

## **R-041. The Diversity of the Genus *Acinetobacter* Determined by AFLP Analysis**

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The genus *Acinetobacter* comprises 31 genomic species delineated by DNA-DNA hybridization. Only 17 of these genomic species have valid species names. Identification of several named and most unnamed species is problematic which hampers their study at species level. In the present study, AFLP analysis, a high resolution genomic fingerprinting method, was applied to assess the diversity among 2100 isolates of *Acinetobacter* collected over a 20 years' period. Most isolates were from human clinical samples; others were from environmental origin or animals. The collection comprised single isolates and 182 sets from outbreaks and sets of multiple isolates from individual patients each. For AFLP, restriction of bacterial DNA was done with EcoRI and MseI; for PCR a Cy-5 labelled EcoRI+A and Mse+C primer (A, C= selective bases) were used. Electrophoresis and laser fluorescent fragment detection were done with a semi-automated slabgel system. Cluster analysis of fingerprints was performed with Pearson's coefficient as a similarity measure and UPGMA as a clustering criterion. Initial analysis of a training set of 267 reference strains of all described species, identified by DNA-DNA hybridization, the gold standard, showed that strains of the same species clustered at  $\geq 50\%$  with all species being well separated from each other. At the thus validated (50%) species delineation level, 1570 isolates could be identified to described species. Among the remaining 263 isolates 31 clusters of multiple isolates and 26 unique strains were distinguished, indicating the possible existence of 57 novel (genomic) species. Multiple isolates from outbreaks or single patients clustered well above 90%, the thus defined strain delineation level. At this level, 1200 unique profiles or highly similar profile groups were distinguished among the 2100 isolates. The study shows that AFLP is a powerful tool to identify *Acinetobacter* species and strains. Results also emphasize the great diversity of the genus *Acinetobacter* with a high number of putative novel species to be analyzed in more detail.