Acaricomes phytoseiuli gen. nov., sp. nov., isolated from the predatory mite *Phytoseiulus persimilis*

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A Gram-positive, rod-shaped, non-spore-forming bacterium, strain CSC^T, was isolated from diseased, surface-sterilized specimens of the predatory mite *Phytoseiulus persimilis* Athias-Henriot and subjected to polyphasic taxonomic analysis. Comparative analysis of the 16S rRNA gene sequence revealed that the strain was a new member of the family *Micrococcaceae*. Nearest phylogenetic neighbours were determined as *Renibacterium salmoninarum* (94·0 %), *Arthrobacter globiformis* (94·8 %) and *Arthrobacter russicus* (94·6 %). Although the predominant fatty acids (anteiso $C_{15:0}$), cell-wall sugars (galactose, glucose) and polar lipids (diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol) are in accordance with those of members of the genus *Arthrobacter*, strain CSC^T can be distinguished from members of the genus *Arthrobacter* by biochemical tests, the absence of a rod–coccus life cycle and the occurrence of the partially saturated menaquinone MK-10(H_2) as the predominant menaquinone. The DNA G+C content is 57·7 mol%. On the basis of morphological, chemotaxonomic and phylogenetic differences from other species of the *Micrococcaceae*, a novel genus and species are proposed, *Acaricomes phytoseiuli* gen. nov., sp. nov. The type strain is CSC^T (=DSM 14247^T=CCUG 49701^T).

The mite *Phytoseiulus persimilis* Athias-Henriot is a specialized predator of spider mites in the genus *Tetranychus* and is one of the cornerstones of greenhouse biological control programmes against spider mites. A novel disease was detected in adult females of *P. persimilis*, the 'non-responding syndrome' (Schütte *et al.*, 1998; Dicke *et al.*, 2000; Bjørnson & Schütte, 2003), that significantly affected various characteristics of the predators that are important for successful biological control of spider mites. The disease was not transmitted vertically from mother to offspring directly via the egg, but was transmitted horizontally among and between generations via leftovers, e.g. faeces and debris of diseased mites (Schütte *et al.*, 1998; C. Schütte and others, unpublished data).

Strain CSC^T was isolated from diseased surface-sterilized female *P. persimilis* mites on LB agar (Difco), incubated for 1 week under aerobic conditions at 25 °C. For observation of colony and cell morphology, the strain was grown on tryptic soy agar (TSA; Difco) for 5 days at 25 °C. The oxidase test and determination of catalase activity were performed with reagent droppers (Becton Dickinson) according to the manufacturer's instructions. The temperature range for growth was determined by incubating inoculated slant agar cultures (TSA, pH. 7·0) at 4, 10, 15, 22, 25, 28, 30 and 37 °C. Growth was also assessed in tryptic soy broth (TSB; Difco) at pH 4·5–9·5. The lower pH values of the test medium were obtained by adding hydrochloric acid and pH values greater than 7·5 by addition of sterilized sodium sesquicarbonate buffer (1 M). Starch hydrolysis was tested by the standard procedure described by Collins *et al.* (1989); the agar plate was flooded with dilute iodine solution after 5 days of incubation.

The ability to utilize a variety of substrates as carbon sources was tested using the GP2 microplates of the MicroLog system (Biolog). Analyses were done in duplicate, in order to check the reproducibility of the results obtained. Bacterial cells were suspended in inoculating fluid (GP; Biolog) at the recommended cell density of 20 % transmission using the Biolog turbidimeter. One hundred and fifty microlitres of the resulting solution was transferred to each well of the GP2 microplate and, after 24 h incubation at 30 °C, reduction of

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the tetrazolium dye was determined using the Microplate reader of the Biolog MicroStation.

