MYCOMEMBRANE ANR-07-blan-0363

Colloque BIOLOGIE & SANTÉ 2011

Laboratoire de Microbiologie Moléculaire et Cellulaire et Laboratoire de Chimie des Protéines
IBBMC Université Paris XI, CNRS UMR 8619, Orsay
Laboratoire de Biologie moléculaire des Corynébactéries, Université Paris XI, CNRS UMR 8621, Orsay
Département Mécanismes Moléculaires des Infections Mycobactériennes, CNRS, IPBS Toulouse
4C-CINA Center For Imaging and NanoAnalytics,University of Basel, Switzerland

Context and objectives

The cell envelope of Mycobacteria is essential for virulence and forms a very efficient permeability barrier that contributes to their high resistance against hydrophilic drugs. Our current view is still essentially based on the model of Minnikin (1982) who was the first to propose the presence of an outer mycolate membrane structure (Mycomembrane) covalently linked to an underlying polymer of arabinogalactan (AG) and peptidoglycan (PG). Several lines of evidence have since firmly established this model using *Corynebacterium glutamicum* as a model. In 2008, cryo-electron microscopy on vitreous sections provided a definitive proof for the presence of the Mycomembrane in these very atypical Gram-positive bacteria. In this project we present the first biochemical isolation and characterization of the Mycomembrane of *C. glutamicum*.

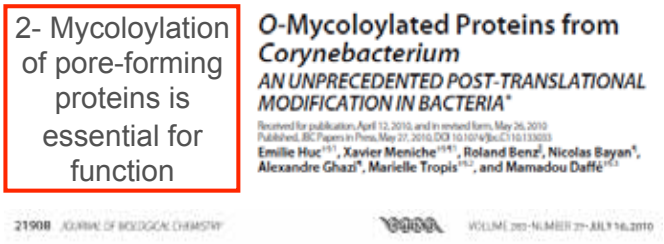
Results and publications

1- Identification of a mutant secreting Mycomembrane fragments

A Deficiency in Arabinogalactan Biosynthesis Affects *Corynebacterium glutamicum* Mycolate Outer Membrane Stability

Corynebacterineae is a specific sub-order of Gram-positive bacteria including *Mycobacterium tuberculosis* and *Corynebacterium glutamicum*. The ultrastructure of their cell envelope is very atypical. It is composed of a heteropolymer of peptidoglycan and arabinogalactan (AG) covalently associated to an outer membrane. Five arabinosyltransferases are involved in the biosynthesis of AG in *C. glutamicum*. AftB catalyzes the transfer of Araf onto the arabinan domain of the arabinogalactan to form terminal b(1→2)-linked Araf residues. Here we show that ΔaftB cells lack half of the arabinogalactan mycoloylation sites but are still able to assemble an outer membrane. In addition, we show that a ΔaftB mutant grown on a rich medium has a perturbed cell envelope and shed a significant amount of membrane fragments in the external culture medium. These fragments contain mono- and di-mycolate of trehalose, PorA/H the major porin of *C.glutamicum* but lack conventional phospholipids that typify the plasma membrane, suggesting that they are derived from the atypical mycolate outer membrane of the cell envelope. This is the first report of outer membrane destabilization in *Corynebacterineae* and it suggests that a strong interaction between the mycolate outer membrane and the underlying polymer is essential for cell envelope integrity. The presence of outer membrane derived fragments (OMFs) in the external medium of the ΔaftB mutant is also a very promising tool towards outer membrane characterization.

2- Mycoloylation of pore-forming proteins is essential for function



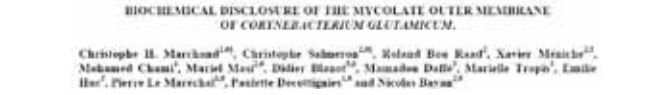
O-Mycoloylated Proteins from *Corynebacterium*
AN UNPRECEDENTED POST-TRANSLATIONAL MODIFICATION IN BACTERIA*

21908 JOURNAL OF BIOLOGICAL CHEMISTRY VOLUME 285 • NUMBER 29 • JULY 16, 2010

3- Lipid composition of the mycomembrane is regulated by ElrF

Identification of a Stress-Induced Factor of *Corynebacterineae* That Is Involved in the Regulation of the Outer Membrane Lipid Composition

4- Biochemical disclosure of the Mycomembrane
(submitted to J. Biol. Chem.)



BIOCHEMICAL DISCLOSURE OF THE MYCOLATE OUTER MEMBRANE OF *CORYNEBACTERIUM GLUTAMICUM*.

The Mycomembrane has been visualized by cryoelectron microscopy of vitreous sections but its biochemical composition is still widely lacking hampering elucidation of its physiological function. In this report, we show for the first time, that the M-AG-PG of *C. glutamicum*, a model organism for *Corynebacterineae* species, is able to float on a gradient and can therefore be easily separated from the inner membrane. Purification of M-AG-PG allowed us to show that the mycomembrane is specifically composed of mycolic acid derivatives without significative amounts of phospholipids suggesting a tight sorting control of membraneous lipids. Proteins associated or inserted in the mycomembrane were extracted from M-AG-PG with LDAO, separated on a Q sepharose column and analyzed by SDS PAGE. 84 bands were analyzed by nano LC ESI MS/MS after in gel trypsin digestion. This procedure allowed us to identify 67 different proteins. Interestingly, we show here that 19 of them were also found in mycomembrane fragments released by the ΔAftB strain described recently. These 19 proteins are almost all predicted to contain a signal sequence and to form a β barrel structure typifying Gram-negative outer membrane proteins. These putative candidates include already known porins (PorA, PorB), 5 mycoloyltransferases (cMytA, cMytB, cMytC, cMytD and cMytF), several lipoproteins and other unknown proteins with a putative C-terminal hydrophobic anchor. They constitute the first experimental database of mycomembrane proteins so far reported.

CONTACT :
Nicolas.bayan@u-psud.fr
Christine.houssin@u-psud.fr
Mamadou.daffe@ipbs.fr
Pierre.le-marechal@u-psud.fr
Mohamed.chami@unibas.ch