Trichococcus patagoniensis sp. nov., a facultative anaerobe that grows at -5°C , isolated from penguin guano in Chilean Patagonia

Elena V. Pikuta,¹ Richard B. Hoover,¹ Asim K. Bej,² Damien Marsic,³ William B. Whitman,⁴ Paul E. Krader⁵ and Jane Tang⁶

Correspondence

Elena V. Pikuta
elenapikuta@hotmail.com

Richard B. Hoover
Richard.Hoover@NASA.GOV

¹NASA/NSSTC, VP-62, Astrobiology Laboratory, 320 Sparkman Dr., Huntsville, AL 35805, USA

²Department of Biology, University of Alabama at Birmingham, AL 35294, USA

³Laboratory for Structural Biology, The University of Alabama in Huntsville, MSB, 203C, Huntsville, AL 35899, USA

⁴Department of Microbiology, University of Georgia, Athens, GA 30602-2605, USA

⁵American Type Culture Collection, 10801 University Blvd, Manassas, VA 20110, USA

⁶Mitretek Systems, 3150 Fairview Park Drive South, Falls Church, VA 22042, USA

A novel, extremely psychrotolerant, facultative anaerobe, strain PmagG1^T, was isolated from guano of Magellanic penguins (*Spheniscus magellanicus*) collected in Chilean Patagonia. Gram-variable, motile cocci with a diameter of $1.3\text{--}2.0\text{ }\mu\text{m}$ were observed singularly or in pairs, short chains and irregular conglomerates. Growth occurred within the pH range $6.0\text{--}10.0$, with optimum growth at pH 8.5 . The temperature range for growth of the novel isolate was from -5 to 35°C , with optimum growth at $28\text{--}30^{\circ}\text{C}$. Strain PmagG1^T did not require NaCl, as growth was observed in the presence of $0\text{--}6.5\%$ NaCl with optimum growth at 0.5% (w/v). Strain PmagG1^T was a catalase-negative chemo-organoheterotroph that used sugars and some organic acids as substrates. The metabolic end products were lactate, formate, acetate, ethanol and CO_2 . Strain PmagG1^T was sensitive to ampicillin, tetracycline, chloramphenicol, rifampicin, kanamycin and gentamicin. The G + C content of its genomic DNA was $45.8\text{ mol}\%$. 16S rRNA gene sequence analysis showed 100% similarity of strain PmagG1^T with *Trichococcus collinsii* ATCC BAA-296^T, but DNA–DNA hybridization between them demonstrated relatedness values of $<45 \pm 1\%$. Another phylogenetically closely related species, *Trichococcus pasteurii*, showed 99.85% similarity by 16S rRNA sequencing and DNA–DNA hybridization showed relatedness values of $47 \pm 1.5\%$. Based on genotypic and phenotypic characteristics, the novel species *Trichococcus patagoniensis* sp. nov. is proposed, with strain PmagG1^T (= ATCC BAA-756^T = JCM 12176^T = CIP 108035^T) as the type strain.

The genus *Trichococcus* was created by Scheff *et al.* (1984) and, after reclassification of some species of the genera *Lactosphaera* and *Ruminococcus*, an emended description of the genus was published (Liu *et al.*, 2002). Currently, the genus *Trichococcus* includes four species: *Trichococcus*

flocculiformis (the type species), *T. palustris*, *T. pasteurii* and *T. collinsii*. All of these species are psychrotolerant mesophiles and are able to grow at low temperatures. All species in this genus have the same morphology, with typical coccoid-shaped cells (sometime elongated ellipses) occurring singularly, in pairs, in short chains or as irregular conglomerates. This pleomorphic nature is a common characteristic within the genus. All species of the genus *Trichococcus* are facultative anaerobes and, like species of the genus *Carnobacterium*, are capable of reducing resazurin in aerobic media during growth. Another interesting characteristic has been noted at the genotypic level: all species of this genus have a highly similar ($99.9\text{--}100\%$) 16S rRNA gene sequence (Liu *et al.*, 2002). In bacteriology, there

Abbreviation: FAME, fatty acid methyl ester.

