Discrimination of clinical and environmental isolates of Burkholderia contaminans, a high prevalent species within Taxon K of Burkholderia cepacia complex, using PCR fingerprinting genotyping and FT-IR spectroscopy-based phenotyping

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Burkholderia cepacia Complex (BCC) species are responsible for devastating lung infections in cystic fibrosis (CF) patients and various infections in inmunocompromised non-CF patients. In common with many other opportunistic pathogens, it appears that severe disease and death depend on the clinical state and predisposition of the patients at the time of infection [(1)]. BCC bacteria used to be mainly patient-to-patient transmitted. Nevertheless, due to the implementation of strict infection control practices in hospitals and to the education of patients the transmission of bacteria among them has been significantly reduced in the last years. However, these stringent infection control measures do not prevent the acquisition of BCC organisms from industrial settings, industrial products, or the natural environments [(1)]. In the last years, bacteria belonging to Group K or Taxon K of BCC have been isolated from sputum cultures of CF patients, in the UK, Italy, Portugal, USA, Canada, Brazil and Argentina, and have also been recovered from environmental samples such as river water, soil, roots, animals, industrial pharmaceutical products, personal care products, and domestic products[(2)]. Particularly, in Argentina Taxon K represents around 13% of the species recovered from sputum samples of CF patients and almost 80% of the BCC isolates. These isolates are characterized by exhibiting yellow-green pigment and *β*-haemolysis and have the *HaeIII recA*-RFLP K pattern being accurate identified by *recA* gene sequencing.

In this work whole cell FT-IR spectroscopy fingerprinting has been used together with hierarchical cluster analysis (HCA) to study clinical and industrial setting isolates belonging to *B. contaminans* species and to compare its typing schemes with genomic profiles based on repetitive intergenic consensus PCR (BOX-PCR).

Fifty clinical isolates and 10 industrial setting isolates identified as *B. contaminans* by *recA* sequencing were used. Like most phenotyping approaches, FT-IR spectroscopy samples had to be prepared under strict standardization culture conditions (growth medium, incubation time and temperature), and particularly for BCC species, we previously reported that spectra had to be acquired avoiding polyhidroxy acids (such as PHB) accumulation and removing pili and/or other protein appendages that might produce interference in IR spectra [(3)].

Molecular typing by PCR fingerprinting revealed a relatively high level of genotypic diversity showing at least 6 different variants for *B. contaminans* clinical isolates. In addition no evidences of genetic similarities were found between *B. contaminans* isolates from industrial settings and clinical isolates. HCA obtained with first derivative spectra analyzed in 3 mid IR ranges successfully confirmed the distinct genotypes within clinical isolates and was also capable to distinguish isolates derived from industrial settings providing therefore, a valuable taxonomic information.

We conclude that both PCR fingerprinting and FT-IR spectroscopy methods can be helpful tools for global epidemiological studies. Particularly, FT-IR spectroscopy can be considered a simple and rapid methodology for differentiating *B. contaminans* strains in sets of isolates obtained during outbreaks contributing to the control of infection spreading, for answering questions of clonality among microbial isolates, and also for allowing the identification of the strains that have the higher degree of transmissibility.

References:

[1] Mahentthiralingam, et al. *J App Microbiol.* (2008), [2] Dalmastri, et al. *Environmmental Microbiol*. (2007), [3] Bosch, et al. *J. Clin Microbiol*. (2008)