Chapter 5

Conclusion and Future Perspective

Bcc species can cause chronic infections in CF patients and although they are not very frequent, the mortality rate among infected patients is very high, mainly due to the ability of these bacteria to produce a wide variety of potential virulence factors and to their high resistance to antibiotics.

In this context, the aim of this thesis was twofold: the main object were the RND proteins, representing a major cause of the high antibiotics resistance of these bacteria, but parallel to this main topic , the type strains of the 18 Bcc species have been characterized from different viewpoints, in order to expand the knowledge of these species, especially of those only recently defined.

Regarding antibiotics resistance, an analysis of the presence and distribution of RND proteins was performed. RND-4 operon, one of the most conserved and involved in resistance to many antibiotics, was characterize from a transcriptomic, proteomic and phenomic viewpoint, proving to be involved in many cellular process (for example motility, chemotaxis, nucleotide and aminoacid metabolism) and not only in antibiotics resistance. The experimental characterization of other RND proteins is still in progress. Indeed, a deep knowledge of the roles of these proteins in the cell represents the starting point for the search of strategies to block their action.

In addition, the 18 Bcc type strains and some RND operons deletion mutants were used for testing their sensibility to six essential oils (*Eugenia caryophyllata*, *Origanum vulgare*, *Rosmarinus officinalis, Lavandula officinalis, Maleuca alternifolia* and *Thymus vulgaris*). All of them are able to inhibit the growth of Bcc strains, but *Thymus vulgaris, Origanum vulgare* and *Eugenia caryophyllata* are the most active in inhibiting Bcc growth. RND operons seem to be involved in the efflux of some of the components of these EOs. These preliminary data are particularly encouraging, since they demonstrate that the use of Essential Oils might represent an alternative way to fight Bcc growth.

Regarding virulence, an analysis of the pathogenicity determinants of the 18 type strains of Bcc species by using the non-vertebrate host *C. elegans* was performed. Data obtained revealed that different Bcc species have a different ability to infect the host and that they are able to kill *C. elegans* with mainly two mechanisms, *i.e*. i) bacterial accumulation/colonisation in the worms intestine, and ii) production of diffusible toxins-virulence factors. A preliminary characterization of one putative toxin/virulence factor was also performed. Moreover the nematode ABC transporters *mrp-3* and *mrp-4* were found to be involved in the Bcc infection process and further studies are required to better understand the role of these transporters. These information will be the basis for future experimental tests aimed to uncover Bcc virulence genes that can be exploited as novel therapeutic target.

All data obtained represent an important basis for the development of new strategy to fight Bcc infections. Indeed, i) a greater knowledge of the physiological function of efflux pumps involved in antibiotics resistance, ii) the use of natural products able to inhibit Bcc growth, and iii) the discovery of new putative virulence factor, that can be used as novel target for antibacterial molecules, are different ways to find an efficient strategy against infections caused by these bacteria

The genome sequencing and the phenotypic characterization of the 18 type strains of Bcc is still in progress and will provide much more information about their virulence factors, antibiotics resistance and mechanisms of infections. All these information will be useful in fighting infections caused by these bacteria.