The GenBank/EMBL/DDBJ accession numbers for the sequences determined in this work are AF394926 (16S rRNA gene sequence of strain PmagG1^T), DQ485314 and DQ485315 (16S rRNA gene upstream regions of strain PmagG1^T and *T. collinsii* ATCC BAA-296^T, respectively) and DQ485316 and DQ485317 (partial sequence of the family A DNA polymerase genes of strain PmagG1^T and *T. collinsii* ATCC BAA-296^T, respectively).

are cases where phylogenetic analysis demonstrates 100 % 16S rRNA gene sequence similarity, but, at the same time, DNA–DNA hybridization shows relatedness values of < 70 % (enough for separation of strains into novel species). This situation is rare, but it raises methodological questions in bacterial taxonomy (Keswani & Whitman, 2001; Stackebrandt *et al.*, 2002; Coenye *et al.*, 2005).

In the present work, we describe a novel species of the genus *Trichococcus* capable of growth at sub-zero temperatures. Phylogenetic analysis of this novel isolate showed an identical 16S rRNA gene sequence (100 % similarity) to that of *T. collinsii* ATCC BAA-296^T, but the phenotypes of these two strains had significant physiological differences. We have previously described the psychrotolerant characteristics of this novel isolate, PmagG1^T (Hoover *et al.*, 2002; Pikuta *et al.*, 2003a; Pikuta & Hoover, 2003, 2004). DNA–DNA hybridization data supported the basis for the separation of this isolate into a novel species.

Strain PmagG1^T was isolated from a sample of guano of the Magellanic penguin (*Spheniscus magellanicus*), an endemic species that inhabits the southern Patagonia region of the South American continent. The guano was taken from the bottom of a shallow marine tidal pool at the Seno Otway Magellanic penguin colony in southern Chile. Guano with surrounding seawater was collected aseptically in a Corning 50 ml centrifuge tube and immediately transferred to a

cooler and maintained at +4 °C during transport to the Astrobiology Laboratory at the NASA Marshall Space Flight Center.

Homogenized sample material (0.5 ml) was injected into Hungate tubes containing the following medium (g l⁻¹): NaCl, 30.0; KCl, 0.3; KH₂PO₄, 0.3; MgSO₄·7H₂O, 0.1; NH₄Cl, 1.0; CaSO₄·7H₂O, 0.0125; NaHCO₃, 0.4; Na₂S·9H₂O, 0.4; resazurin, 0.0001; yeast extract, 0.1; D-glucose, 5.0, with 2 ml vitamin solution (Wolin *et al.*, 1963) l⁻¹ and 1 ml trace mineral solution (Whitman *et al.*, 1982) l⁻¹. The final pH was 7.8. High-purity nitrogen was used as the gas phase. A pure culture was obtained by serial dilution and incubation at 4 °C. The ninth dilution of the morphologically monotypic culture was chosen for growth of colonies on an agar medium. Isolation of colonies was performed on the above-described medium with 3 % (w/v) agar by the roll-tube method. After incubation for 14 days at 4 °C, colonies of strain PmagG1^T were circular (on the surface) or convex lens-shaped (convex–convex lens-shaped in deep agar), white in colour and had a diameter of 0.5–4.0 mm. The surface of the large, circular colonies had an inner raised greyish circle. The margins were entire, smooth and thinner than the centre. The consistency of the colonies was mucoid and slimy. Young colonies were dry, but the older ones were shiny. The morphology of colonies grown at –5 °C was different (Fig. 1d, e). Cells on the surface of the colonies grown at –5 °C were covered by an

Fig. 1. Field emission scanning electron microscopy of strain PmagG1^T. (a) View of a perfectly circular colony grown aerobically on 3 % agar medium at 22 °C. Bar, 1000 µm. (b) Edge of the colony in (a). Bar, 30 µm. (c) Surface of the colony in (a). Note that the cells are not covered by excreted mucopolysaccharides. Bar, 10 µm. (d) Colony grown aerobically on 3 % agar at –5 °C. Bar, 500 µm. (e) Surface of the colony in (d). Note that the cells are covered by mucilaginous matter. Bar, 10 µm. (f) Cells grown at –5 °C covered by mucopolysaccharide film. Bar, 5 µm.

excreted mucoid substance that probably maintains the encapsulated cells in an unfrozen state.