For phylogenetic analysis, genomic DNA was extracted from bacteria and purified as described previously (Pukall *et al.*, 1998). The primer pair 27f (5′-GAGTTTGATCCTGGCTC-AG-3′) and 1527r (5′-AGAAAGGAGGTGATCCAGCC-3′) was used for amplification of the 16S rRNA gene (Lane, 1991). PCR amplification was done as described earlier (Pukall *et al.*, 1999). Amplicons were sequenced by using the dye-labelled dideoxy-terminator cycle sequencing (DTCS) Quick Start kit and the Ceq8000 Genetic Analysis System from Beckman Coulter. The sequence was manually aligned and compared with published sequences from the DSMZ 16S rRNA gene database, including sequences available from the Ribosomal Database Project (Maidak *et al.*, 2001) and EMBL. A manual alignment was constructed with the BioEdit program (Hall, 1999) and used to calculate the distance matrix corrected by the Kimura 2-parameter method (Kimura, 1980). A phylogenetic dendrogram was inferred using the neighbour-joining method and CLUSTAL X (Thompson *et al.*, 1997). Bootstrap analysis was based on 1000 resamplings.

Isolation of DNA (Cashion *et al.*, 1977) for determination of the DNA G+C content by HPLC (Mesbah *et al.*, 1989) followed described procedures. G+C content of the DNA was calculated from six individual measurements.

Fatty acids were determined from cells freshly grown in TSB. Fatty acid methyl esters were obtained from freeze-dried biomass (approx. 10 mg) by saponification, methylation and extraction using the modifications (Kuykendall *et al.*, 1988) of the method of Miller (1982). The fatty acids were separated using the 5898A Microbial Identification System (MIDI) as described previously (Pukall *et al.*, 2003).

Isoprenoid quinones were extracted from strain CSC^T grown in TSB, as described by Collins (1985). Analysis was performed by HPLC (Groth *et al.*, 1996) and the menaquinone structure was determined by electron impact-mass spectrometry using a QP 2000 mass spectrometer fitted with a direct sample inlet device (Shimadzu) as described by Collins (1994). Analysis of the peptidoglycan structure was carried out as described by Schleifer (1985) and Schleifer & Kandler (1972) with the modification that TLC on cellulose was applied instead of paper chromatography. Cell-wall sugars were analysed by using a modification of the method of Staneck & Roberts (1974). Polar lipids extracted by the method of Minnikin *et al.* (1979) were identified by two-dimensional TLC and spraying with specific reagents (Collins & Jones, 1980).

On TSA, smooth, circular, yellowish colonies were formed, 1–2 mm in diameter. Cell morphology of a culture grown in TSB was analysed microscopically using a phase-contrast microscope (Zeiss Axioplot). Cells were small, short rods, 0·5–0·8 × 1–1·5 μm in size. The optimal growth temperature was 25 °C. Growth was observed between 15 and 30 °C and no growth occurred at 4, 10 or 37 °C. Strain CSC^T was able to grow in TSB at initial pH values ranging from pH 6·0 to 9·5, with an optimum range of pH 6·0–8·0. A rod–coccus life cycle, typical of members of the genus *Arthrobacter*, could not be observed during 3 days of incubation. The oxidase test was negative, but catalase activity was detectable. Detailed characteristics obtained from the Biolog GP2 assay after 3 days of incubation at 25 °C are indicated in the species description.

The nearest phylogenetic neighbours, determined by analysis of the nearly complete 16S rRNA gene sequence, were found to be members of the family *Micrococcaceae*. Highest sequence similarities were obtained for *Renibacterium salmoninarum* (94·0 %), *Arthrobacter globiformis* (94·8 %) and *Arthrobacter russicus* (94·6 %). Patterns of selected 16S rRNA gene signature nucleotides defined for the family *Micrococcaceae* (Stackebrandt *et al.*, 1997; Stackebrandt & Schumann, 2000) were consistent with nucleotides determined for the 16S rRNA gene sequence of strain CSC^T. Positions 502–543 and 1310–1327, given as R–Y by Stackebrandt & Schumann (2000), were determined as A–U and G–C, respectively. The position of strain CSC^T relative to its phylogenetic neighbours is shown in Fig. 1.

