BBSRC MEETING ON THE HISTORY OF *STREPTOMYCES* SCIENCE AND THE SEQUENCING OF THE *S. COELICOLOR* GENOME[1]

EXECUTIVE SUMMARY

The Biological and Biotechnological Sciences Research Council (BBSRC) held a one day meeting in London, on 21 January 2010, to review the key historical events that had led to the decision to sequence the *Streptomyces coelicolor* genome, to examine the impact of the sequence so far, and to invite expert commentary on potential future impacts. This report is organised into four sections, mirroring the four discussion sessions at the meeting: Research and Technology, Networks and training, Funding, and Impacts.

S. coelicolor began as an obscure organism, perhaps because it did not seem to possess any industrial promise. In this connection most of Hopwood's early competitors had been interested in antibiotic production and the more important industrial models were *S. rimosus* and *S. aureofaciens*. From the mid-1970s *S. coelicolor* began to enjoy a sudden prestige and was transformed into a highly successful model system with researchers distributed across more than 20 countries. When the decision to sequence *S. coelicolor* was taken in 1997, it took with it the entire budget that the BBSRC Microbial Genomics Working Group had for genome sequencing (£1.5M). This event was a measure of the remarkable transformation of *Streptomyces* and the four discussions that follow provide valuable insights into how *Streptomyces* science has progressed and what has been achieved.

Research and Technology- Important Outcomes

A timeline of milestones in *Streptomyces* genetics leading up to genome sequencing and genetic manipulation of antibiotic biosynthesis was presented at the meeting. Participants were asked to comment and make additions to the list. The discussion focused on areas of science that fell outside timeline recorded events, for example:

- sequencing of the erythromycin gene cluster was seen as a real turning point as once you had the sequence of the genes you could understand, conceptually, without having to do too many experiments, how this molecule was assembled.
- NMR (Nuclear Magnetic Resonance Spectroscopy) and mass spec. (2D techniques) were offered up among the most influential analytical developments.
- development of multi-dimensional spectroscopy revolutionised the way you determined the structure of things.
- X-ray crystallography was thought to be key to the pharmaceutical industry for development and patenting drugs.
- Pulsed Field Gel Electrophoresis allowed very large DNA molecules to be seen.
- One of the first *Streptomyces* proteins characterised and solved was glucose isomerase (D-xylose isomerase) which has been a major gain to the food industry.
- automated screening was an important advance in the pharmaceutical industry. Robot technology was translated into laboratory and other analytical technologies as well.

Discussions also focused on advances in *Streptomyces* genetics and the advantages of *Streptomyces* as a model system, for example:

- the discovery that antibiotic genes were clustered. The key contributions of classical genetics was that it generated plasmids, established vectors etc, and so you can begin to move into the molecular era, and it showed that when you clone the drug resistance marker, you've got the rest of the genes with you.

[1] Initial historical account written by Sarah Wilmot, JIC.

- BAC vector technology (Bacterial Artificial Chromosome) has allowed manipulations in *E. coli* on a massive piece of DNA.
- reporter systems e.g. neomycin resistance on pIJ486/487, and xylE promoters.

Funding

A graph of BBSRC expenditure on *Streptomyces* projects (Appendix 3) was presented and participants were asked to contribute information on the importance of other non-BBSRC funding streams including the John Innes Centre (JIC) Streptomyces Club, Biotechnology Directorate (BTD), EU funding (IGF and EGI initiatives), Wellcome Trust (Beowulf Genomics Initiative), NIH, USDA.

Public funding has assumed even greater importance against the background of a 'disenchantment' of the pharmaceutical industry with the natural products sector and in many cases a withdrawal from R&D investment in this area. The exit of big-pharma, however, has provided a market opportunity for small biotech companies. New industry for natural products would be built around genomics, based on thousands of sequences. Open access to sequence information was considered key in growing the *Streptomyces* community and advancing the field internationally.

Networks and Training

Interaction between academia and industry in *Streptomyces* research has been driven by a number of formal and informal networks for example:

- JIC Streptomyces Club - members were offered 2-3 days lab space.
- Sabbaticals - two-way movement of scientists between academia and industry.
- International Symposia on the Biology of Actinomycetes (ISBA)
- The Society for Industrial Microbiology (SIM), United States.
- The Genetics of Industrial Microorganisms (GIM) meetings, and the GIM international Symposia, held every four years.
- Vereinigung fur Allgemeine und Angewandte Mikrobiologie (Association for General and Applied Microbiology) promotes German interaction with UK groups.
- The Society for General Microbiology (SGM) offer student overseas travel grants.
- European Molecular Biology Organization (EMBO) training courses run by JIC encouraged academic and industry interaction.
- EU fellowships were instrumental in bringing in scientists to the UK (for 6-month periods) with expertise in different aspects of *Streptomyces* biology.
- The BBSRC dissemination meetings for the *Streptomyces coelicolor* sequence were acknowledged as highly significant for establishing peer-to-peer relationships between graduate students.
- Polyketides conferences to bring together leading experts from different disciplines, to provide a comprehensive update of recent progress and to discuss future developments and priorities in polyketides research, which provided an opportunity for graduate students to speak.
- strong networking within natural products chemistry groups.
- European Union initiatives under FP6 (6^{th} Framework Programme) for Integrated Projects have established new collaborations between European labs.
- summer schools (JIC and the Institute Ruđer Bošković, Zagreb) have attracted participants from around 25 nationalities to train in secondary metabolites and genomics.
- The BBSRC-supported functional genomics initiatives, including the 'Investigating Gene Function' research programme for *Streptomyces.*

- Networks established through training initiatives, and through returning PhD students to overseas academic departments.
- Industrial companies are knowledgeable about research networks and will recruit people coming from 'good groups'. An example was given of a company expansion into Europe that has used networks such as this to recruit in France, Germany and Spain (Barrie Wilkinson).
- Society for Actinomycetes Japan (SAJ) provides a focus for the big Japanese interest in Actinomycete biology in all its aspects - taxonomy, ecology, natural products and genetics.

