GROWTH TEMPERATURE DEPENDANCE OF CHANNEL CONDUCTANCE OF THE MAJOR OUTER MEMBRANE PROTEIN OprF IN *PSEUDOMONADS*

Jaouen[a], T., Chevalier[a], S., Dé[b], E.,Burini,J.F, Orange[a], N.
[a]Laboratoire de Microbiologie du Froid-UPRES 2123, Université de Rouen. 55, rue Saint Germain, 27006 Evreux-France
[b] Laboratoire Polymères, Biopolymères, Membranes-UMR 6522. Faculté des Sciences, 76821 Mont Saint Aignan-France

Pseudomonads are well-known for their adaptation capacities in hostile environments that are often submitted to rapid variations. They are able to grow over a wide temperature range, and in particular for psychrotolerant strains that can grow at temperature near 0°C. This ability involves number of modifications that could affect notably their outer membrane, the first adaptative barrier protecting the cell against environment. In the psychrotrophic *Pseudomonas fluorescens* MF0, isolated from raw milk, adaptation to low growth temperature includes a decrease in outer membrane permeability that influences β-lactam resistance. It has been shown that the major outer membrane protein, the porin OprF, participates to this phenomenon: its major conductance values decrease (250 pS to 80 pS) depending on whether it was purified from cultures grown at optimum (28°C) or low (8°C) temperature (1). Similar results were obtained with the psychrotrophic *P. fluorescens* OE28.3 OprF, isolated from wheat rhizosphere.

These proteins appear to have a non specific porin function, constituted of a N-terminal domain, forming an 8-stranded β-barrel (responsible of channel activity), that is linked by an extracellular loop region containing a poly-proline–alanine pattern to a C-terminal periplasmic and peptidoglycan-associated domain. Studies of the channel-forming properties in planar lipid bilayers of OprF's from other species, the psychrotrophic *Pseudomonas putida* 01G3 isolated from polluted soil, and the human opportunistic pathogen, the mesophilic *Pseudomonas aeruginosa*, which contain a cystein-rich loop in place of the proline-rich one, share the same decrease of major conductance values at low growth temperature.

We have isolated and characterized the OprD porins-homologues OprE1 and OprE3 in *P. fluorescens* MF37. These proteins (19-members in *P. aeruginosa*) are described as substrate-specific porins that could serve as non-specific porins for small substrates (2). Their topological model is very different from the OprF one, with a 16-stranded β-barrel organisation. Their channel-properties share very low single channel conductance values leading rapidly to more important aggregates. No channel conductance variability could be observed for the OprE3-homologue with regards to growth temperature.

Our results suggest that the conductance values decrease at low growth temperature is a particular fact of the major non specific porin OprF, probably due to its two-domain organisation, in which the C-terminal part of the protein could modulate the structure of the pore, depending on the temperature. This behaviour must be very important for many *Pseudomonas* either psychrotrophic or mesophilic, in that it is conserved among species from very different environments or growth characteristics.

1. **Dé, E., Orange, N., Sain, N., Guerillon, J., De Mot, R., Molle, G**. (1997). Growth temperature dependence of channel size of the major outer-membrane protein (OrpF) in psychrotrophic *Pseudomonas fluorescens* strain. *Microbiology*, **143**, 1029-1035
2. **Hancock, RE, Brinkman, FS**. (2002) Function of *Pseudomonas* porins in uptake and *efflux.Annu Rev Microbiol. 2002*;**56**:17-38