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Chemotaxonomy: Wasting Time or Useful Tool for Classification of Bacteria?

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Chemotaxonomy is part of phenotypic characterization of bacteria and the need to carry out chemotaxonomic work, particularly when defining new genera and families was emphasized by the 'ad hoc committees' (Wayne *et al.,* 1987; see also Murray *et al*., 1990).

The term chemotaxonomy designates a set of methods dealing with the analysis of small cellular components such as fatty acids, polar lipids, quinones, peptidoglycan composition, polyamines, lipopolysaccharides and pigments for classification of microorganisms. Analyses of whole cells by MALDI-TOFF or FTIR may be covered by this term as well. Fatty acids and quinones are integral parts of the majority of descriptions of novel bacterial species and peptidoglycan composition is provided for description of many Gram-positive species. In contrast, polar lipids, polyamine patterns and pigment analyses are only rarely examined for characterisation of novel bacterial isolates though their importance was demonstrated. All these approaches have advantages and limitations depending on the bacterial lineage an isolate is affiliated and no generalization to which taxonomic level a certain approach is applicable can be made. In this contribution the importance of fatty acids, quinone systems, peptidoglycan composition, polar lipid profiles and polyamine patterns is discussed.

Though fatty acid analysis requires the most expensive equipment this approach is widely applied for classification of bacteria. The importance of fatty acids is based on their high structural variability (length of the carbon chain, straight chain or branched chain, degree of saturation, and presence of modifications such as hydroxy groups or cyclo-propane rings at different positions of the carbon chain) and their application is facilitated by the availability of numerous reference compounds for identification of fatty acids and a software for identification at the species level. In several bacterial lineages the composition of major compounds in the fatty acid profile may be indicative for affiliation of a novel species to an existing genus. However, in certain genera, such as *Deinococcus*, no common characteristics are shared in the fatty acid profile by all species or almost no variability among species of a family is detected

as shown for members of the family *Microbacteriaceae*.

Analysis of the quinone system is one of the earliest chemotaxonomic methods applied for characterization of bacteria. The discriminative power of this approach relies on the detection of either ubiquinone or menaquinone and in differences of the isoprenoid side chain. Among members of *Proteobacteria* and *Bacteroidetes* the quinone system is usually conserved within families or even at higher taxonomic ranks but may even differ between closely related genera. Within actinobacterial taxa numerous related genera can be distinguished based on differences in their quinone systems (varying in numbers of isoprenoid units in the side chain and/or degree of saturation of isoprenoid units). Exceptions are known where species of a single genus differ in their quinone system such as *Microbacterium* and *Mirococcus*.

Polar lipids have been introduced to the classification of bacteria more than 30 years ago but nowadays they are the most underestimated tool for classification of novel bacterial taxa. While there are genera in which polar lipid profiles of the species exhibit none or only minor differences in other genera species show characteristics supporting their assignment to the genus but also contain lipids useful for differentiation between species. Unfortunately many polar lipid profiles exhibit the presence of unknown compounds which can be only characterized during analysis procedure by their chromatographic behaviour and their reaction with specific spray reagents. Despite lack of knowledge of their names or structures they are of the same importance for classification as known lipids are.

The discriminating power of the peptidoglycan composition is apparently restricted to Gram-positive bacteria whereas no variations have been reported among *Proteobacteria* and *Bacteroidetes*. Analyses of the peptidoglycan structure can be performed at different levels. Easy to perform is the determination of the characteristic diamino acid. Analysis of the position of the amino acid in the peptide side chain conferring cross-linkage to the adjacent chain (peptidoglycan type A or B), mode of cross-linkage (direct or interpeptide bridge and amino acids in the bridge) and complete amino acid compositions provides more detailed information. Usually, species of a genus exhibit identical amino acid composition of the peptide side chain including the characteristic diamino acid. Some variability may be detected in the composition of the interpeptide bridge among species of the same genus.

Polyamines can be detected in the majority of prokaryotes. Although only a rather limited number of polyamines (di-, tri- and tetraamines) can be detected the suitability of analysis of polyamine patterns has been demonstrated for classification of *Proteobacteria*. Among *Alpha-* and *Gammaproteobacteria* often they discriminate between related genera whereas the polyamine patterns of *Betaproteobacteria* are rather homogeneous. Even among genera of the Gram-positive family *Microbacteriaceae* some significant differences were shown which appear to be either genus specific or shared by representatives of few related genera.

In conclusion, analyses of the quinone system and polyamines are usually most useful to describe genera, families or even higher taxa and to demonstrate affiliation to these taxa whereas analyses of fatty acid and polar lipid profiles are most suitable for characterization and differentiation of a novel species from close relatives.

References

1. Murray *et al*., 1990. IJSB. 40: 213-215.
2. Wayne *et al*., 1987, IJSB. 37: 463-464.