

This Week's Citation Classic®

Palleroni N J, Kunisawa R, Contopoulou R & Doudoroff M. Nucleic acid homologies in the genus *Pseudomonas*. *Int. J. Syst. Bact.* 23:333-9, 1973.
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This paper demonstrates the power of ribosomal RNA/DNA hybridization experiments as tools for the resolution of the internal complexity of *Pseudomonas*, a genus composed of groups of bacteria that are only distantly related. The methodology has also revealed relationships between some of the groups and species of other bacterial genera, thus forecasting a reexamination of phylogenetic relationships among bacterial groups. [The SCI® indicates that this paper has been cited in over 150 publications.]

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Two papers on *Pseudomonas* taxonomy have qualified as *Citation Classics*, and this is rather surprising for two main reasons. First, *Pseudomonas* is only one in a multitude of bacterial genera, and, more important, bacterial taxonomy often fails to attract the attention of the scientific community, which includes the taxonomists themselves.

The two papers mark, respectively, the beginning and the end of a period dedicated to *Pseudomonas* taxonomy in the Department of Bacteriology of the University of California, Berkeley, an activity grafted as an intermission in a long and distinguished tradition of biochemical research on many strains of the group.

The first *Citation Classic*¹ proposed a system of phenotypic classification of *Pseudomonas* species and included methodological recommendations of interest to taxonomists, but biochemists and geneticists found in it a handy source of information on the physiological properties of many strains. On the other hand, the paper discussed here introduced new approaches to the taxonomy of *Pseudomonas* and has been frequently cited for the proposal of a scheme of internal subdivision of the genus on the basis of ribosomal RNA similarities. However, in my opinion, the significance of the paper has been of a more general nature, reaching beyond the restricted field of taxonomy of a single genus.

The DNA/DNA hybridization work that followed the phenotypic analysis of the strains of *Pseudomo-*

nas had shown negligible DNA sequence similarities among members of different phenotypic groups. On the basis of these results, I proposed the use of conservative regions of the bacterial genome for the hybridization experiments. Among these regions, the ribosomal RNA genes appeared to be the obvious candidates.^{2,3}

The experiments of rRNA/DNA hybridization, carried out with the able collaboration of Riyo Kunisawa and Rebecca Contopoulou, gave the results reported in this paper, clearly defining five RNA similarity groups deserving independent generic allocations. These taxonomic conclusions were later fully confirmed in many other laboratories.⁴ Interestingly, our results also included a free bonus by showing that some *Pseudomonas* groups were closer to members of other genera (*Escherichia*, *Xanthomonas*) than to other pseudomonads. These unexpected findings demonstrated the power of rRNA similarity studies as tools for exploring distant relationships among bacterial groups and suggested routes for future experimentation, namely, to extend the analysis of rRNA similarities to comparisons among members of other bacterial groups.

Unfortunate circumstances that eventually led to the dispersion of the taxonomy team conspired against the continuation of such studies. At the time we were to start our hybridization experiments, Roger Stanier had decided to leave Berkeley and join the staff of the Pasteur Institute, and Mike Doudoroff was fighting a losing battle against poor health. Shortly before his untimely death, Mike managed, however, to collaborate in the writing of the present paper, which was published some months after I had moved to the eastern US. In 1973 I communicated our findings to a receptive audience at the First International Bacteriology Congress in Jerusalem.

As expected, some years later the seminal ideas contained in this paper bloomed in other laboratories either through modification of our competition hybridization technique or the more sophisticated approach of direct comparison of sequences of rRNA components. It is not necessary here to mention the magnitude of the revolution of ideas on bacterial evolution catalyzed by such studies. What remains is the satisfaction that the studies on *Pseudomonas* may have helped in some measure to inspire the present trend in the study of bacterial phylogeny. Seen in retrospect, the *Pseudomonas* example was just the tip of an iceberg whose real dimensions are still being evaluated.

1. Stanier R Y, Palleroni N J & Doudoroff M. The aerobic pseudomonads: a taxonomic study. *J. Gen. Microbiol.* 43:159-271, 1966. (Cited 1,305 times.) [See also: Stanier R Y. *Citation Classic*. (Barrett J T, ed.) *Contemporary classics in the life sciences. Volume 1: cell biology*. Philadelphia: ISI Press, 1986. p. 47.]
2. Doi R H & Igarashi R T. Conservation of ribosomal and messenger ribonucleic acid cistrons in *Bacillus* species. *J. Bacteriology* 90:384-90, 1965. (Cited 80 times.)
3. Dubnau D, Smith I, Morell P & Marmur J. Genetic conservation in *Bacillus* species and nucleic acid homologies. *Proc. Nat. Acad. Sci. USA* 54:491-8, 1965. (Cited 140 times.)
4. Palleroni N J. The taxonomy of bacteria. *BioScience* 33:370-7, 1983. (Cited 5 times.)