Letters to the Editor

Is AFLP Fingerprinting a True Alternative to the DNA-DNA Pairing Method To Assess Genospecies in the Genus *Aeromonas*?

In a recent issue of the *International Journal of Systematic Bacteriology*, Huys and colleagues (9) report a numerical taxonomic study of the genus *Aeromonas* using the band patterns obtained by restriction fragment selective amplification of chromosomal DNA as characters. The aim of this paper was to evaluate the AFLP technique used to discriminate the DNA hybridization groups delineated in the genus *Aeromonas*. The authors justified their method when consistency between AFLP results and DNA-DNA pairing data was observed; however, they were not able to explain satisfactorily the cases of inconsistency. Among them, they reported that *A. encheleia* strains grouped with some *A. eucrenophila* strains and that *A. allosaccharophila* strains clustered with some representatives of *A. veronii*. Nevertheless, the strains of *A. encheleia* and *A. allosaccharophila* were respectively segregated from the type strain of *A. eucrenophila* (NCIMB 7417) and *A. veronii* (ATCC 35624) as expected from the results of DNA-DNA hybridization (3, 4) and 16S rRNA sequencing (10). Despite these genomic data, the authors supposed that additional DNA-DNA hybridizations using *Aeromonas* strains other than the types would give results more in accordance with their proposals. But this consideration leads to confusion in the species definition because phenotypic and genotypic characteristics of the type are the standard to delineate the biological species. Up to now, all DNA hybridization groups in the genus *Aeromonas* have been constituted by comparison with type strains (1-8, 11).

Examination of the paper of Huys et al. (9) suggests that the inconsistency between AFLP results and DNA-DNA pairing data could be caused by other facts. The authors reported a high level of reproducibility of AFLP patterns based on the correlation observed for reference lanes of strain LMG 13459 (95.0 to 98.5%). However, the numerical analysis showed that two replicates of the same *A. allosaccharophila* strain, LMG 14021 (ATCC 35942) and LMG 16184 (CDC 0715-84), were related at a lower correlation level (70%). The authors did not realize that both strains were in fact replicates obtained from different culture collections, which leads to some misunderstanding in the taxonomic position of *A. allosaccharophila*. Likewise, the strains LMG 9075T and LMG 16184, which were joined by two correlation observed for reference lanes of strain LMG 134459, were clustered at a correlation level of 70%, but their genomic relatedness is of 100% as stated by DNA-DNA pairing method determines the relationship between strains on the basis of the homology degree of total chromosomal DNA, which is directly measured from the hybrid DNA.

In the AFLP method the relationship between strains is based only on the size of chromosomal DNA restriction fragments which have been selectively amplified by PCR. Moreover, a numerical analysis is needed to compare AFLP fingerprints, and so the method for estimation of the resemblance might influence the final results (12). In contrast, the DNA-DNA pairing method determines the relationship between strains on the basis of the homology degree of total chromosomal DNA, which is directly measured from the hybrid DNA. For the present, AFLP fingerprinting is less accurate; therefore, DNA-DNA pairing method remains the best technique to assess the genospecies level and thus to refine *Aeromonas* taxonomy.

