

Report Short Term Scientific Mission

COST Action 850 „Biocontrol Symbiosis“

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My visit to Prof. Richard ffrench-Constant's laboratory (Department of Biology and Biochemistry, University of Bath) supported by the STSM program of COST started on 14 October 2001 and lasted for 31 days. Our co-operation is based upon the fact, that both groups investigate proteases secreted by the entomopathogenic bacteria *Photorhabdus luminescens*. The characterisation of extracellular proteases and the dynamics of their secretion can provide important information to advance our knowledge at molecular level of the pathomechanism of *Photorhabdus-Heterorhabditis* symbiotic complexes, as secreted proteases are known to have significant roles as virulence factors of pathogen micro-organisms in invading the host tissues and surviving the host defence systems.

Secretion of proteolytic activity by *Photorhabdus* strains was detected first more than a decade ago. Since then, a number of proteolytic enzymes have been isolated from various strains but their relationship and their role in the infection process have not been explored in detail yet. Our group at the Eötvös Loránd University, Budapest isolated and characterised a 55 kDa metalloprotease from *P. luminescens ssp. laumondii* strain **Brecon**. We determined a 13 amino acid long amino terminal sequence of our protein. An Rtx-like metalloprotease of similar size was detected and sequenced from *P. luminescens* strain **W14** – this protease is investigated by ffrench-Constant's group. The aim of my visit to Bath was to clear the relationship between the *Photorhabdus* proteases investigated by the different groups in Bath and Budapest using the cosmid library from the *P. temperata* K122 strain that was recently constructed in Dr. Richard ffrench-Constant's laboratory.

The first plan was to screen the *P. temperata* **K122** library for protease expressing clones, then probing them with protease specific oligonucleotides. The second plan – for the case, if the screening is negative – was to make a library from *P. luminescens ssp. laumondii* **Brecon**.

My first task in Bath was to screen the **K122** library for protease secretion. I used an X-ray film digestion assay. This method was developed by my Hungarian supervisor Dr. István Venekei using serine protease secreting *Escherichia coli* transformants. Before leaving to Bath I checked the effectiveness of the method on different *Photorhabdus* strains and it proved to be sufficiently sensitive for the detection of *Photorhabdus* protease secretion. However, when I screened the **K122** library, I did not find protease secreting clones. Difficulties with detecting any activities (lipase, hemolysins, bactericins) from the cosmid clones is a usual experience in the ffrench-Constant laboratory and is explained by a different regulatory environment of the cloning vector in the *E. coli* cell.

In a second approach, I screened the **K122** library for the presence of the protease DNA with a labelled oligonucleotide avoiding the technical problem of low expression. A degenerate oligonucleotide was designed on the amino-terminal protein sequence of the 55 kDa protease that we isolated in Budapest. I labelled the 5'-end of the commercially ordered oligonucleotide with P³² using T4 polynucleotide kinase then performed the screen. Unfortunately, it was also unable to find any positive clone. As a control experiment I checked the hybridisation of the probe to genomic DNA that I isolated from several *Photorhabdus* strains including strain **Brecon**. There was no hybridisation in this case either. So the

conclusion from these experiments was that this probe is too short to give an obvious, detectable signal on DNA from any of the clones or genomic DNA samples.

As a next approach to find proteases, I screened the **K122** cosmid library using an oligonucleotide probe specific for the Rtx-like metalloprotease, that was isolated and cloned from *P. luminescens* strain **W14**. This protease is investigated by Dr. French-Constant's group. The oligonucleotide I used as probe was a 200 base pair long sequence from the gene of this protease. I produced the probe by PCR reaction using **W14** genomic DNA as template and primers specific for the terminals of the 200 base pair long sequence from the protease. I labelled the probe with P³² using the Prime-It™ Random Primer Labelling Kit from Stratagene.

I found 14 positive clones that were re-tested in two different ways. First I repeated the PCR reaction that produced the 200 basepair long probe sequence, so that the template was the cosmid DNA isolated from the positive strains. 3 clones proved to be positives by this secondary PCR screen. I also tested culture supernatants for protease activity with a highly sensitive gelatine-zymographic assay using two-day cultures of the positive clones. Two of the clones were positives by this activity assay – this was the first case when protease activity could be detected from this library. Fortunately these two clones were positive with the PCR screen too, so we can be sure that we managed to pick up at least two protease containing clones. The protease secreted by the clones has the same motility in the SDS-PAGE as our protease isolated from the **Brecon** strain, so it seems that the proteases from the two different strains are homologous enzymes.

During my work in Bath I was given the opportunity to construct a library from the **Brecon** strain, the strain which is investigated in our laboratory. I learned how to prepare genomic DNA, how to carry out digestion experiments to generate inserts of appropriate size for a library. I could not complete the work before leaving Bath, but I managed to prepare large amount of insert DNA, so we can complete the library work in our laboratory in Budapest. This is very important for us, because our group is planning to create libraries from several *Photobacterium* strains. The selected strains would represent the main branches of the phylogenetic tree of the genus, and we would like to use these libraries to investigate the occurrence of the most important virulence factors of this pathogen in these strains.

Summarising the results of my work in Bath, after finding out that our degenerate probe was too short for screening with hybridisation, I managed to pick up two protease containing clones from their K122 cosmid library with a longer probe specific for a W14 protease. These can be further analysed by sequencing in Dr. French-Constant's laboratory. I also learned several molecular biology techniques – radioactive labelling, hybridisation, PCR, genomic/cosmid DNA preparation, ligation, electroporation, etc. I have never used these techniques before, but – as our group in Budapest is planning to extend our research arsenal – these technical skills will be essential for the future work in our laboratory too. And at last but not least I really enjoyed to join a dynamic, well-equipped and effective team – even if just for a month.

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