Identification of Coryneform Bacteria and Related Taxa by FT-IR Spectroscopy

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Coryneform bacteria and related taxa occur almost everywhere on living and non-living matter in the environment, in soil, on cellulosic plant fibres, on mammals, on smeared cheeses, even in subsurface sediments, and in antarctic samples. Several strains or species are classified as opportunistic or obligate human pathogens as well as animal and plant pathogenic species. Identification of these high GC gram positive bacteria is therefore not only of ecological, but also of medical and technological relevance.

An extensive Fourier-transformed infrared (FT-IR) spectroscopy database for the identification of bacteria from the two suborders *Micrococcineae* and *Corynebacterineae* (*Actinomycetales, Actinobacteria*) as well as other morphologically similar genera was established. The database consists of averaged infrared spectra from 730 reference strains, covering 220 different species out of 46 genera. 192 species are represented by type strains. The identity of 352 questionably classified reference strains was determined by comparative 16S rDNA partial sequence analysis and, as a result, 224 strains were reclassified accordingly.

All spectra were recorded and evaluated according to Kümmerle *et al.* (1998). The first derivation of the digitized original spectra was used. The optimal discriminatory spectral windows with weights and reproducibility levels determined were 3000-2800 cm^{-1} / 0.8 / 3.3; 1800-1500 cm^{-1} / 0.8 / 5; 1500-1200 cm^{-1} / 0.9 / 20; 1200-900 cm^{-1} / 0.9 / 33; 900-700 cm^{-1} / 0.9 / 116. In each window, the reproducibility level was adjusted such that a valid identification in the identity test could be expected up to a spectral distance of approximately 1.0 to 1.5 for the first hit.

An internal validation which was carried out using 208 newly recorded single spectra from 208 species out of 41 genera yielded values of 93.9 %, 98.1 % and 99.5 % for correct identification at the strain, species and genus level, respectively.

An external validation which was carried out using 544 strains from 54 species out of 16 genera resulted in a correct identification of 87.3 % at the species level and 95.4 % at the genus level. 12.7 % did not result in a correct identification at the species level, 1.3 % of which were not identified (spectral distance $D > 1.5$) while 11.4 % were misidentified. The species included were represented by a minimum of three strains per species in the database.

The performance of this identification system is well within the range of those having been reported in the literature for the identification of coryneform bacteria by different methods. It appears that coryneform and related taxa display a certain degree of overlapping distribution of different taxonomical markers, leading to a limited differentiation capacity of non-genotypical identification methods in general. However, easy handling, rapid identification within 25 h starting from a single colony, a satisfactory differentiation capacity and low cost render FT-IR technology clearly superior over other routine methods for the identification of coryneform bacteria and related taxa.

Kümmerle, M., Scherer, S. & Seiler, H. (1998). Rapid and reliable identification of fermentative yeasts by Fourier-transform infrared spectroscopy. *Appl Environ Microbiol* **64,** 2207-2214.