Following the decision, in the late 1990s, to sequence the genome of Streptomyces coelicolor A3(2), the Biotechnology and Biological Sciences Research Council (BBSRC) of the UK committed substantial funding for a coordinated functional genomics programme aimed at building a detailed understanding of this model actinomycete species. For current information on the S. coelicolor genome and genome-related tools, see: http://jic-bioinfo.bbsrc.ac.uk/S.coelicolor/.

The programme involves a number of UK centres, covering the following post-genomic technologies:

- DNA microarray development, provision and training: Colin Smith (University of Surrey, formerly at UMIST, Manchester).
- Proteomics: Keith Chater (John Innes Centre, Norwich).
- Systematic gene disruption: Keith Chater and Tobias Kieser (JIC) and Paul Dyson (University of Swansea).
- Bioinformatics: Andy Brass (University of Manchester); Douglas Kell (UMIST, formerly at University of Aberystwyth); Chater (JIC); Smith (Surrey).

These technologies are now well established in the respective laboratories and are starting to provide new biological insights. Whole genome DNA microarrays have been produced for gene expression profiling and ‘genomotyping’, more than 1,000 protein spots have been identified, more than 1,000 genes have been knocked out by ‘PCR targeting’ and in vitro transposition technologies, and genome, transcriptome and proteome databases are actively under development.

DNA microarrays and associated training (http://www.surrey.ac.uk/sbms/Fgenomic) (e-mail, microarrays@surrey.ac.uk). We have focussed largely on producing PCR-based DNA microarrays, although we have recently completed successful side-by-side trials with long oligonucleotide arrays; a complete ‘long oligo’ set has been produced in collaboration with MWG Biotech, and are now being used to produce arrays at UniS (Figure 1).

The PCR-generated microarrays have been tested with commercially important streptomycetes such as S. clavuligerus and found to readily detect their respective orthologous genes. This opens the way for exploiting the arrays more broadly in commercial and taxonomic research programmes.

A great advantage of having the in-house capacity to produce spotted arrays is that they can be customised. For example, gene probes representing antibiotic gene clusters from other species can be designed and spotted alongside the ‘core’ S. coelicolor genes, allowing the parallel measurement of species-specific gene expression.

For generating PCR products, we designed an automated primer selection programme. Similarity searches on each

Figure 1. A Streptomyces coelicolor whole genome DNA microarray produced by the UniS Functional Genomics Laboratory. The cDNA is labelled with Cy3 (green) and the genomic DNA is labelled with Cy5 (red). Each spot is approx 150 microns in diameter.
candidate probe predicted potential cross-hybridisations and allowed selection of ‘unique’ PCR products; these were then generated by a two-stage procedure, firstly using gene-specific primers with universal tags and, subsequently, using universal primers, one of them being 5'-amidated. Arrays comprising the majority of open reading frames have been produced and protocols for RNA isolation, cDNA synthesis and hybridisation have been optimised.

We are now exploiting DNA microarrays to study global patterns of gene expression in Streptomyces and are particularly focusing on investigating patterns of gene expression in time-course experiments to investigate changes that correlate with developmental and metabolic transitions. Our analysis has concentrated mainly on ‘surface-grown’ cultures and has revealed dramatic changes in gene expression at the ‘decision’ phase prior to the onset of aerial mycelium and secondary metabolite formation. Several transiently induced novel genes have already been identified that are likely to play roles in the regulation of development and antibiotic production.

In parallel, the proteomics effort has identified (by 2-D PAGE and subsequent MALDI-MS) more than 1,000 protein spots (e.g. see http://qbab.aber.ac.uk/s_coeli/referencegel/ for information), and a significant number of unusual post-translational modifications have been revealed.

**Streptomyces viewed from the inside:** the application of proteomics to a model streptomycete

Streptomyces coelicolor A3(2) has become the model system for this genus of antibiotic-producing bacteria, thanks to the life-time commitment of Sir David Hopwood to its genetic analysis. The determination of its complete genome sequence has made it an even more valuable model, opening up many analytical possibilities that emerge directly from the sequence, and also various functional genomics approaches.

One particularly exciting prospect is the high throughput application of MALDI-ToF mass spectrometry to the analysis of the overall protein content of cell or culture extracts. In such a proteomics approach, proteins separated by multiple fractionations, most typically by isoelectric properties and size in classical 2D gel electrophoresis, are digested with a protease (typically trypsin) before mass spectrometric analysis. Usually, the resulting mass fingerprint reveals about half of the fragments predicted for any one protein, with a mass accuracy sufficient to give unambiguous identification of the cognate gene.

Proteomic analysis of *S. coelicolor* extends the range of global gene expression analysis beyond the