The sequences of the type strains of *Arthrobacter viscosus* and *Arthrobacter siderocapsulatus* were not included in the phylogenetic analysis, because these two species can not be considered to be authentic members of the genus *Arthrobacter* (Stackebrandt & Schumann, 2000). The sequence of the type strain of *Arthrobacter viscosus* clustered with *Rhizobium* species and *Arthrobacter siderocapsulatus* was reported to be a later heterotypic synonym of *Pseudomonas putida* (Chun *et al.*, 2001). The type strain of *Arthrobacter ramosus* (CCM 1646^T) was obtained from the Czechoslovak Collection of Microorganisms in 2004 and used for reanalysis of its 16S rRNA gene sequence.

Cellular fatty acid analysis revealed predominant amounts of the branched fatty acids anteiso-$C_{15:0}$ (53·59 %) and anteiso-$C_{17:0}$ (31·90 %). iso-$C_{16:0}$ (6·28 %), $C_{16:0}$ (3·62 %) and iso-$C_{15:0}$ (1·71 %) were detectable in smaller amounts.

The peptidoglycan contained the amino acids lysine, alanine and glutamic acid in the molar ratio of 1·0 : 4·9 : 1·0. Two-dimensional TLC of the partial hydrolysate revealed the presence of peptides characteristic of interpeptide bridges consisting of several L-Ala residues. Strain CSC^T possesses peptidoglycan of type A3α, L-Lys–L-Ala$_3$ (type A11.6 according to the DSMZ catalogue of strains; also available at http://www.dsmz.de/species/murein.htm). Cell-wall sugars consisted of galactose and glucose. Phosphatidylinositol (PI), phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG) were found as polar lipids.

Strain CSC^T contained the partially saturated menaquinones MK-10(H_2), MK-11(H_2), MK-9(H_2) and MK-8(H_2) with the ratio of HPLC peak areas of 61 : 11 : 8 : 7. The electron impact mass spectrum of the major component

