C. The optimal growth temperature was 30 °C. Salt tolerance was tested by using the following NaCl concentrations (w/v): 0, 3, 5, 10, 15 and 18 %. The pH range for growth was investigated between pH 4.0 and 10.0 at intervals of 0.5 pH units. The results were monitored after 72 h incubation at 30 °C. Catalase activity was determined by the production of bubbles after the addition of 10 µl 3 % (v/v) H_2O_2 onto single colonies. Oxidase activity was observed by oxidation of *N*,*N*,*N*′,*N*′-tetramethyl *p*-phenylenediamine with oxidase discs containing *N*,*N*-dimethyl *p*-phenylenediamine oxalate and α-naphthol (Fluka). A well-isolated colony was spread with an inoculating loop onto an oxidase disc. The reaction was observed within 2 min at 25–30 °C and compared with positive control *P. aeruginosa* ATCC 27853 and negative control *Escherichia coli* ATCC 25922.

Some physiological properties were tested by using API 20E strips (bioMérieux). Results were scored after 5 days at 30 °C. Differential phenotypic, biochemical and physiological properties of strain Tp12^T and related *Brevibacterium* type strains are given in Table 1. To determine the major fatty acids, fatty acid methyl esters were extracted from strain Tp12^T and analysed by gas chromatography (Hewlett Packard 5890 II plus) and the Sherlock Microbial Identification System using version 4.10 of the TSBA40 library (Microbial ID). These analyses were kindly performed by Fikrettin Şahin (Yeditepe University, Istanbul, Turkey).

Table 1. Differential phenotypic characteristics of strain Tp12^T and its close phylogenetic neighbours

Strains: 1, strain Tp12^T; 2, *B. album* YIM 90718^T (data from Tang *et al.*, 2008); 3, *B. samyangense* SST-8^T (Lee, 2006). +, Positive; W, weakly positive; −, negative; ND, no data available. All three strains grow at 40 °C, form circular colonies and test negative for urease and oxidase and positive for catalase.

Characteristic	1	2	3
Colony colour	Yellow	White	Yellow
Motility	−	+	+
Growth at:			
25 °C	+	ND	+
30 °C	+	ND	+
45 °C	−	+	+
pH for growth			
Range	6–10	6–8	6.1–10.1
Optimum	8	ND	ND
NaCl tolerance (%, w/v)	10	10	10*
Fermentation of:			
D-Glucose	−	−	W
Sucrose	−	−	W
Melibiose	−	ND	ND
Utilization of citrate	−	ND	ND
Nitrate reduction	+	−	−
Gelatin hydrolysis	−	+	−
DNA G+C content (mol%)	69.8	70.7	70
Source of isolation	Insect	Saline soil	Beach sediment

*Weak growth at 15 % (w/v) NaCl.

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were done as described by İnce *et al.* (2008). DNA sequencing was performed at Macrogen Inc. (Seoul, Korea). A total of 1402 nt of the 16S rRNA gene sequence from strain Tp12^T were determined. 16S rRNA gene sequence comparisons with entries in the updated GenBank and EMBL databases were performed with the FASTA and BLAST programs (Pearson, 1990; Altschul *et al.*, 1990, 1997). Sequence alignments were performed with the program CLUSTAL W (EMBL European Bioinformatics Institute; http://www.ebi.ac.uk) and edited with the GeneDoc software (Nicholas *et al.*, 1997).

Evolutionary relationships of closely related members of *Brevibacterium* were inferred using three tree-making algorithms: the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. The relationships among taxa were coherent in all tree-making methods

used. A phylogenetic tree was constructed using the neighbour-joining method (Fig. 1). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Evolutionary distances were computed using the Jukes–Cantor method (Jukes & Cantor, 1969) and are in units of the number of base substitutions per site. The bootstrap consensus tree is taken to represent the evolutionary history of the taxa analysed (Felsenstein, 1985). Percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). There were a total of 778 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura *et al.*, 2007).

The cellular fatty acids of strain Tp12^T were predominantly of the branched type, with anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$ accounting for more than 90 % of the total cellular fatty acids. Other fatty acids included iso-$C_{15:0}$ (4.8 %) and iso-$C_{16:0}$ (2.8 %). A comparison of the cellular fatty acid contents of strain Tp12^T, *Brevibacterium album* YIM 90718^T and *Brevibacterium samyangense* SST-8^T is shown in Supplementary Table S1, available in IJSEM Online.

