**Study of the outer membrane permeability of Pseudomonas aeruginosa to β-lactam antibiotics**

**Aims**

- Periplasmic production of BlaR-CTD as a probe
- Quantification of different β-lactams in the periplasm and measure of their permeability coefficients (P)
- Analysis of a PAO1 porin mutants collection to study the role of each single channel in the β-lactams permeability
- Characterization of the influences of the low outer membrane permeability of *P. aeruginosa* PAO1 and TNP065 (ΔoprC, ΔoprD) in the periplasmic proteome composition.

**Introduction**

β-Lactams are the most potent and widely used antibiotics but their activity depend on the presence in the target bacteria of resistances caused by the interplay between four independent factors:

(i) the sensitivity of the target enzymes, the penicillin-binding proteins.

(ii) the properties and concentration of the periplasmic β-lactamases.

(iii) the permeability of the outer membrane.

(iv) the activity of the outer membrane transporters.

On this basis, Zimmermann and Rosselet [1] proposed a model which allowed a quantitative prediction of the MICs for Gram-negative bacteria and it was applied with success to Escherichia coli and Enterobacter cloacae.

This model seems to be not applicable to *Pseudomonas aeruginosa* due to its low outer membrane permeability that is mostly influenced by the combined result between a remarkable reduction of the functional porins expression and an over-expression of the efflux system (e.g. MexA-MexB-OprM, MexC-MexD-OprJ, MexE-MexF-OprM and MexXY-MexP-OprM) [2, 3]; this decrease in permeability causes difficulties in the direct measures of the permeability coefficient resulting in few and highly variable coefficients published for *P. aeruginosa*.

For this purpose BlaR-CTD, the C-terminal domain of a highly sensitive penicillin binding protein derived from *Bacillus licheniformis*, expressed in the periplasmic space has been used for the direct determination of the concentrations of different β-lactam antibiotics in cell compartments and to have reliable measures of the permeability coefficients [4].

**Results**

The reference strain *P. aeruginosa* PAO1 was transformed with CTX240Blal plasmid (fig. 1) in order to produce BlaR-CTD in the periplasm. We also receive a *P. aeruginosa* PAO1 collection of porin mutant strains (tab.1[6]) used for the outer membrane permeability determination (TNP040, ΔoprD) or for the proteomic analysis (TNP065, ΔoprC, ΔoprD).

For the permeability assay *P. aeruginosa* cultures, at their stationary phase, were incubated with β-lactam at room temperature; 1ml samples were collected at different incubation times and the excess of antibiotic was hydrolysed by the addition of 2 µg of VM-4 metal-lactamase for the β-Iactam resistance assays. The soluble crude extract, obtained after sonication, was isolated and incubated in presence of 2.5 µM BlaR. The samples were then analysed by SDS-PAGE and quantified with a densitometric method.

The quantification result refers to the BlaR-CTD-Bocill complex, while the quantification of the BlaR-CTD-β-lactam complex was obtained subtracting the different time values from the quantification of the total BlaR-CTD.

We report here the permeability tests made for Imipenem in PAO1 at 0.01 µM (fig. 2 and 3) and at 2 µM for TNP040 (fig. 4 and 5).

**Proteomics**

Proteomic analysis of the periplasmic fraction following the spheroplasting by lysozyme and sucrose method [9] on:

- i) PAO1 wt;
- ii) PAO1 (Δkt240Blal);
- iii) PAO1ΔTNP067;
- iv) PAO1ΔTNP040;
- v) TNP065 (ΔoprC, ΔoprD);

Spots were selected on the basis of a statistically difference between the different conditions and 76 proteins were identified by mass spectrometry; we here report the comparison between PAO1 and TNP040 (fig. 6).

**Conclusion**

We validated the use of BlaR-CTD for the determination of permeability coefficient (P) in *P. aeruginosa*.

We determine the permeability coefficients of different β-lactams in *P. aeruginosa* PAO1.

We confirmed the specificity of OpoD for Imipenem permeability resulting in a 100 fold decrease between PAO1 and TNP040 (ΔoprD).

Finally, the proteomic analysis of the periplasmic proteome is in progress.

**References**