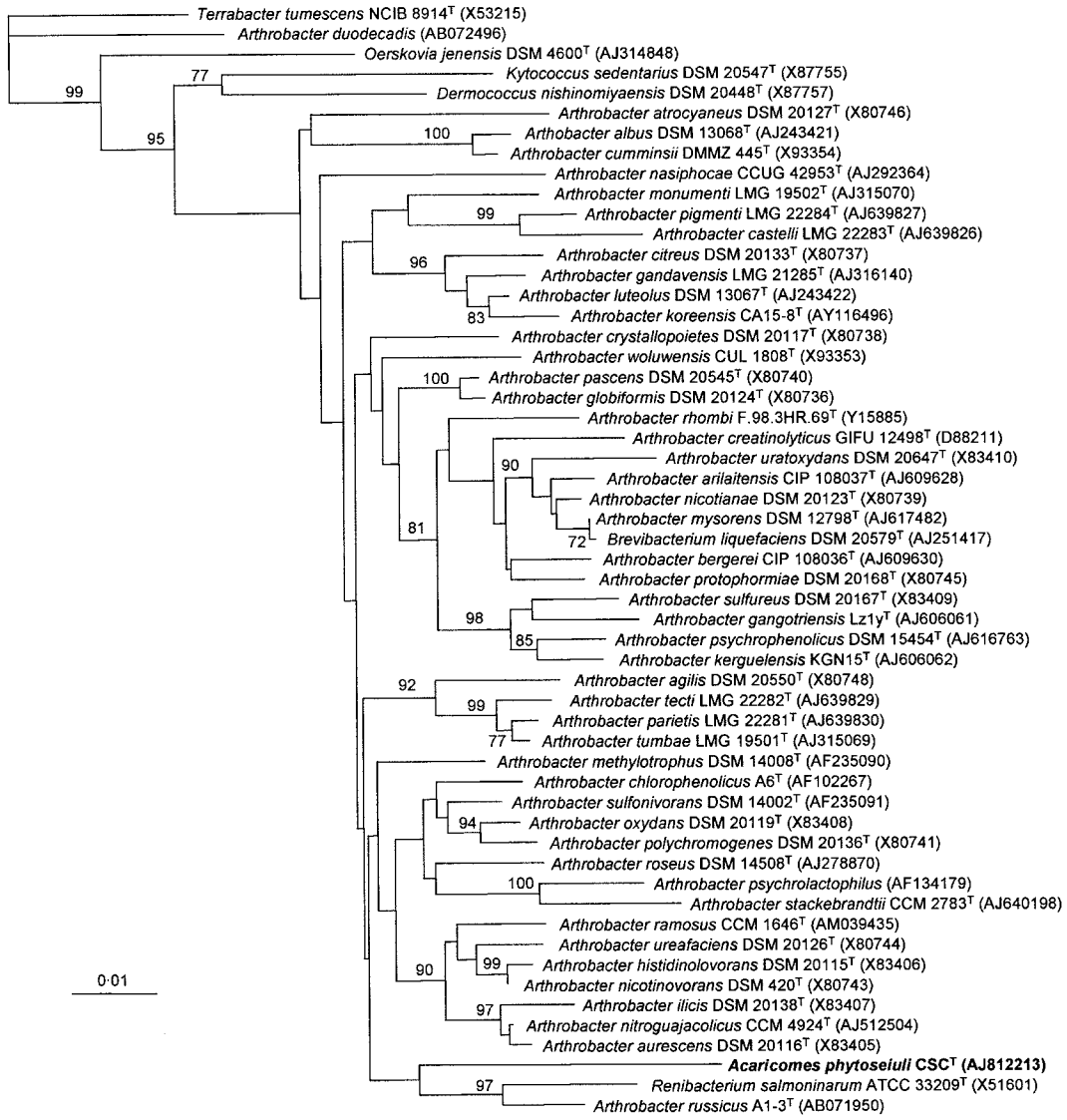

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the phylogenetic position of strain CSC^T among members of the family *Micrococcaceae*, suborder *Micrococcineae* (Stackebrandt *et al.*, 1997). Bootstrap values (percentages of 1000 replications) greater than 70% are shown at branching points. Bar, 1 substitution per 100 nucleotide positions. Note that *Brevibacterium liquefaciens* is a heterotypic synonym of *Arthrobacter nicotianae* (Gelsomino *et al.*, 2004).

revealed the additional presence of an isoprenoid quinone methylated at the aromatic nucleus. From the fragment ions detected it was concluded that, in addition to MK-10(H_2), MMK-10(H_2) also occurred. The G+C content of the DNA was 57·7±0·5 mol%.

Characteristics that differentiate strain CSC^T from other representatives of the nearest phylogenetic neighbours detected by 16S rRNA gene sequence analysis are given in Table 1. 16S rRNA gene sequence analysis, chemotaxonomic properties and the profile of metabolic properties revealed that strain CSC^T represents a new genus and a novel species within the family of *Micrococcaceae*, for which the name *Acaricomes phytoseiuli* gen. nov., sp. nov. is proposed.

Description of *Acaricomes* gen. nov.

Acaricomes (A.ca.ri.co′mes. N.L. masc. pl. n. *acari* the mites; L. masc. n. *comes* companion; N.L. masc. n. *Acaricomes* companion of mites).

Gram-positive, aerobic, non-endospore-forming rods. A rod–coccus life cycle is absent. Mesophilic. The pH optimum for growth is pH 6·0–8·0. Catalase-positive, oxidase-negative. The peptidoglycan type is A3α L-Lys–L-Ala_3. The

Table 1. Comparison of properties of *Acaricomes phytoseiuli* CSC^T and its nearest phylogenetic neighbours

Strains/species: 1, *Acaricomes phytoseiuli* CSC^T; 2, *Arthrobacter globiformis* (unless indicated, data from Keddie *et al.*, 1986); 3, *Arthrobacter russicus* A1-3^T (Li *et al.*, 2004); 4, *Renibacterium salmoninarum* Lea-1-74^T (Sanders & Fryer, 1980). +, Positive, −, negative; W, weak reaction, NA, data not available.