According to the comparison of 16S rRNA gene sequences, the closest relatives of strain Tp12^T were *B. album* YIM 90718^T and *B. samyangense* SST-8^T, both showing 96 % similarity. Levels of similarity between strain Tp12^T and the other recognized *Brevibacterium* species were lower than 96 %. In light of previous studies performed by Stackebrandt & Goebel (1994) and Lee (2006), the 16S rRNA gene sequence comparisons indicate that the isolate can be assigned to a separate genospecies without the need for DNA–DNA hybridization experiments. Since the primary structure of the 16S rRNA gene is easier to determine than hybridization between DNA strands, the strength of the sequence analysis is to recognize the level at which DNA pairing studies need to be performed, which certainly applies to similarities of 97 % and higher (Stackebrandt & Goebel, 1994). A tree depicting the phylogenetic position of strain Tp12^T within the genus *Brevibacterium* is shown in Fig. 1. Based on 16S rRNA gene sequence comparison, strain Tp12^T forms a distinct subclade with *B. samyangense* SST-8^T.

Genomic DNA of strain Tp12^T was prepared for the determination of the G+C content. The DNA base composition of strain Tp12^T was determined by HPLC and calculated according to the method of Mesbah *et al.* (1989) by the Deutsche Sammlung von Mikroorganismen und Zellkulturen as 69.8 mol% G+C, which is high in comparison with most other brevibacteria (Gruner *et al.*, 1994). However, Lee (2006) indicated that the DNA G+C contents of the type strains of *B. samyangense* and *B. album* are 70.7 and 70.0 mol%, respectively, and the DNA G+C content of the type strain of *Brevibacterium marinum* was reported to be 71.4 mol% (Lee, 2008).

Strain Tp12^T can also be discriminated from *B. samyangense* (Lee, 2006) and *B. album* (Tang *et al.*, 2008) according to some biochemical and physiological properties. Significantly, strain Tp12^T cannot grow at 45 °C, while *B. samyangense* and *B. album* can. While *B. samyangense* grows weakly in 15 % NaCl, strain Tp12^T cannot grow at that concentration. Additionally, fermentation tests showed other differences between strain Tp12^T and *B. samyangense* and *B. album* isolates (Table 1).

In spite of the similarity to *B. samyangense* and *B. album*, strain Tp12^T can be readily differentiated from known species of the genus *Brevibacterium* with reference to some physiological and biochemical characteristics, cellular fatty acids, 16S rRNA gene sequence and DNA G+C content. On the basis of the data obtained with our polyphasic taxonomic approach, strain Tp12^T merits recognition as a member of a novel species of the genus *Brevibacterium*, for which we propose the name *Brevibacterium pityocampae* sp. nov.

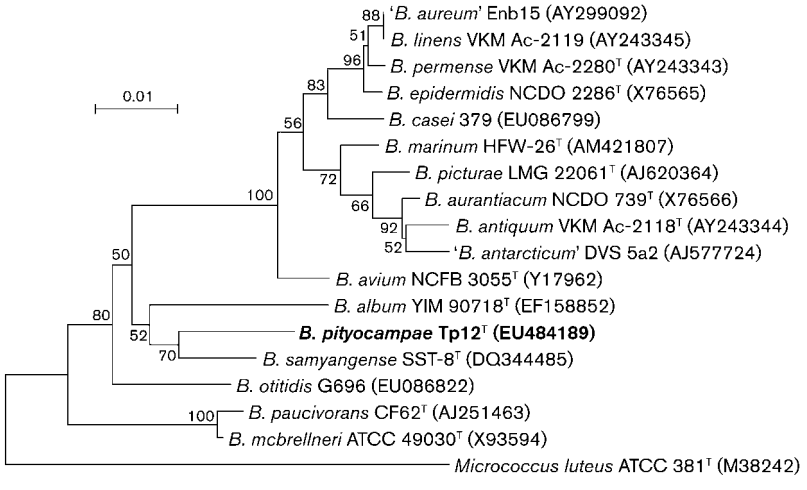

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of *Brevibacterium pityocampae* sp. nov. Tp12^T within its phylogenetic neighbours in the genus *Brevibacterium*. Evolutionary distances for tree construction were calculated using the Jukes–Cantor coefficient. Numbers at nodes indicate percentages of bootstrap support based on a neighbour-joining analysis of 1000 iterated datasets. *Micrococcus luteus* ATCC 381^T was defined as the outgroup. Bar, 1 substitution per 100 nucleotide positions.

Description of *Brevibacterium pityocampae* sp. nov.

Brevibacterium pityocampae (pi.ty.o.cam′pae. L. fem. n. *pityocampa* a pine grub; L. fem. gen. n. *pityocampae* of a pine grub, referring to the isolation of the type strain from larvae of *Thaumetopoea pityocampa*).

Cells stain Gram-positive and are non-motile, non-spore-forming rods, 1.0 μm long and 0.5 μm wide. Catalase-positive and oxidase-negative. Colonies are yellow, circular and convex with entire margins. Growth is observed at 25–40 °C, pH 6–10 (optimum pH 8) and 0–10 % NaCl (w/v). No growth in 15 % NaCl (w/v). Both the API 20E system and conventional tests show that arginine dihydrolase and acetoin production are negative. Nitrate is reduced to nitrite. Tests for *β*-galactosidase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, urease, tryptophan deaminase, indole production and gelatinase and fermentation of D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, sucrose, melibiose, amygdalin and L-arabinose are negative. The fatty acid profile contains predominantly anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$. The G+C content of the type strain is 69.8 mol%.