Economic Impact

What has sequencing the *Streptomyces coelicolor* genome achieved?

1. Development of phage integration systems – a key technology for engineering and started with the work of Natasha Lomovskaya and Keith Chater, Nagaraja Rao's group at pharmaceutical company Eli Lilly, and then Maggie Smith.
2. Revolutionised the way geneticists do their experiments and organise their research, and it provide the scaffolding and the reference point for new investigations.
3. Heightened scientific awareness of the possibilities of new technologies. There is realisation that "cryptic" secondary metabolic gene clusters have the ability to produce many more natural products than had previously been recognised; this has stimulated research worldwide.
4. The *coelicolor* would always represent the 'gold standard' because of the investment in annotation of the sequence; therefore it remains the key starting point.
5. Major impact on research into Mycobacteria (important for understanding the behaviour of the disease-causing bacteria *Mycobacterium tuberculosis* and *M. leprae).*
6. ActinoGEN initiative resulted in the creation of a 'superhost'. A streptomycete was used to express heterologous secondary metabolic gene clusters. Employing microarray and global proteome analyses, the strain was improved as an expression host for the production of small molecules (a 'superhost').

What will it achieve in the future?

1. Better understanding of gene regulation and expression.
2. Deploying new approaches to exploiting Actinomycete genomes.
3. Development of new Antibiotics from actinomycetes.
4. Development of more powerful machines for sequencing may allow sequencing of an entire company strain collection (we might look forward to perhaps a 10-fold increase in sequencing capacity). There will be a need for investment in data storage and bioinformatics.
5. Sequence genomes of bacteria and Archaea from across the tree of life (sequencing organisms that are distantly related from any that have been sequenced so far). This will help with the annotation of existing Streptomycete genomes and may in future stimulate the development of new model systems.
6. New technologies will also bring new model systems to the forefront of molecular microbiology. *Streptomyces venezuelae* will become an incredibly powerful alternative model system in the future, enabling a lot of 'omic techniques to be used in a way where you can synchronise the developmental cycle with antibiotic biosynthesis because sporulation is synchronous in *venezuelae* and occurs in liquid culture.
7. Future trend for sequencing organisms that are actually in the soil will produce information that can be used translationally to express compounds themselves. It is very likely that studies of the ecology of Actinomycetes in soil (for example, Liz Wellington's studies which aim to unravel the mechanisms controlling responses in

bacteria to key environmental signals) will be a key growth area in the future (David Hodgson, Maggie Smith).

The meeting closed with some general reflections on the lessons learnt from the investment in *Streptomyces* genetics and genomics. Returning to the networks that had been built it was stressed that a lot of the work underpinning them was essentially ‘blue sky’ and not applied research. Networks were critical to the successes achieved, and will need maintaining in the future. The long-term nature of the investment in *Streptomyces* genetics was stressed and the timeframe for future advances in this field will also be lengthy, both in academia and for the long lead-in time in industrial research and development before you get to the point of marketing a new antibiotic.

BBSRC MEETING ON THE HISTORY OF *STREPTOMYCES* SCIENCE AND THE SEQUENCING OF THE *S. COELICOLOR* GENOME

HISTORICAL BACKGROUND

Streptomyces coelicolor genetics in the UK began in 1954 when David Hopwood chose this organism for his PhD studies at the School of Botany, University of Cambridge. At the time nobody else in the world was known to be working on *Streptomyces* genetics. Indeed, bacterial genetics was still in its infancy and 'appeared bizarre compared with the genetics of plants and animals' (Hopwood 2007, p.51), while fungal genetics was also a small but developing field. Fascinated by the fundamentally different ways that bacteria and fungi exchanged genes, Hopwood chose to investigate *Streptomyces* (the most important genus of the *Actinomycetes*), because at this time this group of microbes were usually regarded as 'intermediate' between bacteria and fungi. Hopwood did not know that *S. coelicolor* produced antibiotics, though Streptomycetes were becoming pre-eminent in this field; he chose to work on this organism because it could be cultured easily and because he thought that the beautiful blue colour it produced might prove to be a useful genetic marker.

Hopwood later discovered that at least five other scientists or groups internationally had had the same idea of investigating genetic exchange in *Streptomyces* and all six had found recombination between 1955 and 1958. Hopwood had a particularly productive collaboration with Giuseppe Sermonti's group in Rome, spending sabbatical periods there in 1960 and 1961. Most of these scientists (including Sermonti) dropped out within a few years, so that in 1961, when Hopwood moved to a lectureship at the University of Glasgow, he was 'essentially alone in the field'. His small group of researchers at Glasgow: a technician, graduate research assistant and a couple of postdocs moved with him when he made the move to the John Innes Institute in Norwich.

Research Council funding of *Streptomyces* genetics (initially ARC, later AFRC then BBSRC) began at the John Innes Institute (now John Innes Centre) in 1968 when David Hopwood was appointed as Head of Genetics and John Innes Professor of Genetics at the University of East Anglia. Hopwood had by this time developed the basic genetics of *S. coelicolor* A3 (2), using his novel method of linkage analysis (a procedure developed in 1959 which later proved useful for genetic studies of other microbes). At the time Hopwood founded the *Streptomyces* group in Norwich he had just established a detailed circular linkage map of more than 100 *S. coelicolor* genes (1967), pioneering work that confirmed *S. coelicolor* A3 (2) as the model system for the genus.