Characteristic	1	2	3	4
Colony colour	Yellow	Cream/white	Cream/white	Cream/white to yellow
Cell morphology	Short rods	Irregular rods, cocci	Irregular rods	Short rods
Rod–coccus cycle	−	+	NA	−
Cell-wall sugars*	Gal, Glc	Gal, Glc	NA	Gal, Rha
Peptidoglycan type	L-Lys–Ala_3	L-Lys–Ala_3	L-Lys–Ala_2	L-Lys–Ala–Gly
Predominant menaquinone	MK-10(H_2)	MK-9(H_2)	MK-9(H_2)	MK-9
Hydrolysis of starch	−†	+	NA	NA
DNA G+C content (mol%)	57·7	61–66	65·5	52–54
Utilization of:‡				
Dextrin	+	+	−	NA
Maltose	+	+	−	NA
Maltotriose	+	+	−	NA
D-Mannitol	−	+	−	NA
α-Hydroxybutyric acid	−	+	−	NA
Uridine	−	+	+	NA
Xylose	−	+	−	NA
L-Glutamic acid	+	W	W	NA
Glucose 1-phosphate	+	−	−	NA

*Gal, Galactose; Glc, glucose; Rha, rhamnose.

†Test performed according to Collins *et al.* (1989).

‡Biolog GP2 assay data for *Arthrobacter globiformis* are for strain DSM 20124^T and were obtained in this study.

predominant menaquinone is the partially saturated menaquinone MK-10(H_2) with one of the ten isoprene units hydrogenated; in addition, MMK-10(H_2) is detectable. The main fatty acids determined are anteiso-$C_{15:0}$ (12-methyltetradecanoic acid) and anteiso-$C_{17:0}$ (14-methylhexadecanoic acid). The major polar lipids are PG, DPG and PI. The type species is *Acaricomes phytoseiuli*.

Description of *Acaricomes phytoseiuli* sp. nov.

Acaricomes phytoseiuli (phy.to.sei′u.li. N.L. gen. masc. n. *phytoseiuli* of *Phytoseiulus*, the nomenclatural genus name of the host mite).

Shows the following properties in addition to those given in the genus description. Cells are 0·5–0·8 × 1–1·5 μm in size. Colonies on TSA are circular, convex, 1–2 mm in diameter and coloured yellowish. Growth occurs at 15–30 °C with an optimum of 25 °C. Grows at pH 6·0–9·5. Utilizes dextrin, α-D-glucose, α-D-fructose, maltose, maltotriose, D-mannose, sucrose, turanose, L-glutamic acid and glucose 1-phosphate. Only weak reactions for utilization of Tween 80, palatinose, L-pyroglutamic acid and glucose 6-phosphate. The following substrates are not utilized: α- and β-cyclodextrin, glycogen, inulin, mannan, Tween 40, *N*-acetyl-D-glucosamine, *N*-acetyl-D-mannosamine, amygdalin, L-arabinose, D-arabitol, arbutin, D-cellobiose, L-fucose, D-galactose, D-galacturonic acid, gentiobiose, D-gluconic acid, *myo*-inositol, α-D-lactose, lactulose, D-mannitol, D-melezitose, D-melibiose, methyl α-D-galactoside, methyl β-D-galactoside, 3-methyl glucose, methyl α-glucoside, methyl β-D-glucoside, methyl α-D-mannoside, D-psicose, D-raffinose, L-rhamnose, D-ribose, salicin, sedoheptulosan, D-sorbitol, stachyose, D-tagatose, D-trehalose, xylitol, D-xylose, acetic acid, α-, β- and γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α-ketovaleric acid, lactamide, D-lactic acid methyl ester, L-lactic acid, D-malic acid, methylpyruvate, monomethyl succinate, propionic acid, pyruvic acid, succinamic acid, succinic acid, *N*-acetyl-L-glutamic acid, L-alaninamide, D-alanine, L-alanyl glycine, glycyl L-glutamic acid, L-serine, putrescine, 2,3-butanediol, glycerol, adenosine, 2′-deoxyadenosine, inosine, thymidine, uridine, adenosine 5′-monophosphate, thymidine 5′-monophosphate, uridine 5′-monophosphate, fructose 6-phosphate and DL-α-glycerol phosphate. Cell-wall sugars are galactose and glucose. The G+C content of the type strain is 57·7 mol%.

The type strain, CSC^T (=DSM 14247^T=CCUG 49701^T), was isolated from the predatory mite *Phytoseiulus persimilis*.

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