

Infrared spectroscopic and molecular characterization of clinical and environmental atypical Burkholderia cepacia complex strains from Argentina

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Burkholderia cepacia complex (BCC) is a group of bacteria comprising at least nine recognized species or genomovars. These gram-negative bacteria are associated with various opportunistic human infections. Particularly, they have been identified in lung infections of cystic fibrosis (CF) patients. BCC species are also widely distributed in the natural environment as water, soil, plants, industrial settings, and hospitals.

In previous studies we have proved the high reliability and strong potential of ANN-based FT-IR spectrum analysis for a rapid identification of the gram-negative rods suitable for routine diagnosis of BCC isolated from CF patients. Our research carried out with sputum samples of 150 CF patients hospitalized in three different CF health care centers of Argentina between 2004 and 2006 revealed that the majority of BCC infections were produced by genomovars I to IV isolates. Interestingly, almost 80% of these infections were caused by atypical strains, which were identified as *B. cenocepacia* (genomovar III) by *recA* species-specific PCR exhibiting a PCR-RFLP *Hae*III profile different from those previously described^[1]. Considering the high incidence of these *B. cenocepacia* atypical strains among local CF patients, we have tried to elucidate potential environmental reservoirs of these atypical bacteria and applied different FT-IR spectroscopy techniques to discriminate, characterize and phenotypically compare environmental BCC isolates with our local clinical atypical strains.

From the analysis of hospital settings and 65 domisanitary disinfectant products, 10 BCC isolates could be recovered from the latter and among them, 5 were identified as *B. cenocepacia recA* group III-A with PCR-RFLP *Hae*III atypical pattern. In a *Burkholderia recA* sequences based phylogenetic analysis these strains clustered together with the atypical clinical isolates at 97,5 % similarity level. On the other hand, comparative FT-IR spectroscopy studies showed some phenotypic differences mainly in the carbohydrates (1200 – 900 cm⁻¹) region, and in the expression of polyhydroxybutyric acid (PHB) and pili. These differences might possibly be due to clinical isolates evolution driven by lung environmental conditions. Moreover, when we tested the ability of atypical strains to attach and grow on abiotic surfaces by FT-IR spectroscopy^[2], both, clinical and environmental strains, showed the capability to form biofilms. Taking into account these results we hypothesize that the environmental strains could be able to colonize CF patients' lungs.

Our findings suggest that domisanitary products might represent a source of atypical local pathogenic strains for CF patients. The enormous genetic potential for adaptation to different environments that *Burkholderia* species have, corroborates our hypothesis. Therefore, this research should alert on the need of effective microbiological controls measures that ought to be performed on housecleaning products to prevent bacterial spreading.

References

- [1] Mahenthalingam *et al.* 2000. Journal Clinical Microbiology, **38**:3165-3173.
- [2] Serra *et al.* 2007. Analytical and Bioanalytical Chemistry, **387**:1759-1767.