In all experiments for the description of physiology and metabolism, pure cultures were incubated at 4–22 °C. The purity of the culture was checked by microscopy during the study.

The morphology of the novel isolate was observed under a phase-contrast microscope (Fisher Micromaster) and a FEI Quanta 600FEG field emission scanning electron microscope was used to examine the ultramicrostructure of the cell surfaces (Fig. 1). Cells of strain PmagG1^T were coccoid or ovoid (olive)-shaped and at sub-zero temperatures were surrounded by mucoid matter (Fig. 1d, e). Cells occur singly, in pairs, in short chains or gathered in irregular conglomerates. The diameter of cells was 1.3–2.0 µm. Cells were motile and slow moving, with a peritrichous type of locomotion. Gram staining showed a variable reaction. Spores were never observed.

Growth of a pure culture was determined by direct cell counting under a phase-contrast microscope or by turbidimetry by measuring optical density changes at 595 nm (Genesis 5; Spectronic Instruments). Catalase activity was determined by the reaction with hydrogen peroxide (Gerhardt *et al.*, 1994). Growth substrates at concentrations of 3 g l⁻¹ were added separately to the medium, which contained 0.1 g yeast extract l⁻¹. Metabolic end products of glucose fermentation in the liquid phase were determined by HPLC (Pikuta *et al.*, 2005). Separation was done on an Aminex HPX-87H (Bio-Rad) column with 5 mM H₂SO₄ as the mobile phase. Gases were measured using a gas chromatograph 3700 (Varian) equipped with a Porapak Q column and thermal conductivity detector. Nitrogen was used as the gas carrier.

The novel isolate was a facultative anaerobe and grew well under both aerobic and anaerobic conditions. During growth under aerobic conditions, resazurin was reduced (i.e. became colourless). The same effect is observed during cultivation of *Carnobacterium* species (Pikuta *et al.*, 2005; Franzmann *et al.*, 1991). Strain PmagG1^T had a negative catalase reaction. It could grow without NaCl in the medium if sodium salts were replaced with potassium salts, with a prolonged lag phase, indicating that strain PmagG1^T was not dependent on the Na⁺ ion. The NaCl range for growth was 0–6.5 % (w/v) and optimum growth was observed at 0.5 % (w/v). No growth was observed at 10 % (w/v) NaCl. Strain PmagG1^T grew in the pH range 6.0–10.0, with optimum growth at pH 8.5 (Fig. 2b), indicating that it is a slightly alkaliphilic bacterium. The temperature range for growth of strain PmagG1^T was from –5 to 35 °C with optimal growth at 28–30 °C. Growth was never observed at –7 °C or 40 °C (Fig. 2a).

Strain PmagG1^T had a chemo-organoheterotrophic metabolism and was capable of growth on D-glucose, D-fructose, D-mannose, D-maltose, D-arabinose, D-ribose, sucrose,

Fig. 2. Effect of temperature (at optimal NaCl concentration and pH) (a) and pH (at optimal NaCl concentration and at 23 °C) (b) on the growth rate of strain PmagG1^T.

lactose, starch, D-mannitol, pyruvate and citrate. No growth was observed with formate, acetate, lactate, propionate, butyrate, ethanol, methanol, glycerol, acetone, betaine, trimethylamine, triethylamine, peptone, Bacto tryptone, Casamino acids, yeast extract, pectin, D-trehalose and lactose.

Metabolic end products of the culture grown on D-glucose were lactate (3.3 mM), formate (1.5 mM), acetate (0.5 mM) and ethanol (1.3 mM) in the liquid phase and CO₂ in the gas phase.

The sensitivity of strain PmagG1^T to ampicillin, kanamycin, gentamicin, tetracycline and rifampicin (all at 250 µg ml⁻¹) and chloramphenicol (125 µg ml⁻¹) was tested. Strain PmagG1^T was sensitive to all of these antibiotics.