**REFERENCES**


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**Authors’ Reply**

One of the most striking inconsistencies between our AFLP data and the previously reported DNA-DNA hybridization data was the finding of two genotypic subgroups in the species *Aeromonas eucrenophila* (6). The first subgroup contained the type strain of this species, whereas the second subgroup comprised three *A. eucrenophila* strains that were joined by two representatives of DNA hybridization group (HG) 11 (a currently unnamed *Aeromonas* taxon) and a group of four strains which was recently described as *Aeromonas encheleia* and containing its type strain (3). To us, the latter result indicated that the genotypic core of the new species *A. encheleia* may also comprise reference strains of two previously recognized *Aeromonas* taxa. Consequently, we proposed to include more strains other than the type strain to perform additional DNA-DNA hybridizations in the “*A. eucrenophila*-A. encheleia-...
This proposal, so it seems, is being considered by Dr. Esteve to be an attempt by us to question the use of type strains for nomenclatural conclusions. This is certainly not the case. Only because *A. eucrenophila* was found to be genotypically heterogeneous (6), we set out to investigate if the type strain indeed covered all strains with the same species name. As we announced in our paper (6), we have continued with experiments (4) that focus on the apparent inconsistency between our fingerprinting data (6) and previously reported DNA pairing results (3, 8) by (i) including more *A. eucrenophila*-like strains from diverse geographical locations and (ii) thereby using AFLP analysis, ribotyping, protein profiling, and fatty acid analysis. This further polyphasic investigation uniformly confirmed our previous findings (4). In fact, that is why polyphasic taxonomy is so powerful (9), justifying the need for decisive DNA-DNA hybridizations including more than only the type strains of different species (3). Within the "A. eucrenophila-*A. encheleia*-HG11" complex, we have recently obtained new DNA-DNA hybridization results (5) which fully agree with the taxonomic interpretation revealed by AFLP analysis (6).

A second important inconsistency was the finding that the three strains of the species *A. allosaccharophila*, including the type strain, genotypically group together with representatives of *Aeromonas veronii* (including its type strain) in our AFLP cluster VIII (6). DNA-DNA hybridization data (2), on the other hand, indicated that both species constitute separate HGs, although homology values as high as 40% between *A. veronii* biogroup sobria ATCC 9071 and *A. allosaccharophila* ATCC 35942 have been reported. As we proposed in our paper (6), it is obvious that additional DNA-DNA hybridizations are required to elaborate on the taxonomic positions of both species. In this context, it should be noted that the AFLP patterns of the *A. allosaccharophila* replicate strains LMG 14021 and LMG 16184 were linked at a correlation level of 79.3%, not at 70%, as was stated by Dr. Esteve. In spite of this relatively low level of correlation, which resulted from the comparison of two AFLP gels with different electrophoresis times, we believe that repeating these experiments will only confirm the genotypic classification of *A. allosaccharophila* in the *A. veronii* complex. Nonetheless, one would indeed expect a correlation of nearly 100% between the AFLP patterns of two genotypically stable duplicates when samples are run close to each other on the same gel. Between different gels, however, we adopted the rule that the correlation level of the reference tracks should be at least 90% in order to allow a reliable numerical analysis of the digitized AFLP data (7). This criterion clearly affects the final linkage level between strains originating from different gels but, from our experience, has no pronounced effect on their relative taxonomic position in an AFLP-based cluster analysis. In this context, it has also been demonstrated that a limited genomic variation between representatives of the same bacterial clone does not significantly affect the grouping of such strains by AFLP methodology (1). Likewise, we find it not contradictory that two strains showing 100% genomic relatedness (e.g., LMG 9075 and LMG 16183) cluster at only 80.3% (not at 70%, as was noted in Dr. Esteve’s letter) with AFLP.

Two bacterial genomes that differ slightly in genomic size or organization may still exhibit high DNA homology values, whereas their AFLP banding patterns may differ by a significant number of bands. In fact, the reported percentages can be somehow expected when the cutoff levels for the delineation of HGs in the genus *Aeromonas* are compared for DNA-DNA hybridizations (>70% DNA similarity) (10) and AFLP analysis (40 to 50% Pearson product-moment correlation) (6).

Finally, we find it necessary to further clarify the meaning of the term alternative in the conclusion of our paper (6) because this part has apparently created some confusion for Dr. Esteve. The authors fully agree that DNA-DNA pairing remains the standard method to define genomic species in *Aeromonas* and other genera, and we never suggested that AFLP could actually replace this technique for species delineation. Rather, our data (6) indicate that AFLP fingerprinting can be used in (i) genotypic identification of unknown *Aeromonas* isolates and (ii) screening for new genomic groups in the genus *Aeromonas*. In the latter case, it is obvious that any significant taxonomic finding should be confirmed by DNA hybridization studies.

REFERENCES


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