RESEARCH NOTE

A PCR-based method to differentiate between *Acinetobacter baumannii* and *Acinetobacter* genomic species 13TU

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ABSTRACT

A new PCR-based method that exploits differences in *gyrB* gene sequences was developed to distinguish between *Acinetobacter baumannii* and *Acinetobacter* genomic sp. 13TU. Among 118 clinical and reference *Acinetobacter* strains, 102 of which were previously speciated by amplified rDNA restriction analysis as belonging to the *Acinetobacter calcoaceticus–A. baumannii* complex, the method correctly identified 31 *A. baumannii* and 54 *Acinetobacter* genomic sp. 13TU isolates to the species level. The method was rapid, specific and easy to interpret.

Keywords *Acinetobacter*, genomic species, *gyrB* genes, identification, PCR, speciation

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Members of the genus *Acinetobacter* are now recognised as important nosocomial pathogens, with *Acinetobacter baumannii*, in particular, causing serious problems in the intensive care unit setting because of its innate and acquired antimicrobial resistance and its tendency for epidemic spread. The unnamed *Acinetobacter* genomic spp. 3 and 13TU are also involved in nosocomial infections, but to a lesser extent. These three species are commonly grouped with the environmental organism *Acinetobacter calcoaceticus* in the *A. calcoaceticus–A. baumannii* complex because of the difficulty in separating these species using phenotypic methods [1,2]. Manual and semi-automated commercial identification systems, e.g., API 20NE, VITEK 2, Phoenix and MicroScan WalkAway, do not differentiate among these species, resulting in misidentification of *c.* 25% of *Acinetobacter* isolates belonging to the *A. calcoaceticus–A. baumannii* complex as *A. baumannii* [3], and there is no recognised biochemical method to distinguish reliably between *A. baumannii* and genomic sp. 13TU [1]. DNA–DNA hybridisation is the reference standard method to differentiate among these species, but this technique is very laborious and is not used routinely. Of the PCR-based methods, amplified rDNA restriction analysis is recognised as providing good discrimination, but can be time-consuming, while tRNA fingerprinting does not discriminate between *A. baumannii* and genomic sp. 13TU [4,5]. More recently, sequencing of the *rpoB* gene and its flanking spacer regions, and of the 16S–23S rRNA gene spacer region, have been proposed for identification of *Acinetobacter* isolates to the species level [6,7], but it is unlikely that these sequencing techniques will be used routinely, except in a few specialised reference laboratories.

As part of an ongoing project to evaluate a multilocus sequence typing scheme for *A. baumannii* and genomic sp. 13TU, interspecies heterogeneity was observed in the *gyrB* gene. The working hypothesis of the present study was that this could be exploited to develop a PCR-based method to differentiate between these two genomic species.

In total, 118 clinical, type and reference strains were used. These comprised 31 *A. baumannii* and 54 *Acinetobacter* genomic sp. 13TU clinical isolates (epidemiologically unrelated by pulsed-field gel electrophoresis) [3,8], the *A. baumannii* type strain ATCC 19606T^T, *Acinetobacter* genomic sp. 3 (13 isolates), *A. calcoaceticus* (3), *Acinetobacter haemolyticus* (2), *Acinetobacter johnsonii* (2), *Acinetobacter junii* (3), *Acinetobacter lwoffii* (4), *Acinetobacter radioresistens* (3), *Acinetobacter* genomic sp. 6 (1) and *Acinetobacter* genomic sp. 10 (1). The type strains *A. calcoaceticus* ATCC 23055^T, *A. johnsonii* ATCC 17909^T, *A. junii* ATCC 17908^T, *A. lwoffii* ATCC 13509^T, *A. radioresistens* SEIP 12.81, genomic sp. 3 ATCC 19004^T, genomic sp. 6 ATCC 17979^T, genomic sp. 9 ATCC 9957^T and genomic sp. 10 ATCC 17924^T were included. Clinical isolates had been identified previously to the

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species level using amplified rDNA restriction analysis [5] and the simplified phenotypic identification scheme for *Acinetobacter* spp. devised by Bouvet and Grimont [9].

The *gyrB* gene sequences from *A. baumannii* and *Acinetobacter* genomic sp. 13TU were aligned and three primers were designed; two were universal to both species, sp4F (5′-CACGCCGTAAGAGTGCATTA) and sp4R (5′-AACGGAGCTTGTCAGGGTTA), and one differed at the 3′ end from genomic sp. 13TU, sp2F (5′-GTTCCTGATCCGAAATTCTCG). Gradient PCR was performed to determine the optimum annealing temperature for sp2F so that it would yield a PCR product with *A. baumannii*, but not with genomic sp. 13TU; thus, in a PCR with all three primers, both *A. baumannii* and genomic sp. 13TU would yield an amplicon of 294 bp (sp4F to sp4R) but only *A. baumannii* would yield a second amplicon of 490 bp (sp2F to sp4R). The eventual multiplex PCR was performed using *Taq* PCR Master Mix (Qiagen, Hilden, Germany), with a final volume of 25 μL or 50 μL and primer concentrations of 0.2 μM. Amplification comprised 94°C for 2 min, followed by 25 cycles of 94°C for 1 min, 60°C for 30 s and 72°C for 1 min, with a final extension at 72°C for 10 min. PCR products were analysed on agarose 1.2% w/v gels, stained with ethidium bromide, and visualised on a UV transilluminator.

