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A cell array reporting on gene expression in living *Bacillus subtilis*

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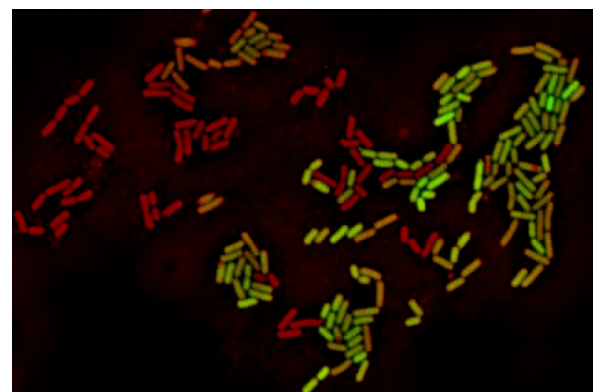
The functions of living cells are determined by the repertoire of genes that are expressed. The chromosome of *Bacillus subtilis* contains more than 4000 genes. Some of these genes have 'house-keeping' roles, which require that they are continuously expressed. Other genes have functions in the adaptation of the bacteria to changing environmental conditions such as an alternative source of nutrients or a specific challenge such as oxidative stress. These genes are expressed only in response to specific signals or cues. Establishing the repertoire of genes being expressed under a given set of circumstances has traditionally involved laborious study of expression gene-by-gene or alternatively involved cell killing. In Workpackage 1.1 of BaSysBio, we are generating an array that can report simultaneously on the expression levels of several hundred genes in living cells.

Each element in the array is a small growing culture of chromosomally-engineered cells in which the level of expression of a particular gene is indicated by green fluorescence. The array will be a collection of several hundred of these cells, growing in parallel, with each culture reporting on the expression of a distinct gene. The living cell array will allow us to determine the global pattern of gene expression under changing growth conditions.

To generate this cell array we are using high throughput methods to construct hundreds of plasmids in which the control region of each gene of interest (promoter) is placed adjacent to the gene encoding green fluorescent protein (GFP). These plasmids are then introduced into *B. subtilis* cells where they integrate into the chromosome without perturbing the expression pattern of other genes. We have completed a pilot study in which 10 plasmids were constructed in York and validated before distribution to the partner laboratories in Dublin, Groningen and INRA for strain preparation and characterisation. This pilot study has shown that it is feasible to construct the cell array and to monitor growth rates and gene expression by parallel growth of (i) small scale liquid cultures in which the fluorescence of the cell population is measured or (ii) cells on microscope slides where gene expression in single cells can be followed (Figure below).

Fluorescence microscopy image of *B. subtilis* expressing the green fluorescent protein gene from a defined promoter (*ackA*). These cells were grown on a microscope slide in a defined medium with 0.3% glucose and 0.3% malate as the carbon source.

Courtesy of Sjouke Piersma and Jan Maarten van Dijl (AZG)



From Work-Package 1.1 : Chromosome engineering and living cell array

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