microbiology Comment

Non-specificity of *Staphylococcus* generic primers

The genus *Staphylococcus* is virtually ubiquitous and, at the time of writing, represented by 37 species and numerous subspecies (http://www.bacterio.cict.fr/s/staphylococcus.html). At least 12 of these staphylococcal species/subspecies are human commensals that can become opportunistic pathogens (Kloos *et al.*, 1992). For example, *Staphylococcus epidermidis* (the most prevalent colonizer of the human skin) can cause nosocomial infections (von Eiff *et al.*, 2002), while *Staphylococcus aureus* (which occupies the anterior nares in 50–75 % of healthy individuals) can cause scalded skin syndrome and toxic shock syndrome (Ellis *et al.*, 2003; Hanakawa *et al.*, 2002).

As part of a programme investigating microbial contamination of pristine Antarctic Dry Valley environments resulting from human activities, we chose *Staphylococcus* as a target organism. PCR amplification of community DNA extracts from human-impacted and pristine Antarctic field sites using a published set of *Staphylococcus*-specific primers [TstaG422 (5′-GGC CGT GTT GAA CGT GGT CAA ATC A-3′) and TStag765 (5′-TIA CCA TTT CAG TAC CTT CTG GTA A-3′)] (Martineau *et al.*, 2001) resulted in strong and reproducible signals in all samples, suggesting that *Staphylococcus* was widely disseminated (L. Ah Tow & D. A. Cowan, unpublished data).

Martineau *et al.* (2001) have reported that the specificity of this primer set, which targets the *tuf* gene, is extremely high. Certain regions in the *tuf* genes are conserved within bacterial species belonging to the same genus. Ke *et al.* (1999) and Martineau *et al.* (2001) have used this characteristic as an effective bacterial phylogenetic marker. The *tuf* gene encodes the elongation factor Tu (EF-Tu), which plays an important role in protein synthesis (Weijland *et al.*, 1992). *tuf* gene sequences have been cloned and sequenced from both Gram-negative and Gram-positive bacteria (Filer & Furano, 1981; Sela *et al.*, 1989). Martineau *et al.* (2001) have shown that a 370 bp PCR product could be amplified from 27 different staphylococcal species, but not from 53 non-staphylococcal species tested. *Macrococcus caseolyticus*, which is phylogenetically closely related to members of the genus *Staphylococcus*, was amongst the 53 non-staphylococcal species that yielded a negative result in the *Staphylococcus*-specific PCR.

To confirm our contention that *Staphylococcus* spp. were widely disseminated in Antarctic Dry Valley soils, samples collected from Bratina Island and McMurdo station, Ross Dependency, Eastern Antarctica, were cultured on mannitol salt agar (a selective medium for the isolation of staphylococci and a few halophilic species; OXOID Manual). Numerous orange-pigmented colonies were observed on the selective medium. When the *Staphylococcus*-specific primers were tested on DNA extracts of the orange-pigmented colonies isolated from the Antarctic Dry Valley soil samples, a 370 bp PCR product was amplified (Fig. 1), indicating that they were possibly staphylococci. However, PCR amplification with 16S rDNA eubacterial primers E9F (5′-GAG TTT GAT CCT GGC TCA G-3′; Farrelly *et al.*, 1995) and U1510R (5′-GGT TAC CTT GTT ACG ACT T-3′; Reysenbach & Pace, 1995) and subsequent sequencing of the 16S rDNA genes revealed that the orange-pigmented colonies showed highest sequence identity with *Planococcus psychrophilus* (99 % sequence identity across 559 bp) and a *Planomicrobium* sp. (99 % sequence identity across 527 bp). To date, there have been no *tuf* gene sequences published for *Planococcus* and *Planomicrobium* spp. *Planococcus psychrophilus*, a Gram-positive, rod-shaped bacterium (Reddy *et al.*, 2002), is one of five *Planococcus* species within the family *Planococcaceae* (http://www.bacterio.cict.fr/p/planococcus.html). *Planococcus* spp. have been isolated from a variety of marine environments and some Antarctic

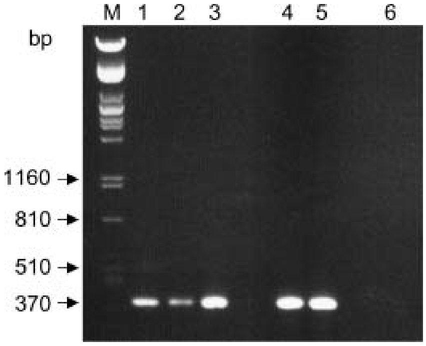

Fig. 1. Agarose gel (1 %) depicting the 370 bp PCR product obtained with the *Staphylococcus*-specific primers TstaG422 and TStag765. Template DNA was obtained from laboratory isolates of *Planococcus* (lane 1), *Planomicrobium* (lane 2) and *Staphylococcus* (lane 3), and *S. aureus* (lane 4) and *S. epidermidis* (lane 5). Lane 6 contains the PCR negative control, which was supplemented with sterile water instead of template DNA. M, molecular size marker.

