

Streptomyces africanus sp. nov., a novel streptomycete with blue aerial mycelium

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An actinomycete with blue aerial mycelium and yellow substrate mycelium was isolated from a suburban soil sample collected in Cape Town, South Africa and named strain CPJVR-H^T. The colour of the substrate mycelium was not sensitive to changes in pH. The organism produced spiny spores in *Spirales* spore chains. Chemical taxonomy indicated that it is a member of the genus *Streptomyces*. Strain CPJVR-H^T grew at 45 °C and did not produce melanin or any diffusible pigments. It exhibited weak antibacterial activity against a clinical isolate of *Enterococcus faecium*, but no antibacterial activity against *Escherichia coli* ATCC 25922 or *Pseudomonas aeruginosa* ATCC 27853. Analysis of its 16S rRNA gene sequence, DNA–DNA hybridization studies and the results of physiological tests showed that this strain represents a novel species of *Streptomyces*, for which the name *Streptomyces africanus* sp. nov. is proposed. The type strain is CPJVR-H^T (=NRRL B-24143^T = DSM 41829^T).

Streptomyces species are abundant in terrestrial environments and are easy to isolate on simple laboratory media. Strains are readily differentiated by differences in the colours of their aerial and substrate mycelia on isolation plates. Many soil streptomycetes also exhibit weak to moderate inhibition of growth of Gram-positive bacteria, making them very well suited for study in short undergraduate microbiology projects. Fifty to a hundred isolates can be tested for antibiotic activity in such a project; of these isolates, the five with the greatest antibiotic activity can be subjected to preliminary physiological characterization. Any isolates that appear to be particularly interesting (e.g. having uncommon aerial or substrate mycelium colours) can be assessed further as candidates for novel species.

Strain CPJVR-H^T was isolated by C. E. Price and J. M. van Rooyen from suburban garden soil in Cape Town, South Africa in September 2001 as part of their 3rd year BSc project on actinomycetes.

The standard morphological and physiological tests recommended for characterizing *Streptomyces* species were carried out as described by Williams *et al.* (1989). International

Streptomyces Project (ISP) media were prepared as described by Shirling & Gottlieb (1966). Antibiotic resistance was determined by incorporation of antibiotics into Bennett's medium agar plates (Atlas, 1993) at the concentrations recommended and not by using antibiotic-impregnated filter discs. Non-standard test antibiotics were tested at the following concentrations: capreomycin (20 µg ml⁻¹), carbenicillin (100 µg ml⁻¹), cefotaxime (100 µg ml⁻¹), chloramphenicol (50 µg ml⁻¹), D-cycloserine (50 µg ml⁻¹), erythromycin (50 µg ml⁻¹), kanamycin (10 µg ml⁻¹), nalidixic acid (25 µg ml⁻¹) and spectinomycin (20 µg ml⁻¹). Antimicrobial activity was determined using 5-day-old colonies of strain CPJVR-H^T grown on nutrient agar (Williams *et al.*, 1989).

Physiological tests were carried out at 28 °C (unless otherwise indicated) and were read after the recommended incubation periods. All carbon sources for carbon utilization tests were filter-sterilized. *meso*-Erythritol, glycerol, maltose, methyl α -D-glucoside, (-)-D-ribose and (-)-L-sorbose were tested as sole carbon sources at a concentration of 1% (w/v). Sodium benzoate, sodium butyrate, sodium formate, sodium DL-malate, sodium maleate, sodium oxalate, sodium salicylate, sodium succinate and sodium (+)-L-tartrate were tested as sole carbon sources at a concentration of 0.1% (w/v). DL-Ornithine and 4-aminobutyric acid were tested as sole nitrogen sources at a concentration of 0.1% (w/v).

Determination of the diaminopimelic acid (DAP) isomer and the whole-cell sugar pattern was as described by Hasegawa *et al.* (1983) with the exception that dried cells

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Abbreviations: DAP, diaminopimelic acid; ISP, International *Streptomyces* Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Streptomyces africanus* CPJVR-H^T is AY208912.

were used instead of colonies from agar plates. Fatty acid methyl esters were prepared by the method of Luquin *et al.* (1991). The base composition of the genomic DNA of strain CPJVR-H^T was determined in 0.1 × SSC according to the method of Mandel & Marmur (1968).