For extraction of fatty acid methyl esters (FAMES), the culture was incubated for 4 days at 22 °C on the liquid medium described above. The extraction and analysis procedures have been described previously (Pikuta *et al.*, 2003b). The major fatty acids for strain PmagG1^T were C_{14:0}, C_{16:1 cis7}, C_{16:0}, C_{18:1 cis9} and C_{18:0}. The percentages of each are shown in Table 1.

Electrophoretic analysis of whole-cell proteins was performed for strain PmagG1^T and *T. collinsii* ATCC BAA-296^T. As a distant relation, the psychrotolerant strain *Carnobacterium pleistocenium* FTR1^T was also tested. Strain PmagG1^T and *T. collinsii* ATCC BAA-296^T were grown on the liquid anaerobic medium described above, with D-glucose added as the substrate. *C. pleistocenium* FTR1^T was grown on the same medium, but with the addition of 3 g yeast extract l⁻¹. Preparation of cell extracts was performed according to the method of Keswani *et al.* (1996) with slight modifications. Each biomass (0.1 g) was resuspended in 1.5 ml TE buffer (pH 8.0) and washed twice following centrifugation at 14 000 g for 5 min. Each pellet was then resuspended in 1 ml TE buffer (pH 8.0) and sonicated for 4 min. Before preparation of the mixture for electrophoresis, each suspension was homogenized for 1 min. The total volume of each mixture was 12 µl, including 1 µl blue/orange 6× loading dye (Promega) and an aliquot of distilled water. Dilutions were as follows: 3 µl protein standard; first dilutions (threefold) contained 4 µl cell extract; second dilutions (sixfold) contained 2 µl of each suspension. SDS-PAGE was performed using a 12% Tris/HCl gel (Bio-Rad), which was subsequently stained with Coomassie blue (Fig. 3). Differences were observed between strain PmagG1^T and *T. collinsii* ATCC BAA-296^T in the intensities of protein bands b and d; strain PmagG1^T (Fig. 3, lanes 2 and 5) contained band b but did not have band d, which was found in *T. collinsii* ATCC BAA-296^T (Fig. 3, lanes 3 and 6). In addition, strain PmagG1^T contained an additional protein band between bands g and h, which was absent in *T. collinsii* ATCC BAA-296^T. Band k was missing in the reference strain but was present in strain PmagG1^T (Fig. 3, lanes 2 and 3).

Table 1. Fatty acid compositions of strain PmagG1^T and type strains of *T. collinsii* and *T. pasteurii*

Strains: 1, strain PmagG1^T; 2, *T. collinsii* ATCC 296^T (data from Liu *et al.*, 2002); 3, *T. pasteurii* ATCC 35945^T. Values are percentages (w/w) of total fatty acids. ND, No data.

Fatty acid	1	2	3
C _{12:0}	0.65	6.00	1.34
C _{14:0}	11.18	46.00	16.00
C _{16:1}	0.00	27.00	0.00
C _{16:1 cis7}	42.66	ND	48.00
C _{16:0}	16.48	18.00	15.02
C _{18:2 cis9,12}	0.72	ND	0.00
C _{18:1 cis9}	21.84	2.00	17.26
Summed feature 10*	1.92	ND	1.17
C _{18:0}	3.31	2.00	1.21
C _{19:1 cyclo 9,10}	0.60	ND	0.00
C _{20:1 cis11}	0.64	ND	0.00

*Summed feature 10 represents unresolved C_{18:1 cis11/trans9/trans6} FAME.

Fig. 3. Comparative gel electrophoretic analysis of cellular proteins of the psychrotolerant PmagG1^T, *T. collinsii* and *C. pleistocenium*. Lanes: 1, protein molecular mass standards (Sigma); 2 and 5, strain PmagG1^T; 3 and 6, *T. collinsii* ATCC BAA-296^T; 4 and 7, *C. pleistocenium* FTR1^T. Samples in lanes 2–4 were diluted threefold; samples in lanes 5–7 were diluted sixfold.

For phylogenetic analysis, the genomic DNA of strain PmagG1^T was isolated by phenol/chloroform extraction of sonicated biomass, followed by ethanol precipitation (Sambrook *et al.*, 1989). The 16S rRNA gene of strain PmagG1^T was amplified and sequenced as described previously (Hoover *et al.*, 2003).