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Chris Thomas, Editor-in-Chief

lakes (Bowman *et al.*, 1997; Engelhardt *et al.*, 2001; Reddy *et al.*, 2002; Sheridan & Brenchley, 2000), and many of these species have been reported to produce an orange/yellow pigment (Engelhardt *et al.*, 2001; Reddy *et al.*, 2002; Sheridan & Brenchley, 2000). *Planomicrobium*, a new genus belonging to the family *Planococcaceae*, contains two previously assigned planococcal species, namely *Planomicrobium mcmeekinii* and *Planomicrobium okeanokoites* (Yoon *et al.*, 2001). Although these species have been assigned to a separate genus, *Planomicrobium* and *Planococcus* are clearly closely related genera (Fig. 2).

Phylogenetically, *Planococcus* and *Planomicrobium* do not belong to the same clade as *Staphylococcus* (Fig. 2). They are, thus, more distantly related to *Staphylococcus* than *Staphylococcus* is to other members of the family *Staphylococcaceae*, such as the genus *Macrococcus*, the latter showing no positive PCR signal in the published studies by Martineau *et al.* (2001).

Our results allow us to conclude that there appears to be significant conservation between the *tuf* genes of *Planococcus*, *Planomicrobium* and *Staphylococcus* spp., and that although the primer set TstaG422/TStag765 has been shown to possess high specificity, its use for the definitive identification of *Staphylococcus* spp. must be treated with some caution.

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Bowman, J. P., McCammon, S. A., Brown, M. V., Nichols, D. S. & McMeekin, T. A. (1997). Diversity and association of psychrophilic bacteria in Antarctic sea ice. *Appl Environ Microbiol* **63**, 3068–3078.

Ellis, M., Serreli, A., Colque-Navarro, P., Hedstrom, U., Chacko, A., Siemkowicz, E. & Mollby, R. (2003). Role of staphylococcal enterotoxin A in a fatal case of endocarditis. *J Med Microbiol* **52**, 109–112.

Engelhardt, M. A., Daly, K., Swannell, R. P. J. & Head, I. M. (2001). Isolation and characterization of a novel hydrocarbon-degrading, Gram-positive bacterium, isolated from intertidal beach sediment, and description of *Planococcus alkanoclasticus* sp. nov. *J Appl Microbiol* **90**, 237–247.

Farrelly, V., Rainey, F. A. & Stackebrandt, E. (1995). Effect of genome size and *rrn* gene copy number on PCR amplification of 16S rRNA genes from a mixture of bacterial species. *Appl Environ Microbiol* **61**, 2798–2801.

Filer, D. & Furano, A. V. (1981). Duplication of the *tuf* gene, which encodes peptide chain elongation factor Tu, is widespread in Gram-negative bacteria. *J Bacteriol* **148**, 1006–1011.

Hanakawa, Y., Schechter, N. M., Lin, C. & 8 other authors (2002). Molecular mechanisms of blister formation in bullous impetigo and staphylococcal scalded skin syndrome. *J Clin Invest* **110**, 53–60.

Ke, D., Picard, F. J., Martineau, F., Ménard, C., Roy, P. H., Ouellette, M. & Bergeron, M. G. (1999). Development of a PCR assay for rapid detection of enterococci. *J Clin Microbiol* **37**, 3497–3503.

Kloos, W. E., Schleifer, K.-H. & Götz, F. (1992). The genus *Staphylococcus*. In *The Prokaryotes*, 2nd edn, pp. 1369–1420. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer-Verlag.

Martineau, F., Picard, F. J., Ke, D., Paradis, S., Roy, P. H., Ouellette, M. & Bergeron, M. G. (2001). Development of a PCR assay for identification of staphylococci at genus and species levels. *J Clin Microbiol* **39**, 2541–2547.