The 16S rRNA gene of strain CPJVR-H^T was amplified by PCR using universal bacterial 16S rRNA gene primers. The forward primer, F1 (5'-AGAGTTTGATCITGGCTCAG-3'; I, inosine), and reverse primer, R6 (5'-AAGGAGGTGITC-CAICC-3'), were modified from primers fd1 of Weisburg *et al.* (1991) and p1525r of Chun & Goodfellow (1995), respectively. The 16S rRNA gene was sequenced using a MegaBACE 500 automated capillary DNA sequencing system (Molecular Dynamics) and a DYEnamic ET Dye Terminator Cycle Sequencing kit for MegaBACE; a 1488 bp sequence was determined. DNA relatedness studies were conducted in 2 × SSC supplemented with 10% (v/v) formamide at 71 °C by the identification service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) using the spectrophotometric method of De Ley *et al.* (1970), as modified by Escara & Hutton (1980) and Huß *et al.* (1983).

Strain CPJVR-H^T was Gram-positive, catalase-positive and did not grow under anaerobic conditions. Light microscopy showed a branched mycelium with *Spirales*-type spore chains and no verticils. Scanning electron microscopy revealed *Spirales*-type spore chains with spiny spore sheaths. The spiny ornaments were generally straight and very long, but with a sharp curve at the tip (Fig. 1).

Chemotaxonomic tests showed that the cell wall was of type I (i.e. containing LL-DAP); no diagnostic sugars were detected in whole-cell hydrolysates. Fatty acid analysis showed that strain CPJVR-H^T contained a high proportion

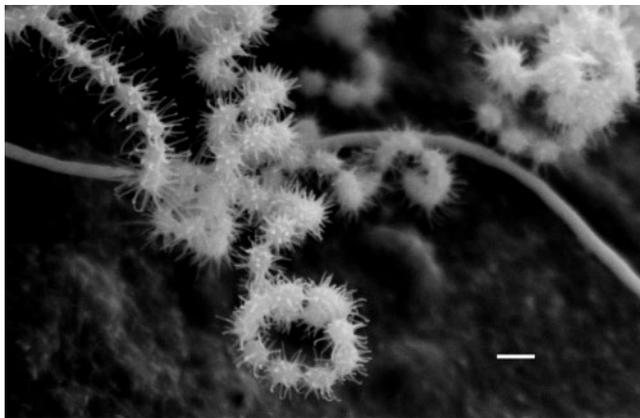


Fig. 1. Scanning electron micrograph of strain CPJVR-H^T, grown on inorganic salts/starch agar (ISP medium no. 4) at 28–30 °C for 14 days, showing *Spirales*-type spore chains. The spiny spore-sheath ornaments are very long with curved tips in some cases. Bar, 1 µm.

of saturated straight-chain, iso and anteiso fatty acids: i-14:0 (4.1%), i-15:0 (20.9%), ai-15:0 (23.2%), i-16:0 (22.4%), 16:0 (4.6%), i-17:0 (5.0%) and ai-17:0 (8.3%). The G+C content of the genomic DNA was 73.2 mol%.

Melanin was not produced on peptone/yeast extract/iron agar (ISP medium no. 6) or on tyrosine agar (ISP medium no. 7). No diffusible pigments were recorded after growth on glycerol/asparagine agar (ISP medium no. 5).

A 1488 bp 16S rRNA gene sequence was determined for strain CPJVR-H^T. A BLAST search (Altschul *et al.*, 1997) of the GenBank database using this sequence showed that it was 99% similar to the 16S rRNA gene sequence of '*Streptomyces steffisburgensis*' strain JCM 4833 (GenBank accession no. AB045889) over 1485 bases and 99% similar to the 16S rRNA gene sequence of *Streptomyces afghaniensis* strain NRRL-ISP 5228^T (GenBank accession no. AJ399483) over 1434 bases. A high degree of similarity (98–99% over 1450–1490 bases) was also recorded to 16S rRNA gene sequences of other members of the *Streptomyces cyaneus* species group. A phylogenetic tree of *Streptomyces* 16S rRNA gene sequences was constructed using the neighbour-joining method of Saitou & Nei (1987) with CLUSTAL W (version 1.81) and MEGA (version 2.1; Kumar *et al.*, 2001) (Fig. 2). This tree shows the close phylogenetic association of strain CPJVR-H^T with certain members of the *S. cyaneus* species group (namely *S. afghaniensis*, *Streptomyces caelestis* and *Streptomyces fumanus*).

All members of the *S. cyaneus* species group produce *Spirales* (sometimes *Retinaculiaperti*) spore chains of spiny or smooth spores. All strains produce melanin on peptone/yeast extract/iron agar (Williams *et al.*, 1989). *S. afghaniensis* is one of seven allied species of *S. cyaneus* that produce spiny, red spores in *Spirales* or *Retinaculiaperti* spore chains (Williams *et al.*, 1989). Strain CPJVR-H^T produces spiny, blue spores and does not produce melanin.