During the 1970s Hopwood's group showed that the fertility system of *S. coelicolor* involves a plasmid sex factor. In the course of these studies they identified the first clear example of plasmid-encoded antibiotic synthesis. These discoveries, along with first tools they developed for strain improvement for antibiotics (protoplast fusion) and genetic engineering (protoplast transformation and transfection), secured JIC's international reputation and began to attract academic and industrial scientists working on natural product pharmaceuticals to Norwich. Particularly with its success in developing cloning methods, the JIC *Streptomyces* group actively embraced a training role, offering practical courses based around its influential *Genetic Manipulation of Streptomyces: a laboratory manual* (1985) and *Practical Streptomyces Genetics* (2000). These initiatives and over 100 alumni working in senior positions in academic institutions or the private sector have carried JIC *Streptomyces* research around the world.

The JIC team was the first to clone a complete set of antibiotic production genes, they engineered the first hybrid antibiotic, and they have led the world in understanding the regulation of antibiotic production and *Streptomyces* development. They oversaw the *Streptomyces coelicolor* A3 (2) genome sequencing, done at the Sanger Centre (published

2002), and the subsequent development of a range of genomic tools by UK consortia. The sequence (and that of the avermectin-producing *Streptomyces avermitilis* published at about the same time by a Japanese group) demonstrated for the first time that *Streptomyces* genomes are very large, and teem with unsuspected gene clusters for potentially interesting metabolites, a discovery made amenable to exploitation by the tools developed at JIC. The substantial investment by the BBSRC in the sequencing of the *S. coelicolor* genome was an outcome of the extent and quality of the genetic work that had already taken place, at the JIC and in JIC-driven collaborations, and recognition of the substantial user-community for the genome around the world.

BBSRC MEETING OF EXPERTS TO DISCUSS *STREPTOMYCES* HISTORY

Dr Celia Caulcott, Director of Innovation and Skills, BBSRC, called a one-day meeting at the Russell Hotel, London on 21st January 2010 to review the key historical events that had led to the decision to sequence the *Streptomyces coelicolor* genome, to examine the impact of the sequence so far, and to invite expert commentary on potential future impacts. The meeting was attended by an international group of leading *Streptomyces* scientists and BBSRC administrators (see Appendix 1). The recording and transcripts of the meeting form the basis of the following report which is not intended as a verbatim account. The report is organized into four sections that mirror the four sessions of the meeting: Research and Technology, Networks and Training, Funding, and Impacts.

To summarise, *S. coelicolor* began as an obscure organism, perhaps because it did not seem to possess any industrial promise. In this connection most of Hopwood's early competitors had been interested in antibiotic production and the more important industrial models were *S. rimosus* and *S. aureofaciens*. From the mid-1970s *S. coelicolor* began to enjoy a sudden prestige and was transformed into a highly successful model system with researchers distributed across more than 20 countries. When the decision to sequence *S. coelicolor* was taken in 1997, it took with it the entire budget that the BBSRC Microbial Genomics Working Group had for genome sequencing (£1.5M). This event was a measure of the remarkable transformation of *Streptomyces* and the four discussions that follow provide valuable insights into how *Streptomyces* science has progressed and what has been achieved.

1. Research and Technology

The meeting was presented with a timeline of milestones in *Streptomyces* genetics leading up to genome sequencing and genetic manipulation of antibiotic biosynthesis. The timeline had been prepared for the DTZ report for BBSRC on the 'Economic Impact of *Streptomyces* Genetics Research' (see Appendix 2). Participants were asked to comment and make additions to the list. The discussion organised into points 1-18 focused mainly on those areas of science that fall **outside** the events recorded in the *Streptomyces* genetics timeline; points 19-51 focus more particularly on advances in *Streptomyces* genetics and the advantages of *Streptomyces* as a model system.

1. Mike Goodfellow made the point that scientific research on *Streptomyces* has a very long history and that it doesn't begin with David Hopwood's work on *Streptomyces* genetics. The story begins pre-1950 with the taxonomic work on which David Hopwood was able to build when he began his PhD researches in the 1950s. There was also significant industry investment in the infrastructure of *Streptomyces* research in the 1960s. The International Streptomyces Project or ISP (which also had NSF funding) studied all the streptomycetes from a taxonomic, morphological, and a few physiological characters. Industrial funding was forthcoming because everyone was hampered by the 'appalling state' of streptomycete systematics. The key figures

in ISP were E.B. Shirling and D. Gottlieb, and their key paper (1966) is still in use. Mike Goodfellow commented that some of the 'secondary' factors you think of in *Streptomyces* history are in fact primary - the establishment of authenticated culture collections was of key importance to the scientific community, leading to solutions about what '*Streptomyces*' is. Taxonomists were able to define the genus based on morphological characters and subsequent work on chemotaxonomy (and later still molecular biology). The chemotaxonomic work of the Lechevaliers and others made an important contribution. Taxonomic work was also important for industry - he pointed out that if your organisms are in the wrong groups you will use the wrong techniques (for example, early attempts at protoplast fusion in *S. erythreus*).

