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Abstract

Comparative and functional genomic approaches are being used to further our understanding of tuberculosis and leprosy. New targets for therapy and potential subunit vaccine candidates have been identified and are currently being characterised. Genomics has now been extended to include *Mycobacterium ulcerans*, an emerging pathogen responsible for Buruli ulcer.

The pathogenesis of *Clostridium difficile* and *Clostridium perfringens* is due to powerful toxins and in *C. difficile* their synthesis shown to be controlled by novel sigma factors.

Annual Report

Functional genomics of mycobacteria (Stewart Cole, Roland Brosch, Priscille Brodin, Caroline Demangel, Brigitte Saint-Joanis)

Mycobacterium tuberculosis, the etiological agent of human tuberculosis shares more than 99,9 % sequence similarity with other members of the so-called *M. tuberculosis* complex, which comprises *Mycobacterium africanum*, *Mycobacterium canettii*, *Mycobacterium microti*, *Mycobacterium bovis* and *M. bovis* BCG. A number of regions of difference (RD1-RD14) that range in size between 2 and 13 kb were identified in the genomes of the members of the *M. tuberculosis* complex by various comparative genome analyses. The study of presence or absence of certain RD regions in 100 strains from the *M. tuberculosis* complex has allowed to redefine the phylogenetic relationship among the members of the complex. These results contradict the often reported hypothesis that *M. bovis* is the progenitor of *M. tuberculosis*. This investigation further has identified some important markers (TbD1, RD9, RD4) for the rapid identification of the tubercle bacilli. Some other regions like RD1 are probably associated with the attenuation of the vaccine strains, *M. bovis* BCG and *M. microti*, the vole bacillus. This hypothesis has been tested experimentally using gene knock-ins of several regions absent from BCG and *M. microti*. Interestingly only complementation with the RD1 region, which contains genes coding for important T-cell antigens, resulted in increased virulence in immune-compromised mice. Immune-competent mice, however were able to control the infection with the BCG::RD1 and *M. microti*::RD1 strains, but increased persistence and immunogenicity was noted.

This finding opens new perspectives for the development of more efficient anti-tuberculosis vaccines, a strategy which is presently investigated in our laboratory.

Isoniazid (INH) is a powerful anti-tuberculous agent whose toxicity results from its transformation into acyl radicals during a reaction catalysed by catalase-peroxidase, KatG. The virulence of selected isoniazid-resistant clinical isolates was assessed in an animal model of tuberculosis. This work revealed that the most frequently-encountered mutation, *katG* Ser315Thr, had no significant effect on the virulence of *M. tuberculosis* but resulted in high level drug resistance.

The post-genomic analysis of *Mycobacterium leprae* (Nadine Honoré, Marc Monot, Romulo Araoz) :

Molecular methods may be the key to understanding the epidemiology of leprosy. We have developed DNA microarrays based on the whole genome of *M. leprae*. Our aim is to try and find genetic differences among *M. leprae* strains from Africa, Asia and America. The control of leprosy would also be improved if rapid diagnostic tests were available. Comparative genomic analyses allowed us to identify 40 candidate genes that may encode *M. leprae*-specific antigens. The recombinant expression of these genes is currently underway and preliminary results indicate that some of the products show seroreactivity with sera from leprosy patients.

The *Mycobacterium bovis* genome sequencing project (Thierry Garnier)

The genome sequence of *M. bovis* has now been fully assembled and annotated. The *M. bovis* genome comprises 4345492 base pairs and encode 3952 genes.

Intergenomic comparisons have been performed between the *M.bovis* and *M. tuberculosis* genomes in two ways; firstly, at the nucleotide level which identifies SNPs (Single Nucleotide Polymorphisms) and secondly, at the genomic level.

These two complementary approaches give us the opportunity to identify genes responsible for host specificity and to explain the differences found in the metabolic pathways between the two species.

Determination of the complete genome sequence of *Mycobacterium ulcerans* (Timothy Stinear)

Mycobacterium ulcerans causes severe, chronic, necrotizing lesions of subcutaneous fat. It is the causative agent of Buruli ulcer, a disease

that, since the 1980s, has become a very significant cause of morbidity throughout the world, but particularly in the countries of central and West Africa. Buruli ulcer is considered the third most common mycobacterial disease of immunocompetent individuals, after tuberculosis and leprosy. Unlike other mycobacterial pathogens, *M. ulcerans* produces a macrolide toxin called mycolactone and appears to remain extracellular during infection. We are adopting the genomic approach to better understand the epidemiology and the pathogenesis of this important disease and to find effective prophylactic and therapeutic treatments.

The determination of the complete genome sequence of *M. ulcerans* has made significant progress this year. An assembly of 43 000 reads from a whole-genome random shotgun library has been obtained. Currently there are 1597 contigs (56%>1kb et 10%>10 kb), representing a total length of 6.032 Mb. These contigs have been made publicly available and can be searched via a BLAST server

(<http://genopole.pasteur.fr/Mulc/BuruList.html>). To assist with the final assembly, a bacterial artificial chromosome (BAC) map of the *M.*

ulcerans genome has been constructed using 800 BAC clones.

Investigation of the pathogenicity in Clostridia (Gilles Reysset, Bruno Dupuy)

Our work on the pathogenicity of toxinogenic strains of Clostridia (*C. perfringens* and *C. difficile*) aims to understand mechanisms of the regulation of the major enterotoxins and to identify and characterize new virulence factors that may be implicated in the pathogenic behaviour of these two organisms.

C. perfringens is responsible for conditions ranging from mild food poisoning to necrotic enteritis or gas gangrene. It seems that adaptation to oxidative stress is an important factor in the pathogenesis of this ubiquitous organism. Analysis of the whole genome sequence of the strain 13 shows that *C. perfringens* possesses genes potentially encoding (i) a classical Mn-superoxide dismutase, (ii) an alkyl peroxide reductase, and (iii) two superoxide reductase systems potentially catalyzing the reduction of O_2^- to H_2O_2 without the production of oxygen, such as those of sulfate-reducing bacteria. In addition, there are three genes encoding potential non-heme rubrerythrin proteins, which may act as the terminal component of NADH peroxidase, catalyzing the reduction of hydrogen peroxide to water. The catalytic properties of the products from all these genes, and the role of each of them in the oxidative stress response in *C. perfringens*, were assessed by complementation experiments in *Escherichia coli* strains impaired in either superoxide dismutase, alkyl peroxidase or catalase activity. Transcriptional analysis of these genes was also performed to investigate their regulation in *C. perfringens*.

In the separate study, the search for new targets to enable the development of strong inhibitors of the initiation and establishment processes of *C. perfringens* during infection, led us to work on the pathogenicity and the immunogenic capacity of four CBP proteins (Choline Binding Protein). These CBP belong to a small family of proteins that have affinities to choline residues. They are present in all toxinotypes of *C. perfringens* strains and are cell membrane associated.

The pathogenic capacity of *C. difficile*, the organism responsible for pseudomembranous colitis and most antibiotic-associated diarrhoeas, is mainly due to the large production of two major toxins, Tox A and Tox B. The transcription of the *tox* genes is regulated through the *txeR* gene product, an alternative RNA sigma factor, that directs transcription from the *tox* promoters and its own promoter in response to growth phase and nutrition. We have shown that UviA, a protein homologous to TxeR, regulates as a sigma factor, transcription of a bacteriocin gene in *C. perfringens*. TxeR and UviA are the first described members of a new family of alternative sigma factors (Class V).

Keywords : Functional genomics , Mycobacterium tuberculosis complex , drug resistance , catalase-peroxidase ; Clostridia

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