

Working with your Hands Tied behind your Back or Throwing off the Shackles: FT-IR in the Identification and Characterisation of Prokaryotes

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It is a paradox that while many scientists consider identification to be an important service function resulting from taxonomy, it also imposes inherent constraints on what we can achieve when we undertake the identification of microbial isolates. Perhaps the greatest difficulty which we face at present, when trying to identify prokaryotes, is the fact that only a fraction of the extant prokaryotic species have been properly characterised, classified and named. Without these three stages we do not have a comprehensive known reference pool against which to identify our unknown organisms. Identification systems are confronted with a problem when challenged with large numbers of novel species. The extent of the difficulties presented by novel organisms depends to a certain degree on the nature of the methods used.

An alternative to the identification of strains is their sorting into groupings which indicate which organisms are "similar" and which are "different". This approach has not been widely used, perhaps because one believed in the past that one could easily identify the vast majority of strains isolated. The realisation that from any one environmental sample we may be able to obtain a wide range of novel species has presented us with other problems. In the past enrichment methods tended to select for a few species, distorting our picture of prokaryotic diversity. In order to gain an overview of those strains which can be isolated it is more appropriate to use a wide range of different media and also to isolate a large number of strains.

It is the problem of handling large numbers of isolates in order to characterise them quickly and efficiently which has begun to present itself as a significant problem within the last few years. A variety of methods have been used, but many of them are either too time consuming or too expensive. The application of FT-IR to environmental isolates is not novel, but the problems presented when dealing with nearly 1,000 strains of various taxa quickly become apparent to the expert. Rather than attempting to identify environmental isolates against an inherently limited database another approach has been taken - that of strain sorting. In addition no attempt has been made to search for the "optimal" presentation of results, but to sort according to "very similar" and "different". An approach analogous to bootstrap methods has been applied manually. The initial results indicate that the method is promising, and that it adds a new dimension to the use of FT-IR analysis in the characterisation of prokaryotes, freeing it from the inherent limitations of being just an "identification system". Comparison against other methods indicates that the resolution of the system is at the species - subspecies - strain level (Tindall *et al*, 2000).

Tindall, B.J., Brambilla, E., Steffen, M., Neumann, R., Pukall, R., Kroppenstedt, R.M., & Stackebrandt, E. (2000) Cultivable Microbial Biodiversity: Gnawing at the Gordian Knot. *Environmental Microbiology* 2: 310-318