2. Peter Leadlay commented that alongside the genetics timeline you could construct a timeline for the development of chemistry as applied to the pharmaceutical industry. He stressed the importance of rapid developments in chemical thinking, not only just in terms of the modification of antibiotics (which are often complex sensitive molecules) but also total synthesis and developments on the analytical side that made the work very much faster and set the bar higher in terms of competition within industry (it became possible to detect faster and more accurately small quantities and more difficult compounds). In academia there was a tremendous burst of creativity directed at understanding the intermediates in building up natural products and increasingly piecing together the whole series of steps that would lead to something like erythromycin. A small number of highly influential biosynthetic groups in Europe and the US (Oxford, Cambridge, Texas, Yale) had got chemistry to the point in 1989/90 where its competing technologies (including high-throughput screening) threatened to overwhelm the extraordinarily powerful genetic tools developed by the John Innes. The pivotal point for Leadlay came with the piecing together of chemical research to reveal how peptide antibiotics are made.

3. Looking also at chemical developments, Greg Challis pinpointed the sequencing of the erythromycin gene cluster as a real turning point and that was done in Peter Leadlay's lab, Cambridge, and at Abbott Labs, Chicago, USA. The reason it was pivotal was that once you had the sequence of the genes you could understand, conceptually, without having to do too many experiments, how this molecule was assembled. He commented that it dawned on everyone that lots of other molecules were going to be assembled in the same kind of way and that was probably a big motivation for moving towards the large-scale sequencing of things. Sequencing turned out to be so powerful for understanding how these molecules were made and it immediately suggested ways you could manipulate the pathways. (see DTZ timeline, reference no. 13). He added that when Mohamed Marahiel sequenced the gramicidin biosynthesis genes, which revealed how non-ribosomal peptides are made and was done at a similar time to the erythromycin sequence, this was in *Bacillus* not in *Streptomyces*, though it fed in.

4. Staying on chemistry, Celia Caulcott invited contributions on analytical developments that have been critical to advancing the field. NMR (Nuclear Magnetic Resonance Spectroscopy) and mass spec. (2D techniques) were offered up among the most influential analytical developments. Opinion was divided on where this technology originated. One view was that the development of NMR was driven by research on protein structure, to look at the structure of big molecules and took place quite outside research on small molecule natural products (though it was picked up quite quickly and has been very powerful in those areas). The increased sensitivity of NMR was mostly linked to bio-molecules; to study these you needed more sensitive instruments and higher resolutions. Barrie Wilkinson suggested NMR technology had come from people like Jim Staunton and methods he picked up from lectures on astronomy to achieve much better quality spectra. These developments really

pushed the field forward for biosynthesis, for stable isotope labelling. Many other groups in the biosynthesis field had an impact on broader methodologies now that impact on many areas of science. Flavia Marinelli felt on the other hand that mass spectra, NMR technologies were developed in industry to define the structure of natural products because in those days academia was more focussed on genetics and genetic manipulation, and understanding biosynthetic pathways, but new products were discovered in industry. She argued that chemical equipment and the analytical technology was more concentrated in companies.

5. It was agreed that the instrumentation probably was an industry-led development because it was too expensive for academia. There is a whole other history of industry / academic collaboration in instrument development. There were very few instrument companies, Bruker is one of the big ones, and there was a very intimate interaction between the academic and the industrial labs to develop those things and make them possible.

6. Another key event for structure-determination was the development of multi-dimensional spectroscopy which 'totally revolutionised the way you determine the structure of things', replacing crystallography and degradation methods. Also hyphenated chromatography methods which changed the timeframe for analytical investigations from a month to overnight.

7. Greg Challis drew attention to the importance of X-ray crystallography. This was absolutely key to the pharmaceutical industry being able to develop and patent drugs because the structures of most natural products are very complex and the only secure patent is one that defines exactly what your molecule is. Dorothy Hodgkin first did this with penicillin, but if you look through whole swathes of 60s and 70s patents on new compounds you will find John Carly's name will be found on most. He was the crystallographer that everyone in academe and industry went to if they wanted to find out what the structure of their compound was. Many of his 7-800 patents are used today in industry.

8. Paul Dyson suggested that a technological development that was critical to *Streptomyces* research was Pulsed Field Gel Electrophoresis. 'Suddenly we were able to see very large DNA molecules and to figure out the fact that they were linear plasmids'.

9. David Hodgson commented, apropos X-ray crystallography, that one of the first *Streptomyces* proteins characterised and solved was glucose isomerase (D-xylose isomerase) which has been a major gain to the food industry (essentially converting the glucose to fructose to increase sweetness and used in the manufacture of high-fructose corn syrup). Industry had attempted to engineer the protein to make it more temperature resistant, investing a lot of company time in the project, but then it was found that the *Streptomyces*- derived protein was five degrees more temperature resistant. This was an important aspect of *Streptomyces* as an industrial organism (but was not a John Innes development). Gilles van Wezel agreed and commented that glucose isomerase was the only reason GenenCor (a big producer of glucozymes) had continued working on *Streptomyces* in the early '90s because you can't express the protein and get it properly active in *Bacillus* or *E. coli.* It was an important development in terms of enzyme production using Streptomycetes which Gilles thought was now five or ten per cent of the turnover of big companies. *Streptomyces* research and the enzyme industry could perhaps form the basis of another history.

10. David Hodgson commented that the combination of chemistry and genetics is very interesting. In the classical genetics era they used things like C^{13} to work out which compounds went into the pathways, and pathways were often worked out in a purely chemical fashion. But there were other examples (for instance, actinorhodin) where the biosynthetic pathway was worked out from the genetics, without using the C^{13}. Some of the pathways you understand a lot better because of the genetic input- 'but it has been a later development, kind of parallel going on'.