A sequence covering 1483 nt of the 16S rRNA gene was obtained, corresponding to positions 27–1492 of the *Escherichia coli* 16S rRNA gene sequence. The G+C content of this sequence was 53.4 mol%. The sequence was compared with all GenBank sequences and appeared to be highly similar to the sequences of *Trichococcus* species. The sequence was aligned with closely related sequences using CLUSTAL W (Thompson *et al.*, 1994). Pairwise distances were computed from 1371 common nucleotide sites with MEGA version 3.1 (Kumar *et al.*, 2004) using the Jukes–Cantor model (Jukes & Cantor, 1969). Unrooted phylogenetic trees showing the position of strain PmagG1^T among all currently known species of the genus *Trichococcus* and some species of the genera *Carnobacterium* and *Isobaculum* were constructed with the same program using neighbour-joining (Fig. 4), minimum evolution and maximum-parsimony methods. All trees shared the same topology. According to the pairwise distance table (not shown), strain PmagG1^T appeared closest to *T. collinsii* ATCC BAA-296^T, *T. pasteurii* ATCC 35945^T, *T. palustris* DSM 9172^T and *T. flocculiformis* DSM 2094^T, with similarities of 100.0, 99.9, 99.4 and 99.0%, respectively.

As the 16S rRNA sequence similarity of the novel isolate was determined to be highest compared with *T. collinsii* ATCC

Fig. 4. Unrooted phylogenetic tree based on 16S rRNA gene sequences showing the position of strain PmagG1^T among species of *Trichococcus*, *Isobaculum* and *Carnobacterium*. The tree was generated by the neighbour-joining method. GenBank accession numbers are shown next to strain names. Bar, 5 substitutions per 1000 nt.

BAA-296^T and *T. pasteurii* ATCC 35945^T, these strains were chosen for DNA–DNA hybridization analysis.

Melting temperatures (T_m) of total genomic DNA from strain PmagG1^T, *T. pasteurii* ATCC 35945^T and *T. collinsii* ATCC BAA-296^T were determined as described previously (De Ley *et al.*, 1970; Gillis *et al.*, 1970). DNA was prepared and purity determined as described by Ausubel *et al.* (1987). The T_m of the genomic DNA was $78 \pm 3^\circ\text{C}$ (mean $\pm$ SD, $n=4$) for strain PmagG1^T, $86 \pm 2^\circ\text{C}$ ($n=4$) for *T. collinsii* ATCC BAA-296^T and $85 \pm 1^\circ\text{C}$ for *T. pasteurii* ATCC 49837 ($n=4$).

DNA–DNA hybridization analysis of genomic DNA of strain PmagG1^T and *T. collinsii* ATCC BAA-296^T and of strain PmagG1^T and *T. pasteurii* ATCC 49837^T was performed at 5°C above the T_m of the DNA from each species, followed by calculation of the initial reassociation kinetics using linear regression analysis (De Ley *et al.*, 1970; Johnson, 1985). The results showed DNA–DNA relatedness values of $47 \pm 1.5\%$ (mean $\pm$ SD, $n=3$) between the genomes of strain PmagG1^T and *T. pasteurii* ATCC 49837^T and $45 \pm 1\%$ ($n=3$) between the genomes of strain PmagG1^T and *T. collinsii* ATCC BAA-296^T.

The G + C content of the genomic DNA of strain PmagG1^T was determined by HPLC as described previously (Pikuta *et al.*, 2003b; Mesbah *et al.*, 1989) and was found to be $45.8 \pm 0.1\%$ mol% (mean $\pm$ SD, $n=4$).

To evaluate further the degree of separation between isolate PmagG1^T and *T. collinsii* ATCC BAA-296^T, additional sequence information was acquired and analysed. A sequence upstream of the 16S rRNA gene was obtained using multiplex restriction site PCR (Weber *et al.*, 1998) with nested primers 5'-GTTACTCACCCGTCGCCAC-3' and 5'-CTCAGTGGAAGCAAGCTTCC-3'. For strain PmagG1^T, the sequence was 435 nt, including 31 nt of the 16S rRNA gene. For *T. collinsii* ATCC BAA-296^T, the sequence was 524 nt long, including 55 nt of the 16S rRNA gene. A pairwise comparison of these sequences revealed 20 differences in the upstream region of the 16S rRNA gene.

