112 Unveiling the roles of small non-coding RNAs and RNA chaperones on the biology of opportunistic pathogens of the *Burkholderia cepacia* complex

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The protein Hfq is RNA chaperone plays an important role in riboregulation, promoting the interaction of small non-coding regulatory RNAs (sRNAs) with their mRNA targets [1]. Previous work from our research group has shown that bacteria of the Burkholderia cepacia complex are among the few prokaryotes that encode two distinct and functional Hfq-like proteins in their genome sequences [2,3]. Although a few studies have identified putative sRNAs from strains of the Burkholderia cepacia complex, to the best of our knowledge the h2cR sRNA remains as the single sRNA from a Burkholderia cepacia complex organism that has been functionally characterized until now [4]. Our research group has initiated an experimental strategy envisaging the identification of sRNAs from *B. cenocepacia* J2315 based on co-precipitation experiments with the bacterium two Hfq-like proteins, Hfq and Hfq2 [3,4]. Details on the experimental approaches used, on the sRNAs identified and on their functional characterization will be presented.

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Acknowledgements: This work was partially supported by Fundação Ciência e a Tecnologia, Portugal (contract PTDC/BIA-MIC/119091/2010) and post doc and PhD grants to GCR, AMG and JRF, respectively.

113 Unveiling the *Burkholderia cenocepacia* J2315 RNA chaperone Hfq2 interactome

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The RNA chaperone Hfq mediates the stability of small non-coding regulatory RNAs (sRNAs) and their interaction with mRNAs target in several bacteria [1]. Recently, we identified and functionally characterized the *hfq* and *hfq2* genes encoding the two RNA chaperones of *B. cenocepacia* J2315 [2,3]. Hfq2 comprises two distinct domains, an N-terminus domain highly homologous to the *E. coli* Hfq and *B. cenocepacia* Hfq proteins, and an atypical C-terminus, composed of 5 times repeated sequence RE(P/S)RRXX(E/G)(G/S) [3].
In *E. coli*, Hfq was shown to interact with proteins that are involved in mRNA decay, such has poly(A) polymerase I, polynucleotide phosphorylase and ribonuclease E [1]. Using far-western blotting, we observed that Hfq2 and not Hfq, interacts with other Bcc proteins.
The Hfq2-protein interacting partners were purified by affinity chromatography, proteomics and identified by mass spectrometry. The proteins identified are involved in central carbon metabolic pathways, purine metabolism, signal transduction mechanisms, and regulation of transcription and translation. Our results suggest that Hfq2 is not a merely RNA chaperone, but plays additional roles in cell physiology, contributing to the overall virulence of these bacteria.
Supported by FCT (Project PTDC/BIA-MIC/119091/2010).

Reference(s)

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114 Role of BceF BY-kinase in host–pathogen interaction, biofilm formation and survival to stress by the cystic fibrosis pathogen *Burkholderia cepacia* IST408: Transcriptomic and phenotypic approaches

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Bacterial tyrosine kinases (BY-kinases) were first described as controlling exopolysaccharide biosynthesis, but now they are known to have a role in several cellular pathways. In this work we aim to study of the role of BceF BY-kinase in the physiology and virulence of bacteria from *Burkholderia cepacia* complex.
To accomplish this we used dual-custom Affymetrix microarrays to access global transcriptomic differences between the *B. cepacia* IST408 and its derivative mutant *bceF*::Tp. The data obtained showed that 630 genes were differentially expressed in the two strains. The mutant had decreased expression in genes involved in stress response, motility, cell adhesion, outer membrane composition and increased expression in genes related to intracellular signaling and type VI secretion. Accordingly, we have conducted several phenotype assays that seem to confirm the results obtained, as the mutant was more susceptible to heat shock stress and UV exposure, presented less swimming motility and increased intracellular levels of c-di-GMP. Also, the parental IST408 forms mature 3D biofilm structures, while the mutant is unable to form biofilms. We also tested the importance of BceF in host–pathogen interactions, using *Galleria mellonella* as an acute infection model and by assessing attachment, invasion, tight junction disruption and interleukin production in CFBE41o- cell lines upon infection with each strain. These results indicate that *bceF*::Tp mutant has strong virulence attenuation compared to the parental IST408. Overall, our results confirm that the BY-kinase BceF is involved in many cellular processes, having a crucial role in both *Burkholderia* virulence and physiology.

115 Phenotypic variation of *Burkholderia dolosa* along 5.5 years of chronic colonization with decline of lung function of a cystic fibrosis patient

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A 2 decade-long epidemiological survey of *Burkholderia cepacia* complex (Bcc) bacteria involved in chronic respiratory infections at the major Portuguese Cystic Fibrosis (CF) Treatment Center at Santa Maria Hospital, in Lisbon, has been carried out by our research group covering over 700 clinical isolates, where *B. cepacia* and *B. cenocepacia* are the predominant species [1,2]. The sole infection with *B. dolosa* registered is on the focus of this retrospective study. The CF patient had been chronically colonized for 5.5 years until death following severe pulmonary deterioration. A co-infection with *B. cenocepacia* (*recA* lineage III-B) was registered, nine months before the patient's death, with isolates being retrieved during a three month period. A systematic phenotypic assessment of clonal variants of *B. dolosa* (14 isolates) and *B. cenocepacia* (4 isolates) obtained during chronic colonization was carried out and involved susceptibility assays against different classes of antimicrobials, cell motility, colony morphology, fatty acid composition, cell hydrophobicity, biofilm size and growth performance under iron limitation/load conditions. The results obtained are consistent with the idea that the CF lung can be persistently infected for years by one lineage of a Bcc species with development of clonal expansion and emergence of phenotypic variants, and are in line with those reported before for *B. cenocepacia* long-term colonization of another CF patient [3].

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