PCR with the three-primer mix identified all 32 *A. baumannii* isolates correctly. In every case, two clear bands were visible on agarose gels, while all 54 genomic sp. 13TU isolates yielded only the lower 294-bp band (Fig. 1). The remaining 32 *Acinetobacter* isolates (i.e., belonging to genomic species other than *A. baumannii* and genomic sp. 13TU) failed to produce any PCR products. No false-positive or false-negative reactions were observed. Results were obtained in <2.5 h from an agar plate to a finished gel, and identification could be achieved with either purified DNA or crude cell lysates.

The use of *gyrB* as a means to identify *Acinetobacter* isolates to the species level is not new. It was demonstrated over a decade ago that nucleotide and amino-acid sequences can both be used for taxonomic purposes, and that they correlate with DNA–DNA hybridisation [10]. It was also shown that *A. baumannii* and *Acinetobacter* genomic sp. 13TU cluster together [11]. The *gyrB* gene sequences of *A. calcoaceticus* and *Acinetobacter*

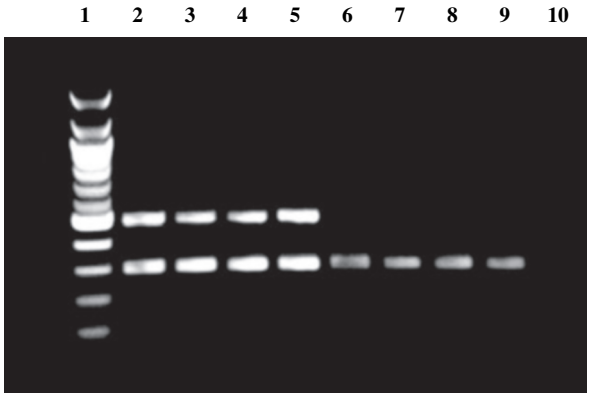

Fig. 1. Example of agarose gel showing *Acinetobacter* isolates speciated by PCR using *gyrB*-directed primers. Lanes: 1, 100-bp ladder; 2, *Acinetobacter baumannii* ATCC 19606; 3–5, *A. baumannii* clinical isolates; 6–9, *Acinetobacter* genomic sp. 13TU clinical isolates; 10, negative control with no DNA template.

genomic sp. 3 also cluster together [11], thereby allowing the possibility of expanding the present PCR method to achieve complete differentiation of the *A. calcoaceticus–A. baumannii* complex in a single reaction.

The species that form the *A. calcoaceticus–A. baumannii* complex have been grouped together because they are genetically closely related and phenotypically very difficult to differentiate from each other. However, there are considerable epidemiological and clinically relevant differences among these species. *A. calcoaceticus* is an environmental organism that, to our knowledge, has never been involved in serious human disease, and which should therefore not be misidentified as *A. baumannii*. The natural habitats of *A. baumannii* and genomic sp. 13TU are unknown, as are the differences in their epidemic behaviour, resistance mechanisms and pathogenicity. *Acinetobacter* genomic sp. 3 can be found regularly on human skin, as well as in aquatic environments. Genomic sp. 3 has been implicated in nosocomial infections, but its tendency for epidemic spread and resistance development is far less pronounced than that of *A. baumannii* [8,12]. For epidemiological and clinical purposes, it is therefore highly desirable to differentiate among these species correctly. At present, sufficient *gyrB* sequences for genomic sp. 3 are not available to allow the expansion of the *gyrB* PCR method to identify genomic sp. 3. For the time being,

isolates that are identified as *A. baumannii* by commercial identification methods, but that fail to yield a PCR product, may be identified tentatively as genomic sp. 3 if they grow at 41°C but fail to grow at 44°C.

The *gyrB* PCR method is robust and reproducible, and can yield a result in <2.5 h. Its simplicity means that it can be employed readily in most laboratories, where it should contribute to a better understanding of the epidemiology and clinical significance of the two most important *Acinetobacter* species.

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RESEARCH NOTE

Specific detection of bla_{VIM} and bla_{IMP} metallo-β-lactamase genes in a single real-time PCR

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ABSTRACT

This study describes the development of a real-time PCR protocol for rapid detection of the most common bla_{VIM} (bla_{VIM-1}, bla_{VIM-2}, bla_{VIM-3}, bla_{VIM-4}, bla_{VIM-5}, bla_{VIM-6}, bla_{VIM-10}, bla_{VIM-11}, bla_{VIM-12}) and bla_{IMP} (bla_{IMP-1}, bla_{IMP-2}, bla_{IMP-6}, bla_{IMP-8}, bla_{IMP-10}, bla_{IMP-15}, bla_{IMP-19}, bla_{IMP-20}) genes in a single reaction. The genes were specifically detected and clearly differentiated into four groups, i.e., (i) bla_{VIM-1}-like (bla_{VIM-1}, bla_{VIM-4}, bla_{VIM-5}, bla_{VIM-12}); (ii) bla_{VIM-2}-like (bla_{VIM-2}, bla_{VIM-3}, bla_{VIM-6}, bla_{VIM-10}, bla_{VIM-11}); (iii) bla_{IMP-1}-like (bla_{IMP-1}, bla_{IMP-6}, bla_{IMP-10}); and (iv) bla_{IMP-2}-like (bla_{IMP-2}, bla_{IMP-8}, bla_{IMP-15}, bla_{IMP-19}, bla_{IMP-20}), by melting curve analysis of the real-time PCR products. The protocol was used to screen positive bla_{VIM-1}, bla_{VIM-2} and bla_{IMP-1} control strains, 70 Gram-negative isolates resistant to carbapenems, and 30 Gram-negative isolates susceptible to carbapenems (negative controls).

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