Room 314 13:30 - 18:00

"Bacillus biotechnology and its prospective future"

The intimate EU-JP cooperation in ***Bacillus*** research was materialized in 1990 when the international consortium for genome sequencing was established and has been maintained involving the successful projects such as the EU-funded BACELL. In this session, five speakers present their expertise along with their international cooperative experiences and discuss about the future prospects.

PROGRAMME

13:30	Opening address: Prof Ken-ichi Yoshida, Kobe University
13:35	Prof Ken-ichi Yoshida, Kobe University "Functional genomics on ***Bacillus***: strategy and application"
14:20	Prof Bernard Joris, Université de Liège "Engineering ***Bacillus subtilis*** genome"
15:05	COFFEE BREAK
15:30	Prof Colin Harwood, University of Newcastle "The ***Bacillus*** cell factory: stress and productivity"
16:15	Prof Jan Maarten van Dijl, University of Groningen
	"Challenges and opportunities for Bacillus research on protein production"
17:00	Dr Philippe Noirot, INRA, CRJ "***Bacillus*** research in systems and synthetic biology"
17:45	Closing remarks: Prof Chiharu Nakamura, Kobe University

Functional genomics on *Bacillus*: strategy and application

Ken-ichi Yoshida
Department of Agrobioscience, Graduate School of Agricultural Science, Kobe University, Japan

The entire genome of ***Bacillus subtilis*** comprising more than 4,000 genes was determined by the successful collaboration amongst European and Japanese researchers. Since then, this organism representing the ***Bacillus*** genus has been regarded as one of the model systems to study functional genomics both in basic and applied aspects. The genome is the entirety of hereditary information of an organism, which is in fact divided into two categories of the "known" and "unknown" genes, and many of the researchers in the field of functional genomics are making efforts to turn the "unknown" into the "known". In the case of ***B. subtilis*** studies, the researchers took a cooperative strategy to increase the "known" genes, and now almost 60% of the genes are "known" as assigned a specific role whilst the rest 40% are yet "unknown" for their function. Then, how can we use functional genomics for application? The "known" genes are predicted to be assigned to "known" metabolic pathways, which can be modified to improve their original capacity to produce desired compounds. On the other hand, the "unknown" genes are functionally identified to discover "new" pathways, which can be enhanced and extended to produce new and additional targets. Our expanding knowledge of ***B. subtilis*** functional genomics now allows us to attempt possible rational approaches to develop biotechnology using the ***B. subtilis*** cell factory to produce a variety of chemicals to fulfill our demands in materials, food, and health.

Engineering *Bacillus subtilis* genome

Bernard Joris
Centre d' Ingéierie des Protéines
University of Liége - Belgium

The completion of the sequencing and annotation of the ***Bacillus subtilis*** 168 genome supply a complete view of the ***B. subtilis*** protein machinery, and this knowledge stimulates new approaches to analyze biochemical pathways. This postgenomic study requires genetic tools that allow the combination of several gene manipulations in the same strain. Classically, these chromosomal modifications could be achieved by a method using a positive selection marker, usually an antibiotic resistance marker generated by the insertion of a selection marker gene in the ***B. subtilis*** chromosome. In this strategy, the introduction of a second chromosomal modification requires a second resistance gene, or, if the same resistance gene is used, the eviction of this gene by a single crossover event prior to further genetic manipulation. To overcome the problems listed above we have developed a novel method that combines the use of ***blaI***, which encodes a repressor involved in ***Bacillus licheniformis*** BlaP β-lactamase regulation, a ***B. subtilis*** strain that is conditionally auxotrophic for lysine and DNA cassette containing blaI and the spectinomycin resistance genes and two short direct repeat DNA sequences, one at each extremity of the cassette. This strategy was successfully used to inactivate a single gene and to introduce a gene of interest in the ***B. subtilis*** chromosome. Two examples will be presented: the first will involve the production of lipopeptides from ***Bacillus*** and the second, the characterization of ***B. subtilis*** YoaJ protein involved in the interaction of ***Bacillus*** with plants.