Reddy, G. S. N., Prakash, J. S. S., Vairamani, M., Prabhakar, S., Matsumoto, G. I. & Shivaji, S. (2002). *Planococcus antarcticus* and *Planococcus psychrophilus* spp. nov. isolated from cyanobacterial mat samples collected from ponds in Antarctica. *Extremophiles* **6**, 253–261.

Reysenbach, A.-L. & Pace, N. R. (1995). Reliable amplification of hyperthermophilic archaeal 16S rRNA genes by the polymerase chain reaction. In *Archaea: a Laboratory Manual – Thermophiles.* Edited by F. T. Robb & A. R. Place. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Sela, S., Yogev, D., Razin, S. & Bercovier, H. (1989). Duplication of the *tuf* gene: a new insight into the phylogeny of eubacteria. *J Bacteriol* **171**, 581–584.

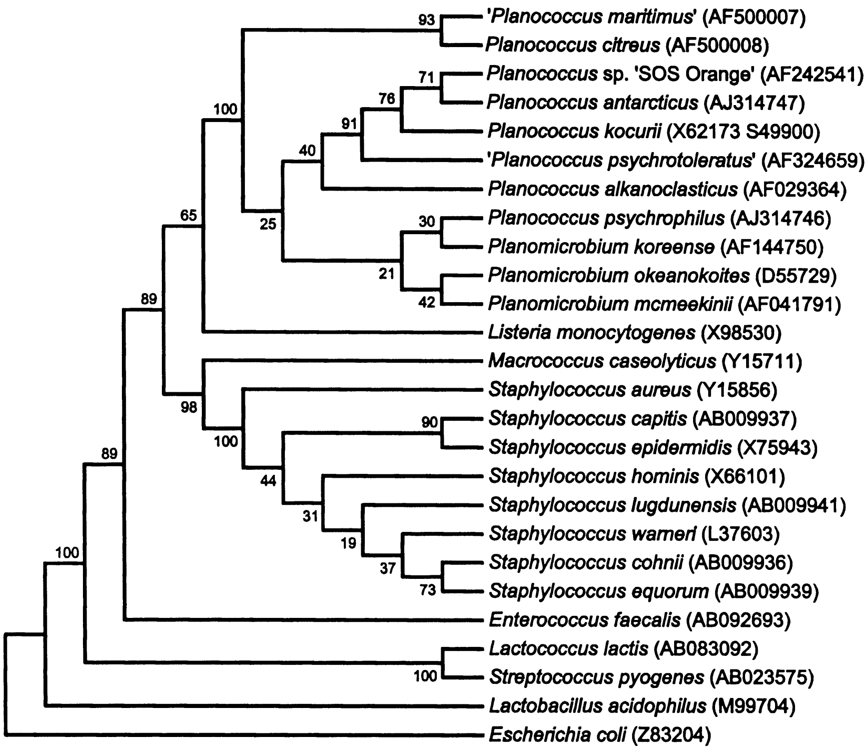

Fig. 2. Phylogenetic tree based on maximum-parsimony analysis (MEGA2) of the partial 16S rDNA sequences (1426 bp) of members of the genera *Planococcus*, *Planomicrobium* and *Staphylococcus*. Branch support is indicated at the nodes as a percentage of 1000 bootstraps.

Sheridan, P. P. & Brenchley, J. E. (2000). Characterization of a salt-tolerant family 42 β-galactosidase from a psychrophilic Antarctic *Planococcus* isolate. *Appl Environ Microbiol* **66**, 2438–2444.

von Eiff, C., Peters, G. & Heilmann, C. (2002). Pathogenesis of infections due to coagulase-negative staphylococci. *Lancet Infect Dis* **2**, 677–685.

Weijland, A., Harmark, K., Cool, R. H., Anborgh, P. H. & Parmeggiani, A. (1992). Elongation factor Tu: a molecular switch in protein biosynthesis. *Mol Microbiol* **6**, 683–688.

Yoon, J.-H., Kang, S.-S., Lee, K.-C., Lee, E. S., Kho, Y. H., Kang, K. H. & Park, Y. H. (2001). *Planomicrobium koreense* gen. nov., sp. nov., a bacterium isolated from the Korean traditional fermented seafood jeotgal, and transfer of *Planococcus okeanokoites* (Nakagawa *et al.* 1996) and *Planococcus mcmeekinii* (Junge *et al.* 1998) to the genus *Planomicrobium. Int J Syst Evol Microbiol* **51**, 1511–1520.

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