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 CTG CTA G-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reproducible signals in all samples, suggesting that Staphylococcus was widely disseminated (L. Ah Tow & D. A. Cowan, unpublished data).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′; Farrell et al., 1995) and U1510R (5′-GTT TAC CTT AGT AGC 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...
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 Planococaceae, contains two previously assigned planococcal species, namely Planomicrobium mcmeekinii and Planomicrobium okeanoikotes (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 Macroccus, 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.

Lemese Ah Tow and Don A. Cowan

Advanced Research Centre for Applied Microbiology, Department of Biotechnology, University of the Western Cape, Bellville 7535, Cape Town, South Africa

Correspondence: Lemese Ah Tow (lah-tow@uwc.ac.za)


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.


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