

# ***Insights into Burkholderia cepacia Complex Long-term Infections through FT-IR Spectroscopy***

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*Burkholderia cepacia* complex (Bcc) species are opportunistic human pathogens capable of causing chronic and often severe respiratory tract infections in cystic fibrosis (CF) patients. The excessive production of thick and sticky mucus in CF patients clogs their airways and provides a favourable environment for bacterial infection. This environment characterized by, a heterogeneous distribution of nutrients, the presence of host immune response agents and therapeutic antibiotics, makes bacteria face continuous adaptive challenges. For *P. aeruginosa* both the genetic adaptations and phenotypic variations that occur during long-term CF lung infection are very well characterized: conversion to the mucoid phenotype, inactivation of quorum sensing signals, resistance to antibiotics, alterations in lipopolysaccharides (LPS), loss of Type III secretion system, and a rise in the mutation rate (hypermutator phenotype)<sup>[1]</sup>. In contrast, the behavior of Bcc species due to adaptation and persistence in CF lungs is mostly unknown.

A collection of 155 isolates belonging to Bcc species recovered since 2004 from 95 patients treated at different CF Reference Centers and hospitals in Argentina was analyzed. In 47 % of this CF population Bcc infection was transient while, in the remaining 53 % patients the infection evolved to a chronic colonization<sup>[2]</sup>. Our aim here is to show the use of FT-IR spectroscopy as a powerful tool to contribute in the understanding of several aspects associated to the adaptation of Bcc bacteria to persist in the hostile lung environment of CF patients leading to chronic infections.

Over these 9 years we have established that the genotypic diversity of our whole population – including isolates obtained from both new and chronically infected CF patients- has decreased. Nevertheless, the phenotypic diversity has increased as indicated by a significantly high number of FT-IR spectral types encountered in the last years of our surveillance. In this work we analyzed subsequent isolates recovered from several chronic infected patients searching for spectral markers associated to phenotypic changes, possible due to adaptations of Bcc during chronic lung infection. For *B. contaminans* species we found: i) loss of Type IV pili (Amide I, 1450- 11350; 1280-950  $\text{cm}^{-1}$ )<sup>3</sup>, ii) changes in the capability of accumulation of polyhydroxy-alkanoate storage material such as PHB (1740, 1383, 1182, 1303, 1182 and 1059  $\text{cm}^{-1}$ ), iii) variations in carbohydrate content which could be associated with an adaptive conversion from the mucoid to the non-mucoid variants. In the case of *B. cenocepacia* consecutively isolates presented changes in the spectral signals assigned to lipids (3000-2800  $\text{cm}^{-1}$ ). This later result is in agreement with membrane chemical composition modifications reported for *B. cenocepacia* isolates recovered from a chronic lung infection in Portugal<sup>[4]</sup>.

As FT-IR spectroscopy is sensitive to the presence of different cell components and/or virulence factors, it constitutes a very powerful and rapid tool to analyze chemical modifications associates with phenotypic variations among the different isolates retrieved over long-term infections. Various approaches have been applied to study the “chronic infection phenotypes” in different genera and species. Nevertheless, this investigation represents the first work which contributes to elucidate the “microbial evolution” in chronic lung infection of CF patients through FT-IR spectroscopy.

## References:

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- [3] Bosch et al., *J. Biophotonics* **3** (8, 9), 522-33 (2010).
- [4] Couthinio et al., *Infect Immun* **79**, 2950–60 (2011).