DNA secretion in *Neisseria gonorrhoeae*

Projects are available for enthusiastic and motivated MSc or HBO students for a period of 5-6 months. The projects will include a combination of diverse techniques used in the molecular biology in both *E.coli* and *N.gonorrhoeae*, and different techniques employed in protein purification and the characterization of an isolated protein.

**Introduction**

Antibiotic resistance is a global problem, confronting communities and nations worldwide. In particular, bacteria causing infectious diseases have become resistant to multiple antibiotics and the number and spread of these resistant bacteria continues to increase. Multi Drug Resistance (MDR) has spread rapidly via horizontal and vertical DNA transfer. Resistance in *Neisseria gonorrhoea*, the pathogenic organism which causes the sexually transmitted disease gonorrhoea, to antibiotics such as penicillins and quinolones has spread to a historically high level that these antibiotics can no longer be used. Even gonococci with decreased susceptibility to third generation cephalosporins start to appear, causing a serious threat to the effectiveness of current therapies.

Ciprofloxacin-resistant *Neisseria gonorrhoeae* (MIC, 1 mg/L) as a percentage of all gonococcal isolates isolated in Sydney, Australia, over the course of 20 years, 1984–2003. (obtained from www.svinfectologia.org/36162web.pdf)

Transfer of MDR occurs via plasmid conjugation, transduction, or transformation. Conjugation is the most important mechanism of DNA transport and occurs via a type IV secretion mechanism. Transport via such type IV secretion mechanisms is used by many pathogenic micro-organisms, causing various diseases like *e.g.* gastritis, formation of ulcers, whooping cough, pneumonia (Legionnaires diseases), bacteremia, brucellosis, Q fever, typhus, spotted fever and river blindness.

We study the mechanism of DNA transported via these type IV secretion mechanisms. Well studied examples of conjugal type IV DNA transport systems are the F-plasmid of *Escherichia coli* and the Ti-plasmid of *Agrobacterium Tumefaciens*. The conjugal transport is mediated by a supramolecular structure called the mating pair formation (Mpf) complex. This pilin-like structure can be extended to contact a recipient cell, and retracted to form a close mating-pair, and is then used to transfer the DNA to the

(Figure from http://www.medmicro.wisc.edu/department/faculty/images/T4SSmodel.jpg)
recipient cell. Before DNA is transferred, it is nicked, unwound and coupled to the relaxase-protein (TraI). An ATP-hydrolysing protein located in the inner membrane then transfers the relaxase-bound DNA to the Mpf complex. The relaxase-bound DNA is then transferred from the host to the recipient. Recently, a variable genetic island has been described in *N. gonorrhoeae* and *Neisseria meningitides* which encodes a conjugation-like DNA secretion system. Remarkably, this secretion system was shown to secrete the DNA directly into the medium. The DNA is then presumably taken up by transformation. Although *N. gonorrhoeae* is a human pathogen, techniques to grow and genetically modify and biochemical study this organism are available. Our aim is to obtain insight in the transport mechanism of this DNA transport system.

**Project 2: Function of the coupling protein.**
The coupling protein fulfills an essential function in conjugation. It transfers the DNA/relaxase complex to the Mpf complex. In this project we want to characterize the function of the coupling protein, by over-expression, purification and a biochemical characterization. If the purification of the protein is possible, the crystal structure will be determined in collaboration with the group of Prof. L. Schmitt (University of Dusseldorf, Germany).

**Techniques used:**
Molecular biological and different cloning techniques in both *Escherichia coli* and *Neisseria gonorrhoeae*, optimization of overexpression of the protein in both *Escherichia coli* and *Neisseria gonorrhoeae*, purification of the protein using a wide range of purification techniques, and finally biochemical characterization using different techniques.

**Host institute**
The research will be performed in the Department of Molecular Microbiology headed by Prof. Driessen, within the group of Dr. C van der Does. The group participates in the Groningen Biomolecular Sciences and Biotechnology Institute (GBB) and the Material Sciences Center (MSCplus), a centre of excellence. Research is focused on the molecular mechanisms of bacterial protein export, membrane protein integration and solute transport, specifically multidrug and antibiotic transporters in bacteria, archaea and lower eukaryotes. The emphasis is on the energetics and kinetics of the translocation processes, the structural analysis of the membrane proteins, and the role of transport processes in the physiology of micro-organisms. The group has a long-standing experience in the overproduction, purification, reconstitution, and the functional characterization of membrane proteins. The laboratory is well equipped; including molecular biology and protein purification (AKTA and FPLC) equipment, large scale fermentation and (ultra)centrifugation facilities, continuous cell lysis, fluorimetry, imaging for in gel fluorescence detection and (2D) electrophoresis equipment and a fully equipped isotope laboratory. A well-equipped ML-II laboratory has been established with all facilities needed to grow and manipulate *N. gonorrhoeae*. The group comprises 5 technicians, 5 post-docs and 15 PhD students. The group was rated excellent by the past two international (VSNU) quality assessment committees of Life sciences research in the Netherlands (1993, 1998) and the recent (2005) assessment carried out according to the standard evaluation protocol (SEP).
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