The type strain is Tp12^T (=DSM 21720^T =NCCB 100255^T), isolated from healthy larvae of *Thaumetopoea pityocampa* from the Middle Black Sea region of Turkey.

Acknowledgements

This work was supported by the T. R. Prime Ministry State Planning Organization (21.111.004.1). The authors would like to thank Professor Dr Fikrettin Şahin (Yeditepe University, Istanbul, Turkey) for his help with the fatty acid methyl ester study.

References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z. & Miller, W. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.

Bahar, A. A. & Demirbağ, Z. (2007). Isolation of pathogenic bacteria from *Oberea linearis* (Coleoptera: Cerambycidae). *Biologia* **62**, 13–18.

Bhadra, B., Raghukumar, C., Pindi, P. K. & Shivaji, S. (2008). *Brevibacterium oceani* sp. nov., isolated from deep-sea sediment of the Chagos Trench, Indian Ocean. *Int J Syst Evol Microbiol* **58**, 57–60.

Breed, R. S. (1957). Family IX. *Brevibacteriaceae* Breed, 1953. In *Bergey's Manual of Determinative Bacteriology*, 7th edn, pp. 490–505. Edited by R. S. Breed, E. G. D. Murray & N. R. Smith. London: Baillière, Tindall & Cox.

Case, C. L. & Johnson, T. R. (1992). *Laboratory Experiments in Microbiology*, 3rd edn. Menlo Park, CA: Benjamin/Cummings.

Claus, M. (1992). A standardized Gram staining procedure. *World J Microbiol Biotechnol* **8**, 451–452.

Collins, M. D. (1992). The genus *Brevibacterium*. In *The Prokaryotes*, 2nd edn, vol. 2, pp. 1351–1354. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.

Collins, M. D., Jones, D. R., Keddie, M. & Sneath, P. H. A. (1980). Reclassification of *Chromobacterium iodinum* (Davis) in a redefined genus *Brevibacterium* (Breed) as *Brevibacterium iodinum* nom. rev.; comb. nov. *J Gen Microbiol* **120**, 1–10.

Demir, İ., Sezen, K. & Demirbağ, Z. (2002). The first study on bacterial flora and biological control agent of *Anoplus roboris* (Sufr., Coleoptera). *J Microbiol* **40**, 104–108.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Gavrish, E. Y., Krauzova, V. I., Potekhina, N. V., Karasev, S. G., Plotnikova, E. G., Altyntseva, O. V., Korosteleva, L. A. & Evtushenko, L. I. (2004). Three new species of brevibacteria, *Brevibacterium antiquum* sp. nov., *Brevibacterium aurantiacum* sp. nov., and *Brevibacterium permense* sp. nov. *Mikrobiologiia* **73**, 218–215 (in Russian).

Gruner, E., Steigerwalt, A. G., Hollis, D. G., Weyant, R. S., Weaver, R. E., Moss, C. W., Daneshvar, M., Brown, J. M. & Brenner, D. J. (1994). Human infections caused by *Brevibacterium casei*, formerly CDC groups B-1 and B-3. *J Clin Microbiol* **32**, 1511–1518.

Heyrman, J., Verbeeren, J., Schumann, P., Devos, J., Swings, J. & De Vos, P. (2004). *Brevibacterium picturae* sp. nov., isolated from a damaged mural painting at the Saint-Catherine chapel (Castle Herberstein, Austria). *Int J Syst Evol Microbiol* **54**, 1537–1541.

İnce, İ. A., Demir, İ., Demirbağ, Z. & Nalcacıoglu, R. (2007). A cytoplasmic polyhedrosis virus isolated from the pine processionary caterpillar, *Thaumetopoea pityocampa*. *J Microbiol Biotechnol* **17**, 632–637.

İnce, İ. A., Katı, H., Yılmaz, H., Demir, İ. & Demirbağ, Z. (2008). Isolation and identification of bacteria from *Thaumetopoea pityocampa* Den. and Schiff. (Lep., Thaumetopoeidae) and determination of their biocontrol potential. *World J Microbiol Biotechnol* **24**, 3005–3015.

Ivanova, E. P., Christen, R., Alexeeva, Y. V., Zhukova, N. V., Gorshkova, N. M., Lysenko, A. M., Mikhailov, V. V. & Nicolau, D. V. (2004). *Brevibacterium celere* sp. nov., isolated from degraded thallus of a brown alga. *Int J Syst Evol Microbiol* **54**, 2107–2111.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Kandler, O. & Weiss, N. (1986). Regular, nonsporing Gram-positive rods. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1208–1209. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.

