Burkholderia contaminans in cystic fibrosis: FT-IR spectroscopy-based study of phenotypic variation and diversity among isolates in long-term infection

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Burkholderia cepacia complex (Bcc) species are capable of causing chronic and often severe respiratory tract infections in cystic fibrosis (CF) patients. Among the 17 Bcc species currently described, a high prevalence of *B. contaminans* (80 %) followed by *B. cenocepacia* is observed in Argentina[1].

The excessive production of thick, sticky mucus in CF patients clogs their airways providing an environment in which bacteria thrive. This environment, characterized by a heterogeneous distribution of nutrients, exposure to host immune response, and continuous therapeutic antibiotic treatments, makes bacteria face continuous adaptive challenges. In *P. aeruginosa* the genetic adaptations leading to phenotypic variation that occur during long-term CF lung infection are very well characterized[2]. In contrast, the diversity due to Bcc bacterial persistence in CF lungs is mostly unknown.

The aim of the present work was to get insights into the genetic and phenotypic diversity of *B. contaminans* long-term infection in CF patients using PCR-BOX fingerprinting and FT-IR based technologies.

We analyzed 65 Bcc isolates recovered in a 7-year -period (2004-2010) from serial sputum cultures of 21 CF chronic patients attending at 3 CF treatment centers in Argentina. Clinical isolates were identified by *recA*-PCR sequencing and *recA*-RFLP-HaeIII restriction analysis, and genotyping by BOX-PCR fingerprinting. Phenotypic diversity among *B. contaminans* isolates was analyzed by FT-IR spectroscopy applying a hierarchical cluster analysis used as an explorative technique without any *a priori* class assignment. Cultivation conditions, sample preparation, and spectrum measurement parameters were previously reported[3].

In 8, out of 21 chronic patients studied, we isolated more than one *Burkholderia* species along the course of the respiratory infection, accounting either for species replacement or co-infected patients. In the remaining 13 patients we could identify only *B. contaminans* isolates along the chronic infection. For these latter patients, 9 different BOX subtypes were found among their isolates recovered during the first years of our surveillance (2004-2007). However, from 2007 on, the genotypic diversity decreased and only 2 Box subtypes were found. In contrast to this genotyping diversity evolution, FT-IR results showed for the same population, 17 different spectral types which changed and kept diversity along the whole period of our study.

Up to our knowledge our results represent the first evidences of phenotypic diversification in *B. contaminans* long-term infections. As reported for *P. aeruginosa,* this would represent a specific bacterial adaptation to the hostile and changing CF lung environment that allows long-term persistence[2]. The understanding of *Burkholderia* adaptation might facilitate the identification of novel targets for better treatments of chronic infectious in CF patients.

References

[1] Miñán et al., *Analyst* 134, 1138–1148 (2009).
[2] Smith, et al., *Proc Natl Acad Sci USA* 103, 8487–8492 (2006).
[3] Bosch et al., *J Clin Microbiol* 46, 2535–2546 (2008).