Thus, despite the high 16S rRNA gene sequence similarity between CPJVR-H^T and members of the *S. cyaneus* species group, morphological and physiological characteristics of CPJVR-H^T indicate that it is not a strain of *S. cyaneus*. DNA–DNA hybridization studies confirmed that strain CPJVR-H^T is unique. The DNA relatedness between strain CPJVR-H^T and *S. afghaniensis* NRRL B-5621^T (=NRRL-ISP 5228^T) is 46.2 ± 0.9% and that between strain CPJVR-H^T and '*S. steffisburgensis*' strain NRRL ISP 5547 (=JCM 4833) is 38.4 ± 0.5%.

Comparison of the characteristics of strain CPJVR-H^T with other streptomycetes that produce blue aerial mycelia shows that it also differs from these species. These differences are discussed below.

Streptomyces amakusaensis produces smooth, blue spores in *Spirales* spore chains and a pH-sensitive, yellow–brown substrate mycelium. This species does not grow at 45 °C

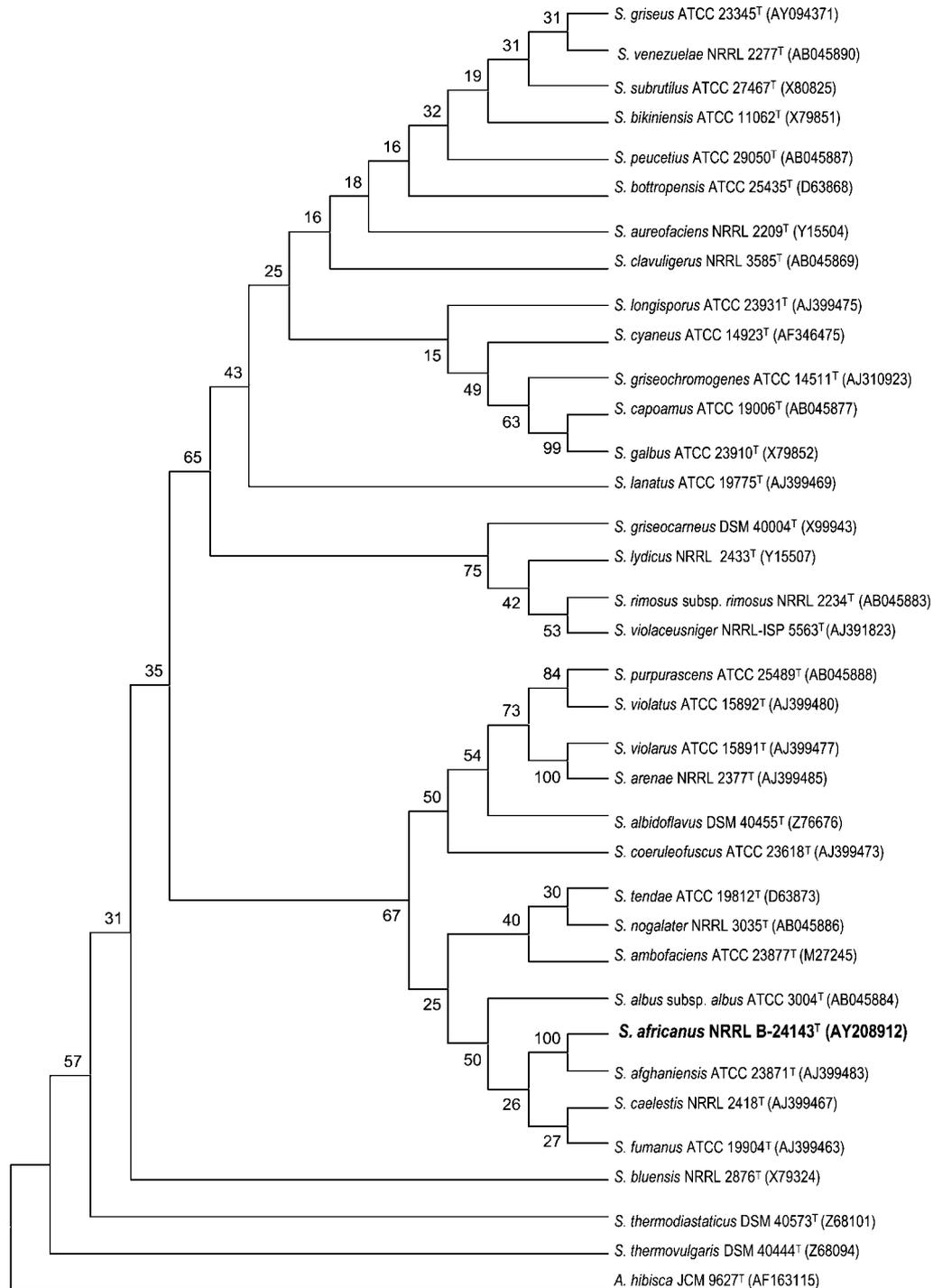


Fig. 2. Unrooted phylogenetic tree constructed from *Streptomyces* 16S rRNA gene sequences showing the relationship between *Streptomyces africanus* NRRL B-24143^T and recognized species of *Streptomyces* belonging to the major, minor and single-member clusters defined by Williams *et al.* (1983). A 16S rRNA gene sequence of *Actinomadura hibisca* strain JCM 9627^T was used as an outgroup. All sequences were edited to produce the longest sequence region common to all sequences (1440 bp). GenBank sequence accession numbers are indicated in parentheses after the strain names. The tree was generated using the neighbour-joining method (CLUSTAL W version 1.81 and MEGA version 2.1) and includes the percentage bootstrap values generated (based on analysis of 1000 resampled datasets).

and is sensitive to penicillin G (10 IU ml⁻¹). It is unable to grow in the presence of 7 % NaCl or 0.1 % phenol. Sucrose and (+)-L-rhamnose are not used as sole carbon sources. L-Histidine cannot be used as the sole nitrogen source.

Streptomyces glaucescens produces a red–orange substrate mycelium and red–orange diffusible pigments. Melanin is produced. Proteolytic activity is absent. It is able to grow in the presence of rifampicin (50 µg ml⁻¹), but is sensitive to penicillin G (10 IU ml⁻¹) and cannot grow in the presence of 7 % NaCl or 0.1 % phenol. It cannot use *meso*-inositol, raffinose or (+)-D-melibiose as sole carbon sources (Williams *et al.*, 1989).