Kollöffel, B., Meile, L. & Teuber, M. (1999). Analysis of brevibacteria on the surface of Gruyère cheese detected by *in situ* hybridization and by colony hybridization. *Lett Appl Microbiol* **29**, 317–322.

Lee, S. D. (2006). *Brevibacterium samyangense* sp. nov., an actinomycete isolated from a beach sediment. *Int J Syst Evol Microbiol* **56**, 1889–1892.

Lee, S. D. (2008). *Brevibacterium marinum* sp. nov., isolated from seawater. *Int J Syst Evol Microbiol* **58**, 500–504.

Leifson, E. (1960). *Atlas of Bacterial Flagellation*. London: Academic Press.

Lipa, J. J. & Wiland, E. (1972). Bacteria isolated from cutworms and their infectivity to *Agrotis* spp. (Lepidoptera, Noctuidae). *Acta Microbiol Pol B* **4**, 127–140.

McBride, M. E., Ellner, K. M., Black, H. S., Clarridge, J. E. & Wolf, J. E. (1993). A new *Brevibacterium* sp. isolated from infected genital hair of patients with white piedra. *J Med Microbiol* **39**, 255–261.

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.

Murray, P. R., Baron, E. J., Pfaller, M. A., Tenover, F. C. & Yolken, R. H. (editors) (1999). *Manual of Clinical Microbiology*, 7th edn. Washington, DC: American Society for Microbiology.

Nicholas, K. B., Nicholas, H. B. & Deerfield, D. W. (1997). GeneDoc: analysis and visualization of genetic variation. *EMBnet News* **4**, 1–4.

Osborn, F., Berlioz, L., Vitelli-Flores, J., Monsalve, W., Dorta, B. & Lemoine, V. R. (2002). Pathogenic effects of bacteria isolated from larvae of *Hylesia metabus* Crammer (Lepidoptera: Saturniidae). *J Invertebr Pathol* **80**, 7–12.

Palleroni, N. J. (1986). Gram-negative aerobic rods and cocci. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, p. 140. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.

Pascual, C. & Collins, M. D. (1999). *Brevibacterium avium* sp. nov., isolated from poultry. *Int J Syst Bacteriol* **49**, 1527–1530.

Pearson, W. R. (1990). Rapid and sensitive sequence comparison with FASTP and FASTA. *Methods Enzymol* **183**, 63–98.

Poinar, G. O., Jr & Thomas, G. M. (1978). *Diagnostic Manual for the Identification of Insect Pathogens*. New York & London: Plenum.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sezen, K., Demir, İ. & Demirbağ, Z. (2004). Studies of the bacterial flora as a biological control agent of the *Agelastica alni* L. (Coleoptera: Chrysomelidae). *Biologia* **59**, 327–331.

Sezen, K., Demir, İ., Katı, H. & Demirbağ, Z. (2005). Investigations on the bacteria as potential biological control agent of summer chafer, *Amphimallon solstitiale* L. (Coleoptera: Scarabaeidae). *J Microbiol* **43**, 463–468.

Sezen, K., Demir, İ. & Demirbağ, Z. (2007). Identification and pathogenicity of entomopathogenic bacteria from common cockchafer, *Melolontha melolontha* (Coleoptera: Scarabaeidae). *N Z J Crop Hort Sci* **35**, 79–85.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Tang, S.-K., Wang, Y., Schumann, P., Stackebrandt, E., Lou, K., Jiang, C.-L., Xu, L.-H. & Li, W.-J. (2008). *Brevibacterium album* sp. nov., a novel actinobacterium isolated from a saline soil in China. *Int J Syst Evol Microbiol* **58**, 574–577.

Wauters, G., Charlier, J., Janssens, M. & Delmée, M. (2001). *Brevibacterium paucivorans* sp. nov., from human clinical specimens. *Int J Syst Evol Microbiol* **51**, 1703–1707.

Wauters, G., Avesani, V., Laffineur, K., Charlier, J., Janssens, M., Van Bosterhaut, B. & Delmée, M. (2003). *Brevibacterium lutescens* sp. nov., from human and environmental samples. *Int J Syst Evol Microbiol* **53**, 1321–1325.

Wauters, G., Haase, G., Avesani, V., Charlier, J., Janssens, M., Van Broeck, J. & Delmée, M. (2004). Identification of a novel *Brevibacterium* species isolate from humans and description of *Brevibacterium sanguinis* sp. nov. *J Clin Microbiol* **42**, 2829–2832.

Yılmaz, H., Sezen, K., Katı, H. & Demirbağ, Z. (2006). The first study on the bacterial flora of the European spruce bark beetle, *Dendroctonus micans* (Coleoptera: Scolytidae). *Biologia* **61**, 679–686.