

Director : Jean-Marc GHIGO ([jmghigo@pasteur.fr](mailto:jmghigo@pasteur.fr))

## Abstract

Biofilms are communities of microorganisms, which develop on surfaces in natural and artificial environments. In medical settings, biofilms are found in association with catheters and prosthetic devices and may constitute an important source of nosocomial infections. Bacterial biofilms display specific biological properties that distinguish them from their planktonic counterparts. Our work focuses on the genetic identification of the bacterial factors necessary for the formation and the maintenance of mature *E. coli* biofilms. These approaches may lead to a better understanding of the biofilm lifestyle as well as to strategies to control pathogenic biofilms.

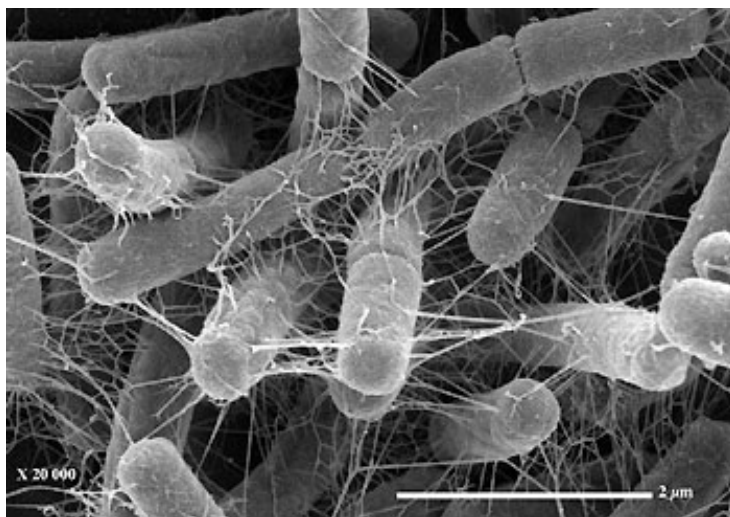
## Annual Report

### Bacterial biofilm formation

#### I- Global impact of mature biofilm lifestyle on *Escherichia coli* K12 gene expression

( Christophe Beloin, Patricia Latour-Lambert, Jean-Marc Ghigo)

The formation of biofilm results in a major lifestyle switch that is thought to affect the expression of multiple genes and operons. We used DNA arrays to study the global effect of biofilm formation on gene expression in mature *Escherichia coli* K12 biofilm. We show that when biofilm formation is compared to planktonic growth phases, up to 10% of the *E. coli* genome is significantly differentially expressed. Using gene disruption of 54 of the most biofilm-induced genes followed by a detailed phenotypic study, we validated the biological



relevance of our genomic analysis and showed that 20 of these genes are required for the formation of mature biofilm. This group includes 11 genes of previously unknown function. This opens new prospects for the characterization of physiological pathways developed in sessile bacterial communities.

• Beloin, C J. Valle, P. Latour-Lambert, P. Faure, M. Kzreminski, D. Balestrino, J. Haagensen, S. Molin, G. Prensier, B. Arbeille And J.-M. Ghigo (2003) Global impact of mature biofilm lifestyle on *Escherichia coli* K12 gene expression. *Mol Microbiol.* in press.

#### II- Role of RfaH in biofilm formation in *E. coli* ( Christophe Beloin, Jean-Marc Ghigo)

Bacterial pathogens express virulence factors that distinguish them from their closely related commensals. The expression of these virulence factors is often tightly genetically controlled. Among known virulence regulators, we investigated the role of RfaH, which acts at the level of transcriptional anti-termination of many extra cytoplasmic determinants. Within a close collaboration with Dr U. Dobrindt's group of Prof. J. Häcker laboratory (Wurzburg University, Germany), we investigated the influence of RfaH on biofilm formation. We showed that, in *E. coli* K12 MG1655, the autotransporter protein AG43 is responsible for a "hyper-biofilm" phenotype. The molecular mechanisms behind this new relationships between the virulence regulator RfaH, AG43 and biofilm formation is currently under investigation. It is a new illustration of the suspected overlap between bacterial virulence and biofilm formation functions.