11. Peter Leadlay said that he had to pick **one** development, for its ability to be subversive and lead to you thinking up new experiments, it would be DNA sequencing. We can't get away from its central importance. Remember that in the '90s the technology involved phosphorous 32 manual sequencing on gels by human beings. For a very long time you would be scorned by your more experienced colleagues for daring to suggest that double-stranded DNA could be sequenced. Brian Rudd agreed, it was 'one studentship, one gene, wasn't it!' Leadlay suggested it wasn't until the early 90s that a protocol for sequencing double-stranded was established in academic labs.

12. Greg Challis adds that when in 1995 the shot-gun sequence of the *Haemophilus influenzae* genome was done, that was obviously pivotal.

13. Mark Paget made the point that when you sequence DNA you need to interpret it and so you need bioinformatic tools. An influential package was FramePlot which is a tool for predicting protein-coding regions in bacterial DNA with a high G + C content, such as *Streptomyces*. The output provides for easy distinction of protein-coding regions from non-coding regions. The Frame analysis is based on codon bias: genes of bacteria which have a high G+C content genome DNA such as *Streptomyces* possess biased codon usage. FramePlot was first developed by Mervyn Bibb *et al.* in 1984, and it is one of the essential tools for studying *Streptomyces* genetics. It is implemented now in Artemis (Sanger's DNA sequence viewer and annotation tool).

14. Iain Hunter introduced the developments in automated screening as an important advance in the pharmaceutical industry. Robots were brought in to do a lot of the manipulation for screening. Dick Baltz reported that Eli Lilly had automated their screening operation in the mid-1970s and were at the cutting edge of automation. For UK companies the development (based on custom-built systems initially) was more early 1980s. Iain Hunter pointed out that robot technology was translated into laboratory and other analytical technologies as well. David Hodgson pointed out there was collaboration between industry and academia to improve screening technologies (e.g. David Hopwood was on the scientific advisory board of Cetus - the first biotech company- and was consulted on prototype screening technology).

15. Another area introduced by participants as under-represented in the DTZ timeline but nevertheless important to the advance of *Streptomyces* science is physiology, including David Hodgson's work, and Gene Seno who worked on glycerol. Gene Seno took this work to Eli Lilly after his PhD work with Keith Chater at the John Innes. Czech scientists were also very strong in this field (physiology was not a John Innes area, with the exception of Seno) and people built on the work by the Czechs.

16. David Hodgson emphasised the enormous strength of work on basic Actinomycete biology in the UK which often doesn't get mentioned because it isn't genetics. He drew attention to the work of Stan Williams, Tom Cross, and Grace Alverton, including their teaching/ evangelizing role for the organisms. He reminded the meeting that the International Symposium on the Biology of Actinomycetes (ISBA)

was originally a network for research on the basic biology of the Actinomycetes before it was taken over by the geneticists.

17. Mike Goodfellow emphasised the importance of work in ecology of Stan Williams and Tom Cross. Williams' papers on the parameters that were driving the ecology of *Streptomyces* had not been bettered and that takes us back to the 1960s. Ecologists have shown us that *Streptomyces* are able to grow not just at pH 7.2 but there are alkaliphyllic *Streptomyces,* there are acidophyllic *Streptomyces*, there are halophyllic *Streptomyces*. This has been influential because people who have screened 'rare' Streptomycetes have found new metabolites. Taxonomy and ecology, he suggested, can hold the key to revealing an organism's ability to produce particular compounds. Greg Challis added that there are extremely important compounds which are used clinically from strange niches. For example, rapamycin was collected from Easter Island [Rapa Nui] by a holidaying industrialist. At one time it was the fashion for industrial researchers to be given kits to go and collect samples on their travels. In general, though, Mike Goodfellow argued that the neglect of ecology in the UK has held back the development of biogeography. Brian Rudd supported Mike Goodfellow's appreciation of Stan Williams's work. His group had a long collaboration with Williams and relied on him a lot for collections of strains and for methods to isolate new *Streptomyces* cultures for screening.

18. Today Liz Wellington is 'flying the flag' for ecology and appreciation was expressed of her work, her achievements have been 'phenomenal'. Liz was a student of Stan Williams. She has had a very successful collaboration over a long period with a Danish enzyme company (Novo) through which, using a very extensive collection of *Streptomyces*, Liz had successfully identified an extremely unusual Streptomycete that produces a novel compound. David Hodgson thought that the company had gone on to exploit it. Novo had funded her laboratory at Warwick for basic soil microbiology. Soil biology has declined as a scientific area in the UK, but Liz's work, looking at genetic exchange in the environment, and her knowledge of the ecology of the organisms, has been an important influence. He argued that you couldn't understand the biology of *Streptomyces* unless you realised it is in an oligotrophic environment, used to fighting for its living in that really cruel world- a very different environment to *E. coli*'s. Her input has been extremely important, including electron-microscopy, and 'classic good, old-fashioned environmental science'. Using these methods, for example, she confirmed that in the environment the plant pathogen *S. scabies* went through the developmental cycle observed in the lab. The cycle could have been a laboratory artefact but she showed it to be a real phenomenon.

19. Turning to the classic genetics milestones in *Streptomyces* research, one correction to the DTZ timeline was suggested by David Hodgson. He said that the discovery of the A-factor should be in the 1970s, not the 1980s where it appears as the discovery at the University of Tokyo. A. S. Khokhlov in Moscow in the 1970s discovered a small molecule, the so-called A-factor, which is secreted by a streptomycete and, when it accumulates in the medium, induces both sporulation and antibiotic production by its own cells. The Tokyo group discovered the A-factor independently and then found out about Khoklov's work. This discovery helped build understanding of the way bacterial hormones control antibiotic production.