Streptomyces inusitatus produces smooth, blue–grey to grey–blue spores in *Spirales* spore chains and a colourless or pale-brown substrate mycelium. It cannot use (+)-L-arabinose, (–)-D-fructose, *meso*-inositol, D-mannitol, raffinose, (+)-L-rhamnose, salicin, sucrose or (+)-D-xylose as sole carbon source. *Streptomyces ipomoeae* produces smooth, blue spores in *Spirales* spore chains and a pale yellow or greyish-yellow substrate mycelium. Faint yellow or green diffusible pigments are produced. *Streptomyces lomondensis* produces warty to spiny, blue spores in *Rectiflexibiles*, *Retinaculiaperti* or *Spirales* spore chains. The substrate mycelium is brick-red, rust-brown or straw-coloured and a brown or pink diffusible pigment is produced. Melanin is also produced. Casein is not degraded and salicin cannot be used as the sole carbon source. *Streptomyces viridochromogenes* produces blue, spiny spores in *Spirales* spore chains and a pH-sensitive, green substrate mycelium. Green, pH-sensitive diffusible pigments are produced. This species produces melanin and is able to grow in the presence of rifampicin (50 µg ml⁻¹) and 0.01 % sodium azide, but is sensitive to penicillin G (10 IU ml⁻¹) and cannot grow in the presence of 7 % NaCl. It cannot use sucrose as sole carbon source or L-phenylalanine as sole nitrogen source (Williams *et al.*, 1989).

These results support classification of strain CPJVR-H^T as a representative of a novel species of *Streptomyces*, for which the name *Streptomyces africanus* sp. nov. is proposed. Additional phenetic characteristics of the strain are presented under the species description.

Description of *Streptomyces africanus* sp. nov.

Streptomyces africanus (af.ri.ca'nus. L. masc. adj. *africanus* of Africa).

Aerobic, Gram-positive, catalase-positive actinomycete that forms a blue aerial mycelium and a yellow substrate mycelium. The colour of the substrate mycelium is not pH-sensitive. Verticils are not present. The mycelium does not fragment. *Spirales*-type spore chains with spiny spore sheaths are produced. No diffusible pigments are produced on glycerol/asparagine agar or on any other medium tested. Melanin pigment is not produced on peptone/yeast extract/iron agar or on tyrosine agar. The cell wall contains

