Use of Fatty Acids for the Identification of Phytopathogenic Bacteria

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Whole-cell fatty acid (FA) profiles have been used in bacterial classification for over 20 years and have become increasingly important in bacterial identification. Any microbiologist with access to a gas chromatograph (GC) can perform FA analysis.

Under standardized conditions, bacterial FAs are stable and reproducible within taxa (though they are modified in predictable fashion with environmental changes.) The FA profiles are highly diagnostic for a given species regardless of geographical source, though for some groups of pathogens there are subgroups related to host range. Fatty acid-based groupings have so far agreed with DNA homology, where DNA homology data are available.

The FAs between nine and 20 carbon lengths are most useful in identification of plant-associated bacteria. Presently, over 200 FAs are used for identification purposes, and these include saturated, unsaturated, hydroxy, cyclopropane, iso, and anteiso FAs (3).

Methods. For pure cultures, approximately 40 mg of wet cells is placed in a screw-capped test tube, and the FAs in whole cells are saponified, methylated, extracted into hexane/ether, and chromatographed (2,3).

It is possible to extract bacterial FAs directly from infected plant tissue for identification to the genus level and often to the species level, taking care to exclude as much plant lipid as possible. Since the bacteria grew in plant tissue, their FA profiles will be slightly different from their counterparts grown under controlled laboratory conditions. Improved methods for this extraction technique are being developed.

Any GC can be used for identification of bacteria, but capillary column GC (5% phenyl methyl silicone) gives the best resolution of peaks. Without a computer, chromatograms of unknowns can be compared with those of type or known strains by placing one chromatogram over the other and holding both up to a light source to see if the patterns are similar or different. This will distinguish genera—for example, *Xanthomonas* from *Pseudomonas*—but may not permit resolution at the species or pathovar level. Additionally, the FAs responsible for each peak are not identified or quantified. The individual component FAs can be identified by comparisons with FA standards purchased from fine chemical supply houses. FA names, peak relative retention time, and overall profiles for known strains published in the literature can also be used as references. For most genera, qualitative differences in FAs can be used for identification. For best results at the species or subspecific levels, the ratios between FAs that are most stable (i.e., the straight chain, iso, and anteiso FAs) should be calculated.

Over 17,000 samples per year can be analyzed on an automated GC system with an automatic sampler, an integrator, and a computer (Microbial ID, Inc., Newark, DE). A computer program calculates the peak areas and compares the unknown FA profiles with those of known reference strains in the data base. A similarity index expresses how nearly the profile of the unknown bacterium fits the closest species match in the data base. At present, the data bases contain FA profiles of over 300 species of aerobic bacteria and over 100 species of anaerobic bacteria. The software allows the user to expand or create data bases by adding FA profiles of additional species.

Examples of fatty acids in phytopathogenic bacteria. Within *Agrobacterium tumefaciens*, the three biotypes can be distinguished primarily by relative amounts of cyclopropane FAs. The tumor-inducing plasmid does not affect whole-cell FA profiles.

Clavibacter michiganense subsp. *michiganense* has a simple profile characterized by iso and anteiso FAs. To delineate subspecies, ratios between major peak areas should be calculated.

Most members of the Enterobacteriaceae contain the FA 14:0 3OH. *Erwinia carotovora* subsp. *atroseptica* is distinguished from *E. c.* subsp. *carotovora* by the ratios of 12:0 to 14:0, of 16:0 to 12:0, and of 16:1 to 18:1 (1).

The fluorescent pseudomonads seldom contain more than a trace amount of 14:0 3OH (a major peak in *Pseudomonas cepacia* and related nonfluorescent bacteria) but do contain 10:0 3OH. Within *P. syringae*, it is possible to distinguish pathovars such as, for example, *P. s.* pv. *tomato* and *P. s.* pv. *papulans* from other pathovars of *P. syringae* on the basis of presence (others) or absence (*P. s.* pv. *tomato* and *P. s.* pv. *papulans*) of 17:0 cyclopropane.

In the genus *Xanthomonas*, 11:0 ISO, 11:0 ISO 3OH, 13:0 ISO 3OH, 15:0 ISO, and 15:0 ANTEISO are important components of a very complex profile, while calculation of ratios between 15:0 ISO and 15:0 ANTEISO is necessary to determine the species. *X. campestris* pathovars are by definition indistinguishable using biochemical tests, yet xanthomonads can often be discriminated at the pathovar level using ratios of FAs.

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