20. David Hodgson considered that the major contribution of classic bacterial genetics mapping was the discovery that antibiotic genes were clustered [in David Hopwood's lab]. In Hodgson's undergraduate classes he summarises the key contributions of classical genetics as two things: 1) it generated plasmids, established vectors etc, and so you can begin to move into the molecular era; 2) it showed that when you clone the drug resistance marker, you've got the rest of the genes with you. The

participants at the meeting agreed that this knowledge, which came entirely from classical genetics, was not particularly intuitive. The only exception to the rule had been oxytetracycline, which at first misled researchers into thinking it was in two regions of the chromosome, when it turned out that the second cluster encoded an antibiotic co-factor.

21. The isolation of the first *Streptomyces* plasmid (SCP2) by Hildgund Schrempf – on sabbatical from the University of Würzburg in David Hopwood's lab- by standard methods, in 1975, was identified as important because it led to 'the sort of genetics instability phenomenology' and work on plasmid curing and amplified DNA (Maggie Smith). Work on amplified DNA was not a John Innes area but was developed elsewhere for example, Ralph Hütter and John Cullum's work.

22. Another suggestion for the DTZ timeline was the Japanese group who promoted the idea that antibiotics are encoded on plasmids [Haruyasu Kinashi, Miyuki Shimaji, and Akira Sakai at Mitsubishi-Kasei Institute of Life Sciences, Tokyo, 1987]. This group at the same time discovered the linearity of some of the plasmids and methods for isolating them, solving one of the problems that Hopwood's lab had been working on since the mid-1970s (that is, to isolate SCP1 from *S. coelicolor*).

23. Several participants (including Dick Baltz, Maggie Smith and Brian Rudd) highlighted work on the *Streptomyces* phage called phiC31 as an important landmark. This phage was discovered by a Russian group (Natasha Lomovskaya at All-Union Research Institute for Genetics and Selection of Microorganisms in Moscow). Taking parts from the phiC31 attachment integration system brought important developments in vector technology. Later coupling that with the discoveries of Julian Davies' group on conjugation from *Streptomyces* to *E. coli* improved the technology even further. There was a lot of interaction between industry and academia in this area, and with the John Innes Institute in particular during that time. Keith Chater at JI made some vectors, but the group led by Nagaraja Rao at Eli Lilly built some of the best vectors and a number of them are still used today, pSet152 being an example. That set of vectors really enabled a lot of work, not just in *coelicolor* but in industrial strains where the ability to transform may be very difficult because of restriction/ modification systems. The conjugation and site-specific integration took care of that and it really took the whole field to a much higher level. The conjugal integrated vectors built by Rao should also be on the DTZ timeline. Maggie Smith added that it needs emphasising that this was a completely original idea – it hadn't been done in any organism then, it has since been copied in all our organisms and so was a 'really, really novel and enabling technology'. Building on from this, the idea was to do as much as you can in *E. coli* and then introduce it into *Streptomyces*- these vectors don't replicate in *Streptomyces*, they have to integrate and they go in in low copy number and they were 'just incredibly easy to use'. It is now the method of choice for GM of actinomycetes (Brian Rudd).

24. Dick Baltz put forward BAC vector technology (Bacterial Artificial Chromosome) as an important development- a more recent methodology that uses the same concept. Here you do all your manipulations in *E. coli* but now you do it on a gigantic piece of DNA. So to be able to manipulate full pathways on BAC vectors that have the ability to conjugate and site-specifically integrate- that technology made it so much easier for the type of work Dick Baltz and others were doing at Cubist on combinatorial biosynthesis where you want to make module changes. Taking that to the next level, through lambda red, phiC31 integration and conjugation, and putting that together with BAC vectors was a significant advance. Those original BAC vectors were produced at Chromaxome, where Paul Brian, formerly at John Innes, was involved. They were then bought by Terragen and acquired by Cubist from Terragen. These

super BAC vectors are now used all the time and are ‘tremendous’ (Dick Baltz). Anna Maria Puglia also made BAC vectors at Biosearch Italia (Mervyn Bibb).

25. Dick Baltz drew attention to Julian Davies as an important figure in the development of antibiotic resistance markers. Nagaraja Rao was the first to do that successfully but he got the idea from Julian Davies- the idea was to have a marker that would express in *E. coli* and in *Streptomyces* so that you wouldn’t have to waste two, just use one.

26. David Hodgson highlighted the work of Eric Cundliffe (University of Leicester) who developed research on thiostrepton, explored the mechanisms of thiostrepton resistance, and developed cloned antibiotic resistance markers with Charles Thompson. These worked not just for *Streptomyces*, but for other actinomycetes, and had a major impact. Thompson was a postdoc at the John Innes using antibiotic resistance genes from *Streptomyces* species as selectable markers- in time those he isolated proved crucial for the future development of *Streptomyces* cloning vectors.

27. A very important breakthrough in terms of *E. coli* conjugation was the development of Dam^- Dcm^- strains by Doug MacNeil (at Merck). That was really a very important barrier going from *E. coli* into *Streptomyces* (because *E. coli* DNA was methylated and was restricted by *Streptomyces coelicolor and S. avermitilis*, though not by most streptomycetes), so you could then develop strains of *E. coli* for conjugation.

28. Gilles van Wezel argued for the importance of reporter systems, for example, neomycin resistance on pIJ486/487. Also the xylE promoters developed by Jan Westpheling in the United States.