LL-DAP (cell wall type I); there are no diagnostic sugars. Excellent growth occurs on inorganic salts/starch agar (ISP medium no. 4). Very good growth occurs on yeast extract/malt extract agar (ISP medium no. 2), oatmeal agar (ISP medium no. 3) and Czapek solution agar (Atlas, 1993). Growth is good on Bennett's agar. Growth on glycerol/asparagine agar is moderate. Weak antibiotic activity is exhibited against *Enterococcus faecium*, but no antibiotic activity is observed against *Escherichia coli* ATCC 25922 or *Pseudomonas aeruginosa* ATCC 27853. Grows in the presence of 2-phenylethanol (0.3 %), sodium chloride (7 %, but not 10 %), carbenicillin (100 µg ml⁻¹), cefotaxime (100 µg ml⁻¹), D-cycloserine (50 µg ml⁻¹), nalidixic acid (25 µg ml⁻¹), oleandomycin (100 µg ml⁻¹) and penicillin G (10 IU ml⁻¹). Grows at pH 4.3 and 45 °C, but not at 4 °C or in the presence of sodium azide, capreomycin (20 µg ml⁻¹), cephaloridine (100 µg ml⁻¹), chloramphenicol (50 µg ml⁻¹), erythromycin (50 µg ml⁻¹), gentamicin (100 µg ml⁻¹), kanamycin (10 µg ml⁻¹), lincomycin (100 µg ml⁻¹), neomycin (50 µg ml⁻¹), phenol (0.1 %), rifampicin (50 µg ml⁻¹), spectinomycin (20 µg ml⁻¹), streptomycin (100 µg ml⁻¹), tobramycin (50 µg ml⁻¹) or vancomycin (50 µg ml⁻¹). Utilizes adonitol, (+)-L-arabinose, (+)-D-cellobiose, (–)-D-fructose, (+)-D-galactose, glycerol, *meso*-inositol, inulin, lactose, maltose, D-mannitol, (+)-D-mannose, (+)-D-melibiose, methyl α-D-glucoside, raffinose, (+)-L-rhamnose, (–)-D-ribose, salicin, sodium acetate, sodium butyrate, sodium citrate, sodium DL-malate, sodium malonate, sodium propionate, sodium pyruvate, sodium salicylate, sodium succinate, sucrose, trehalose and (+)-D-xylose as sole carbon sources, but not *meso*-erythritol, (+)-D-melezitose, sodium benzoate, sodium formate, sodium maleate, sodium oxalate, sodium (+)-L-tartrate, (–)-L-sorbose or xylitol. Utilizes 4-amino-n-butyric acid, DL-α-amino-n-butyric acid (weak growth), L-arginine, L-cysteine, L-histidine, L-hydroxyproline (weak growth), L-methionine, DL-ornithine, L-phenylalanine, potassium nitrate, L-serine, L-threonine and L-valine as sole nitrogen sources. Tests for nitrate reductase and H₂S production are positive. Pectin is hydrolysed, but hippurate is not. Protease, lipase and lecithinase activities are produced on egg-yolk agar (the proteolytic reaction is weak). The organism degrades adenine, aesculin, arbutin, casein, DNA, gelatin, hypoxanthine, starch, Tween 80 and L-tyrosine, but not allantoin, cellulose, guanine, urea, xanthine or xylan.

The type strain is CPJVR-H^T (=NRRL B-24143^T=DSM 41829^T).

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References

- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- Atlas, R. M. (1993). *Handbook of Microbiological Media*. Edited by L. C. Parks. Boca Raton, FL: CRC Press.
- Chun, J. & Goodfellow, M. (1995). A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. *Int J Syst Bacteriol* **45**, 240–245.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). Quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Escara, J. F. & Hutton, J. R. (1980). Thermal stability and renaturation of DNA in dimethyl sulfoxide solutions: acceleration of the renaturation rate. *Biopolymers* **19**, 1315–1327.
- Hasegawa, T., Takizawa, M. & Tanida, S. (1983). A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* **29**, 319–322.
- Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. (2001). MEGA2: Molecular Evolutionary Genetics Analysis software. Arizona State University, Tempe, Arizona, USA (<http://www.megasoftware.net>).
- Luquin, M., Ausina, V., López Calahorra, F., Belda, F., García Barceló, M., Celma, C. & Prats, G. (1991). Evaluation of practical chromatographic procedures for identification of clinical isolates of mycobacteria. *J Clin Microbiol* **29**, 120–130.
- Mandel, M. & Marmur, J. (1968). Use of ultraviolet absorbance-temperature profile for determining the guanine plus cytosine content of DNA. *Methods Enzymol* **12B**, 195–206.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. & Sackin, M. J. (1983). Numerical classification of *Streptomyces* and related genera. *J Gen Microbiol* **129**, 1743–1813.
- Williams, S. T., Goodfellow, M. & Alderson, G. (1989). Genus *Streptomyces* Waksman and Henrici 1943, 339^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, pp. 2452–2504. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697–703.