

Chapter 2

Aims and presentation of the work

Among the more than 70 species that are currently embedded in the *Burkholderia* genus, Bcc bacteria have become known as opportunistic pathogens in humans as they can be very harmful for specific patient groups such as CF patients. The Bcc is a group of genetically distinct but phenotypically similar bacteria that up to now comprises 18 closely related bacterial species, some of which have only recently been defined. Their chronic infections prevalence is not high compared to other CF pathogens, but Bcc infections often correlates with poorer prognosis, longer hospital stays and an increased risk of death. The reasons for this high mortality rate include the ability of these bacteria to produce a wide variety of potential virulence factor and their high resistance to antibiotics, which is mainly due to the outer membrane barrier and the expression of efflux systems, in particular those belonging to RND superfamily.

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.

Chapter 3 has focused on RND proteins. In particular an analysis of the presence and distribution of all the eight RND families was performed, and was used as a basis for the choice of some proteins that were further characterized by an experimental point of view. In particular two operons were chosen to be further characterized in the model systems *B.cenocepacia* J2315: RND-4 operon, one of the most conserved of those belonging to HAE-1 family, and SecDF operon. RND-4 operon has been studied from a transcriptomic, proteomic and phenomic points of view, in order to better understand its role in antibiotics resistance of Bcc species and the possible other roles that it could plays in the cell.

The experimental characterization of SecDF operon is still in progress, but is very interesting for the high conservation of these proteins in *Burkholderia* species and

because it has been demonstrated in other bacteria that these proteins complex is involved in antibiotics resistance and secretion of virulence factors.

In addition, the susceptibility of Bcc strains and RND mutants to some essential oils (EOs) was evaluated to find an alternative to antibiotics in the treatment of infections caused by these bacteria. Indeed, EOs consist of a complex blend of substances with antibacterial, antifungal and antiviral properties that have been screened worldwide as potential sources of novel antimicrobial compounds. Moreover, it is likely that it will be more difficult for bacteria to develop resistance to the multi-component EOs than to common antibiotics that are often composed of only a single molecular entity. In addition, over the past few years, several natural compounds acting as efflux pump inhibitors have been investigated and some of them able to restore the activity of usual antibiotics on resistant clinical bacteria isolated during therapeutic treatment.

On the other site, the characterization of the type strains of 18 Bcc species included the study of the pathogenicity determinants of these strains by using the non-vertebrate host *Caenorhabditis* (Chapter 4).

The study of the interaction between Bcc members and the nematode host *C. elegans* has had the aim of obtaining a knowledge of their pathogenicity traits and of identify strains with high virulence. These information will be the basis for future experimental tests aimed to uncover Bcc virulence genes that can be exploited as novel therapeutic target.

The genome sequencing and the phenotypic characterization of these 18 strains 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.