29. The genetics milestones at the John Innes Institute, and the Institute’s interactions with industry, are well-represented in the DTZ timeline. The meeting nevertheless elicited interesting additional information on the significance of JI- led developments. Brian Rudd thought that a very important point is that David Hopwood recognised at a very early stage the potential importance of the genetics that he was doing for the biosynthesis of secondary metabolites and he had collaborations from very early on with chemists in the United States, in Japan, Europe, who were all focused on biosynthesis of some of the metabolites.

30. David Hodgson commented that what made the John Innes Institute become the centre for *Streptomyces* research was David Hopwood and colleagues’ willingness to move rapidly on from their original interest in classical genetics (unlike Sermonti in Italy who was an important partner and originator in the classical genetics era) and into molecular genetics. Hodgson also emphasised the importance of the evangelical qualities of David Hopwood and Keith Chater, and that often meant them actually going out to industries and making them listen. Hodgson always found American industry much more open, than some in the UK industry, especially some of the older style, but it was actually David and Keith who a lot of the time would say ‘take this vector’, kind of forcing it on them rather than nowadays. He commented that there was some resistance from industry and a lot of that disappeared really because of the efforts of David and Keith at that time.

31. Giving the industrial viewpoint, John Hodgson noted that industry had hit an impasse- their practice was to use mutagenesis (applied at random) to come up with higher producing strains of *Streptomyces*. David Hopwood’s laboratory offered another way through genetic manipulation: ‘it was hugely attractive’.

32. Iain Hunter said that by the 1970s pharmaceutical companies were getting into the genetics of *Streptomyces* based on what was coming out of David Hopwood's lab (companies entering the field at this time included Pfizer, Eli Lilly, Beecham and Glaxo). In particular, the mutagenesis that Hopwood's lab achieved in the 1970s provided the impetus for companies to begin working on the fundamental science of *Streptomyces* (driven by interest, for example, in Mervyn Bibb's work with plasmids). But Pfizer was also interested in the group's research on primary metabolism (namely David Hodgson's PhD, 1979, which was funded by Pfizer). Hopwood's lab was recognised as the centre of interesting developments, and as Iain Hunter put it 'we were translating [Hopwood's lab's work] like fury'. The belief was strong that industrial applications would follow on from the research.

33. Dick Baltz reported having become interested in protoplast fusion and rDNA technology independently, inspired by the 1973 Cohen and Boyer paper, the first recombinant DNA experiment, and by reading a paper on protoplast fusion in *Bacillus* in 1976. His objective to try and make these technologies work in *Streptomyces* led his interests at Eli Lilly to converge with the John Innes Institute.

34. Iain Hunter commented on 'the rapidity with which we were able to translate in industry what was happening at the JI'. In 80/81-ish 'we were taking stuff out of the John Innes, we were taking pre-prints of papers, we were taking plasmids, were taking constructs. No tech transfer offices, no MTA's, nothing.' It'll never happen again 'but the rapidity with which we were able to grab it and exploit it was just amazing'. He made the point that there was also the element of trust which is intangible. 'To walk out of the John Innes with one of Charles Thompson's constructs, or one of Toby's [Tobias Kieser's] constructs in your pocket, you know…. If it did work there is a few million dollars down the line, you know!' The trust and rapidity referred to were facilitated by the '*Streptomyces* Club' at the John Innes (see Section 2: Networks and Training, below).

35. Mervyn Bibb, speaking as part of the John Innes group, noted it was JI policy to get the technology out there to the 'real world' so to speak of industrial environments. He notes that IP would just have hindered that. Industrialists did pay for it to the extent that they subscribed to the funds of the '*Streptomyces* Club'.

36. Dick Baltz spoke with enthusiasm about the benefits of Club membership which included an entitlement to vectors and other products as soon as they were available. His company Eli Lilly participated in the Club in the mid to late 1970s.

37. It was acknowledged that there were some facts of life that made it difficult for industrial and academic viewpoints to work together. In the 1970s Keith Chater was keen to promote phage vectors (for transfection) but industry was extremely resistant. [Keith Chater subsequently explained this was apropos JII development of a process called mutational cloning that made it possible to clone antibiotic production genes very quickly…The process had been validated for academic antibiotics and Keith wanted the industrial antiobiotic producers to try them out. Keith still thinks that several years were lost because of the 'short-sightedness' of industry]. Industrialists saw phages as dangerous contaminants which had to be kept away from their sites; their interest in them was only in making their strains phage-resistant since phage could seriously crash their fermentations and make them unable to produce their products. Phages were something of an industrial secret; you didn't talk about it in public. This lack of openness prevented a dialogue with academics to solve problems.

38. Several interesting contributions were made on the past and future advantages of *Streptomyces* as a model system. Looking to the past there was some suggestion that 'real streptomycetes' were more difficult to work with than *S. coelicolor* – *coelicolor* is a good laboratory organism but industrialists were faced with more genetic instability in their industrial laboratories which might have made technology transfer difficult. On the other hand *Streptomyces coelicolor* proved to be a tractable organism for the new technologies. David Hodgson said that one of the reasons that they had gone down the protoplast route was the fact that you could get very large amounts of DNA in, so you could clone an entire antibiotic cluster in one go which you wouldn't have been able to do with any other system until you developed BACs. This was a 'fortuitous accident'- Hodgson commented that he couldn't think of anywhere else where you could clone an entire metabolic pathway and put it in.