A partial sequence of a family A DNA polymerase gene was amplified by PCR from PmagG1^T and *T. collinsii*

ATCC BAA-296^T using the degenerate primers 5'-AARACNAARACNGGNTAYWSNAC-3' and 5'-ATRTCNGCNGCNSWNCCYTGDATNGG-3', which were designed based on available family A DNA polymerase sequences from 11 members of the order *Lactobacillales*. PCR products were cloned into a sequencing vector (StrataClone PCR cloning kit; Stratagene) and sequenced in both directions using vector-specific sequencing primers. Primer sequences were removed from the consensus sequences. For both strains, a sequence of 722 nt was obtained, encoding a 240 aa sequence. Pairwise comparison revealed 48 differences at the nucleotide level and five differences at the amino acid level.

Our novel isolate demonstrated 100% similarity to the reference strain *T. collinsii* ATCC BAA-296^T by 16S rRNA gene sequence analysis, but had a DNA–DNA relatedness value of $<70\%$ by hybridization analysis. This situation is not unique and has been discussed previously (Liu *et al.*, 2002). Of course, this situation suggests the need for additional differentiating experiments. Here, we added data from gel electrophoresis of total cell proteins and a comparison of the sequence upstream of the 16S rRNA gene, which were also different. In addition, the partial sequence of a family A DNA polymerase gene from the two compared strains showed differences at the nucleotide and amino acid levels.

According to Cohan (2001), strain PmagG1^T and other species of the genus *Trichococcus* may have experienced the same environmental stress during evolution and perhaps developed a strong conservative mechanism in their genomes that prevented the development of separate 'ecotypes' within this group.

From all checked strains of psychrophilic and psychrotolerant bacteria in our laboratory collection, only strain PmagG1^T grew at sub-zero temperatures. This is the first bacterial representative known to be able to grow at -5°C in pure culture (Hoover *et al.*, 2002). Strain PmagG1^T was obtained from guano of the Magellanic penguin, an endemic bird species that has evolved over a long period of time in the same geographical area. Table 2 shows a comparison of the distinguishing features of all known species of the genus *Trichococcus*. The pH and NaCl maxima were found to be highest for strain PmagG1^T. In addition, the optimum pH

for growth of strain PmagG1^T was higher than that of *T. collinsii* and *T. flocculiformis*. Notwithstanding the identical 16S rRNA gene sequences of the novel isolate and *T. collinsii* ATCC 296^T, their metabolic features showed significant differences: strain PmagG1^T was incapable of growth on D-trehalose, whereas *T. collinsii* ATCC 296^T and *T. pasteurii* ATCC 35945^T both fermented this sugar and, in contrast to *T. collinsii* ATCC 296^T, strain PmagG1^T could grow on D-ribose and maltose. The G + C content of the genomic DNA of strain PmagG1^T and *T. collinsii* ATCC 296^T differed by 1.2 mol% and the *T*_m differed by 8 °C. In comparison with *T. pasteurii* ATCC 35945^T, strain PmagG1^T differed in its *T*_m (by 7 °C), its ability to ferment D-ribose and its ability to grow on D-trehalose.

The composition of FAMES in strain PmagG1^T was similar to that of *T. pasteurii* ATCC 35945^T, but significantly different from that of *T. collinsii* ATCC 296^T (differing in the following FAMES: C_{12:0}, C_{14:0}, C_{16:1} and C_{18:1 cis9}; Table 1). DNA–DNA hybridization of the genomic DNA of strain PmagG1^T and *T. collinsii* ATCC 296^T and of strain PmagG1^T and *T. pasteurii* ATCC 35945^T exhibited DNA–DNA relatedness values of < 50 %.

Initially, we thought that the low minimum temperature for growth was exclusive to strain PmagG1^T, but it was determined that reference strain *T. collinsii* ATCC 296^T

was also capable of growth at –5 °C, requiring the species description to be emended.