The *Bacillus* cell factory: stress and productivity

Colin R. Harwood
Centre for Bacterial Cell Biology, Institute for Cell and Molecular Biosciences, Newcastle University, UK

Bacillus subtilis and its close relatives have been used extensively for the production of industrial enzymes, fine biochemical, vitamins and flavour enhancers. As a result of its commercial importance, and the fact that 50 years ago it was the first non-pathogenic bacterium to be successfully genetically transformed with isolated DNA, *B. subtilis* has become one of the best-studied living organisms. Both European and Japanese scientists have a long and distinguished track record for studying the genetics and molecular biology of this bacterium and the extensive collaborations between these scientists that developed in the 1990's have helped to accelerate progress towards the potential for an even greater exploitation of this bacterium in the field of white biotechnology.

A key element in maximizing productivity is a clear understanding the behaviour of this bacterium during its cultivation. In particular how it behaves in response to the stresses imposed by manipulating its ability to overproduce proteins and metabolites and by the fermentation conditions used to maximise growth. To this end, *B. subtilis* has been subject to extensive studies aimed at understanding how it controls the production of its proteins at both the level of gene expression (transcriptomics) and translation (proteomics). Knowledge of the master cell regulators, and how their behaviour is coordinated, is key to optimising the *Bacillus* cell factory, and examples from the work of European and Japanese will be given to show the progress in this field.

Challenges and opportunities for *Bacillus* research on protein production

Jan Maarten van Dijl
Department of Medical Microbiology, University Medical Center Groningen and University of Groningen, the Netherlands

Bacteria secrete numerous proteins into their environment for growth and survival under complex and ever-changing conditions. The highly different characteristics of secreted proteins pose major challenges to the cellular protein export machinery and, accordingly, different pathways have evolved. These secretion pathways have been the focus of fundamental and applied research both in Europe and Japan. While the main secretion (Sec) pathway transports proteins in an unfolded state, the twin-arginine translocation (Tat) pathway can transport fully folded proteins. For a long time, these pathways were believed to act in strictly independent ways. In recent studies, we have employed proteogenomic approaches to investigate the secretion mechanism of the esterase LipA of *Bacillus subtilis*, using hyper-producing strains. While LipA is secreted via Sec under standard growth conditions, hyper-produced LipA is secreted predominantly via Tat through an unprecedented overflow mechanism. This overflow secretion mechanism raised the possibility that the secretion pathway choice can be determined not only by intracellular conditions, but also by environmental conditions. This idea was challenged by determining the effects of environmental salinity on Tat-dependent protein secretion by *B. subtilis*, since this soil bacterium can encounter widely differing salt concentrations in its natural habitats. The results show that environmental salinity determines the specificity and usage of the *B. subtilis* Tat pathway. Interestingly, the studies even identified an essential function of the Tat system for growth of *B. subtilis* under low-salinity conditions. Taken together, our findings show that both intracellular and environmental factors can determine the specificity, substrate spectrum and biological function of bacterial protein secretion via the Tat pathway. This opens up new avenues for biotechnological applications of *B. subtilis* in protein production.

Bacillus Research in Systems and Synthetic Biology

Philippe Noirot
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Bacillus subtilis is one of the best studied bacterium, second only to *Escherichia coli*, and is the model organism for Gram-positive bacteria, a large group including pathogens causing diseases in humans as well as soil-dwelling bacteria heavily used in biotechnology. A vast body of knowledge about gene functions, cellular pathways, regulatory networks, and cell architecture has been accumulated by the scientific community. Rapidly developing high throughput technologies provide information virtually on all cellular components. In combination, these facts have enabled the emergence of systems biology in *Bacillus* by attracting mathematicians and engineers who could build mathematical models based on existing data and test the model predictions by new experiments. A potent facilitator of systems biology in *Bacillus* is the long-standing and fruitful collaboration between European and Japanese researchers in the fields of functional genomics and tool development.

Here, I will review some of the milestones in the development of systems biology and more recently of synthetic biology in *Bacillus*. This research holds the promises of understanding how the cellular processes functionally integrate at the cell level and of rationally designing pathways to exploit the biosynthetic and degradative capabilities of microbes for human benefit.