• Beloin C. ; Michaelis, K ; Häcker J. ; Ghigo, J.M. and U. Dobrindt. Role of RfaH in biofilm formation in *E. coli* through Ag43 regulation. *En préparation*

#### Identification of adhesion factors carried by bacterial plasmids

(Patricia Latour-Lambert, Agnès Roux, Jean-Marc Ghigo)

Mobile genetic elements such as plasmids and transposons represent as much as 10% of the total bacterial DNA. However, with the exception of antibiotic resistance spread, the ecological role of plasmids in bacterial ecology has been largely overlooked. We previously showed that conjugative plasmids directly contribute to the capacity of the bacterial host to form a biofilm through the expression of conjugative pili. This general connection between conjugation and biofilms suggests that medically relevant plasmid-bearing strains are more likely to form or enter into microbial communities. We investigated this hypothesis further and showed that, on the F plasmid, non-conjugative factors related to autotransporter adhesin genes can induce biofilm formation. This findings are in agreement with the hypothesis that, whereas many chromosomal genes have been shown to be involved in different stages of biofilm development, the contribution of the extra-chromosomal plasmid gene pool to biofilm biology may be a widespread phenomenon.

• Latour-Lambert, P.; Roux, A., and J.M. Ghigo. Identification of two new autotransporter adhesins encoded by the F conjugative plasmid : contribution to *E. coli* biofilm formation.. *En préparation*

#### **Molecular analysis of biofilm formation by pathogenic yeast *Candida albicans* et *Candida glabrata*** (Jean-Marc Ghigo)

In a Pasteur collaborative project (Programme Transversal de Recherche- PTR) with Christophe d'Enfert's laboratory (Unité Postulante Biologie et Pathogénicité Fongique) and Françoise Dromer's lab (Unité Postulante de Mycologie Moléculaire), we developed a model system allowing the production and study of *Candida albicans* biofilm. This model was used to analyse the expression profile of *C. albicans* biofilms using macro and micro-arrays. This approach led to the identification of genes whose expression is triggered within *C. albicans* biofilms (for more informations, see:

• Susana García-Sánchez , Sylvie Aubert, Ismaïl Iraqui, Guilhem Janbon, J-M. Ghigo and Christophe d'Enfert. Biofilms of *Candida albicans*: a developmental state associated with specific and stable gene expression patterns. *Eukaryotic Cell*, *in press*.

#### **Development of rapid gene inactivating methods in Gram-negative bacteria.**

(Jean-Marc Ghigo)

In collaboration with E. Carniel's group, (Unité des Yersinias), we developed a rapid PCR-based method to inactivate target genes. This method allows the rapid and cloning-free disruption of genes in many enterobacteriaceae genera such as *Escherichia*, *Yersinia*, *Serratia*, *Salmonella*, *Shigella* and *Klebsiella*. (cf [http://www.pasteur.fr/recherche/unites/Ggb/methodes\\_ang.html](http://www.pasteur.fr/recherche/unites/Ggb/methodes_ang.html) ).

• Derbise, A ; B. Lesic, D. Dacheux, J.M. Ghigo and E. Carniel . (2003) " A rapid and simple method for inactivating chromosomal genes in *Yersinia*". *FEMS J. Med Microbiol.* Sep 22;38(2):113-6

#### **Photo :**

Photo 1 : *Escherichia coli* MG1655, biofilm détail, (X20 000, SEM). Photo Brigitte Arbeille, *Laboratoire de Biologie Cellulaire et Microscopie Electronique, UFR Médecine, 37032 Tours Cedex, France*.

**Keywords:** Biofilm, DNA-arrays, candida albicans, Escherichia coli, plasmid

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## Staff

Office staff

Researchers

Scientific trainees

Other personnel

Christine Naubron

Ghigo Jean-Marc, Researcher  
IP, [jmghigo@pasteur.fr](mailto:jmghigo@pasteur.fr)

Beloin Christophe, Post-doc.\_  
[cbeloin@pasteur.fr](mailto:cbeloin@pasteur.fr)

Da Re Sandra, Post-doc.\_  
[sdare@pasteur.fr](mailto:sdare@pasteur.fr)

Roux Agnès, PhD student, 1st  
year, [agroux@pasteur.fr](mailto:agroux@pasteur.fr)

Latour-Lambert Patricia,  
Technician. IP\_  
[lambertp@pasteur.fr](mailto:lambertp@pasteur.fr)

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