On the basis of phenotypic and genotypic characteristics (cell morphology, facultatively anaerobic and fermentative metabolism, psychrotolerant and slightly alkalitolerant physiology, independence from NaCl, 16S rRNA gene sequence, DNA–DNA hybridization, a sequence upstream of the 16S rRNA gene and a region of the family A DNA polymerase gene), strain PmaG1^T is proposed as the type strain of a novel species of the genus *Trichococcus*, for which the name *Trichococcus patagoniensis* sp. nov. is proposed.

Emended description of *Trichococcus collinsii* Liu *et al.* 2002

The minimum temperature for growth is –5 °C (under both aerobic and anaerobic conditions).

Description of *Trichococcus patagoniensis* sp. nov.

Trichococcus patagoniensis (pa.ta.go.ni.en'sis. N.L. masc. adj. *patagoniensis* pertaining to Patagonia, the region of South America where the sample for the type strain was collected).

Cells are motile cocci with a diameter of 1.3–2.0 µm. Cells are spherical, ovoid or olive-shaped and occur as single cells,

Table 2. Phenotypic characteristics of strain PmagG1^T and type strains of *Trichococcus* species

Strains: 1, strain PmagG1^T; 2, *T. collinsii* ATCC 296^T (data from Liu *et al.*, 2002); 3, *T. pasteurii* ATCC 35945^T (Schink, 1984; Janssen *et al.*, 1995); 4, *T. flocculiformis* DSM 2094^T (Scheff *et al.*, 1984); 5, *T. palustris* DSM 9172^T (Zhilina *et al.*, 1995; Liu *et al.*, 2002). ND, No data; +, positive; –, negative; *T*_m, melting temperature (°C). All species grow on D-glucose, D-fructose, D-mannitol and pyruvate.

Characteristic	1	2	3	4	5
Temperature for growth (°C)					
Range	–5 to 35	–5 to 36	0–42	4–40	0–33
Optimum	28–30	23–30	25–30	25–30	ND
pH for growth					
Range	6–10	6–9	5.5–9.0	5.8–9.0	6.2–8.4
Optimum	8.5	7.5	ND	8.0	7.5
NaCl concentration for growth (% w/v)					
Range	0–6.5	ND	0–2	ND	ND
Optimum	0.5	0.1*	ND	0.5	0.1
Growth on:					
D-Trehalose	–	+	+	+	–
Lactate	–	–	–	–	+
Citrate	+	+	+	+	ND
D-Ribose	+	–	–	–	–
Maltose	+	–	+	+	–
α-D-Lactose	–	–	+	+	–
G + C content (mol%)	45.8	47	45	47	48
<i>T</i> _m (°C)	78	86	85	ND	ND
Source of isolation	Penguin guano, Patagonia, Chile	Hydrocarbon spill site, USA	Septic pit, Germany	Activated sludge, Germany	Swamp, Russia

*Concentration of NaCl in growth medium.

in pairs, in chains or as irregular conglomerates. Gram-variable. Growth occurs between -5 and 35°C (optimum $28-30^{\circ}\text{C}$). Slightly alkalophilic: pH range for growth is $6.0-10.0$, with optimum growth at pH 8.5 . Range of NaCl for growth is $0-6.5\%$ (w/v); optimum growth occurs at 0.5% NaCl. Facultative anaerobe and catalase-negative. Heterotrophic growth occurs with D-glucose, D-fructose, maltose, D-mannitol, D-mannose, D-ribose, D-arabinose, sucrose, starch, pyruvate and citrate. The metabolic end products are lactate, formate, acetate, ethanol and CO_2 (in the gas phase). Sensitive to ampicillin, kanamycin, gentamicin, tetracycline, rifampicin and chloramphenicol. The G + C content of the genomic DNA of the type strain is $45.82\text{ mol}\%$.

The type strain, strain PmagG1^T (=ATCC BAA-756^T=JCM 12176^T=CIP 108035^T), was isolated from a guano sample of Magellanic penguins (*Spheniscus magellanicus*) inhabiting the southern region of Chilean Patagonia.

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