Genotyping of isolates included in the description of a novel species should be mandatory

The ad hoc committee for the re-evaluation of the species definition in bacteriology encouraged researchers to base a species description on more than a single strain and to apply DNA profiling (i.e. genotyping) as a method of discrimination at the subspecies level (Stackebrandt *et al.*, 2002). Genotyping avoids a species being defined on the basis of isolates that could belong to the same strain. This may be critical for isolates recovered from the same source and/or site, where different strains or multiple isolates of the same strains may co-exist. This letter aims to support the committee's recommendation by highlighting the need for the use of genotyping techniques in the description of novel taxa. To illustrate this, we investigated the three most recently described species of *Aeromonas*. These are: *Aeromonas culicicola*, described on the basis of the type strain MTCC 3249^T, isolated from the midgut of the mosquito *Culex quinquefasciatus*, and strains SH and SLH, both from the mosquito *Aedes aegyptii* (Pidiyar *et al.*, 2002); *Aeromonas simiae*, described on the basis of two strains (CIP 107798^T and CIP 107797) recovered from the faeces of the monkey *Macaca fascicularis* (Harf-Monteil *et al.*, 2004); and *Aeromonas molluscorum*, described on the basis of five strains recovered from bivalve molluscs (Miñana-Galbis *et al.*, 2004). In the studies describing *A. culicicola* and *A. simiae*, genotyping techniques were not applied, while they were in the study of *A. molluscorum*.

We genotyped all available strains of the species mentioned above using enterobacterial repetitive intergenic consensus sequence (ERIC)-PCR, a method that has been shown previously to be highly discriminatory for *Aeromonas* (Sechi *et al.*, 2002; Soler *et al.*, 2003; Szczuka & Kaznowski, 2004; Aguilera-Arreola *et al.*, 2005). The primers and conditions were those of Soler *et al.* (2003). This method proved that the two strains SH and SLH of *A. culicicola* showed

the same pattern (Fig. 1a), as was also the case for the two strains of *A. simiae* (Fig. 1b). Random amplification of polymorphic DNA (RAPD) analysis and pulsed-field gel electrophoresis (PFGE) (data not shown) further confirmed this. Furthermore, a re-evaluation of the hydrolysis of aesculin showed that the two strains of *A. simiae* are indistinguishable, contradicting the findings of Harf-Monteil *et al.* (2004). In contrast, the five strains of *A. molluscorum* showed individual ERIC-PCR patterns (Fig. 1c), which confirmed previous amplified fragment length polymorphism (AFLP) results (Miñana-Galbis *et al.*, 2004). In conclusion, in the cases of *A. culicicola* and *A. simiae*, where no typing methods were included in the original publications (Pidiyar *et al.*, 2002; Harf-Monteil *et al.*, 2004), two isolates of a single strain were included in the species description.

Fig. 1. ERIC-PCR patterns of *Aeromonas* strains. (a) *A. culicicola* strains MTCC 3249^T, SH and SLH; (b) *A. simiae* strains CIP 107797 and CIP 107798^T; (c) *A. molluscorum* strains CECT 5864^T, CECT 5867, CECT 5865, CECT 5866 and CECT 5868 (from left to right).

Since the discriminatory power of typing methods varies among bacterial genera or species, the genotyping technique(s) employed should be that proven to be useful for the specific taxa under investigation. If no previous comparative analysis of typing methods exists, several techniques should be applied simultaneously, introducing a control strain to recognize the most discriminatory method (Buczolits *et al.*, 2002).

Acknowledgements

The Spanish Ministry of Health (FIS03/1183) supported this research. We would like to thank Drs Pidiyar, Harf-Montiel, Esteve and Lorén for kindly providing isolates.

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