39. *Streptomyces coelicolor* continues to have several advantages that promise to keep *Streptomyces* science vibrant and productive in the post-genomics era. Brian Rudd pointed out that *Streptomyces* are not just tremendously talented at producing secondary metabolites but they also have a very catholic metabolism. They are able to modify all sorts of compounds that they come up against. They have a battery of enzymes which are very useful for bio-transformations and for other industrial applications, so the enzymes are the second area in Actinomycetes where the heterogeneity produces a tremendous resource. That an important thing that has come out of genome sequencing, just how many enzymes there are, particularly cytochrome P450 oxidases, a tremendous battery of enzymes. David Hodgson added that he knew there had been a big research group at Monsanto using P450s for herbicide breakdown (from *Streptomyces*).

40. Maggie Smith drew attention to *Streptomyces* as an important model for understanding sporulation and development. These are big subjects in themselves but it is also associated with antibiotic biosynthesis. *Streptomyces* provided an alternative model to various other sporulating micro-organisms, in particular *Bacillus* endospores, because they weren't endospores and so the work of Keith Chater, and the isolation of white mutants and bald mutants was something that was fundamental science and incredibly important and enabling. Mervyn Bibb agreed, *Streptomyces* is a good model system because it is a complex but tractable organism- 'it stands alone in a sense'.

41. Maggie Smith put forward the discovery of the UUA codon as another completely original and uniquely (at that time) Streptomycete story which was and still is really interesting.

42. Brian Rudd and Mervyn Bibb added that the link between secondary metabolism and differentiation in *Streptomyces*, issues of compartmentalised gene expression (some genes are only expressed in the hyphae) were really interesting fundamental phenomena. The technology is there now to develop this science.

43. Maggie Smith argued that in future scientists will understand the link between development and sporulation and this will be a great advantage to making new antibiotics. Greg Challis widened the point further – this area is key to the post-genomic aim of exploiting genomics to find new compounds and to understanding regulatory networks.

44. Mike Goodfellow introduced pathogenic *Streptomyces* species as an interesting area for future investigation (it is becoming quite clear that quite a considerable number are pathogenic, though they tend not to cause problems in Europe and America). He suggested that we shouldn't focus only on comparing the genomes of *Streptomyces,*

Mycobacterium and Corynebacterium, but we should also sequence pathogenic *Streptomyces* to find out how they operate and why they are so different. Greg Challis reported that he is currently working with the Environment Agency on some *Streptomyces* belonging to the *Streptomyces griseus* group which are responsible for fish kills in rivers and lakes (through a toxin made by the streptomycetes).

45. Gilles van Wezel noted that *Streptomyces* is, as far as we know, the only organism where it is possible to knock out cell division without killing the organism (work done in Rich Losick's lab by Joe McCormick). This goes against all we have learnt bout cells, but in principle you can have hyphae without any cell division at all. Understanding this will have a really broad impact for understanding cell division in general.

46. Mervyn Bibb commented that regulation of biosynthesis and differentiation is complex in *Streptomyces* and we're now beginning to understand how complex it is. Regulation of genes is at a level above what people have seen in *E. coli* and the knowledge built on this model system is going to go beyond *Streptomyces*, it's almost a eukaryotic development.

47. David Hodgson agreed and said that he had just prepared a compilation of all the known regulators of *Streptomyces*: there's 147 Tet R regulators, 66-69 sigma factors depending exactly on what you include and you've got 500 or so regulatory genes, of which there's only one or two of a particular class in *E. coli*- 'a massive cornucopia'. Hodgson argued that the only way you are really going to understand how this interaction works is with systems biology, and that is all coming directly from the genome, in terms of what information there is. So we know the complexity of the biology of *Streptomyces*, and it's really been confirmed by the complexity of the genetic content. It was agreed generally that the genome is providing a wealth of information, matched by a wealth of questions about, for example, what the environmental/ chemical signals for particular antibiotics to be made in the organism are. Colin Smith pointed out that it could well be that a lot of these antibiotics are only produced in complex communities, and Mike Goodfellow suggested there should be more investment in ecological studies. It was agreed that the sheer resourcefulness of Streptomycetes as organisms had always been a strength for the science in this area and will continue to be so.

48. Drawing this section to a close, Celia Caulcott asked if there were any real issues in science arising from work on *Streptomyces* that the meeting had not covered.

49. Peter Leadlay said that there's been a continuing debate which has been transformed by the work on *Streptomyces* and other bacteria which is about the nature of natural biodiversity molecules and the debate is still going on but I think that that is one of the *key* issues for end users.

50. Greg Challis asked whether prior to the genome sequence, were people aware that there was likely to be so much hidden potential metabolic diversity in *Streptomyces.* Brian Rudd said those working with *S. clavuligerus* were not greatly surprised at the number of metabolic pathways. Greg Challis thought that *clavuligerus* could have been a special case in that lots of the pathways are expressed, but pointed out that in the 1980s the prevailing view of industry was that everything had been discovered and natural products were not worth persevering with. David Hodgson pointed out that if you look at the Bergey categorization it is still the Actinomycetes who are the main producers of antibiotics.

51. Peter Leadlay suggested that one of the issues that need to be sorted out is whether other bacteria that we haven't explored can be hypothesised now really to be as rich. It seems to be perfectly acceptable to say that certain tribes of micro-organisms are actually better than others and it is not even restrictive. Another important point (that goes right back to Darwin) is that not only are we in possession of only a small fraction of what is now available to us on the planet but there is a great virtual set of molecules with exactly the same properties and advantages of the extant set of natural products which are accessible to us because we now understand how to make such things. We don't know which ones to make but they are out there. And those reasonably accessible chemical entities are likely to be of interest to us.

Funding

Celia Caulcott presented a graph of BBSRC expenditure on *Streptomyces* projects (Appendix 3). This documents the annual spend since 1997 and distinguishes the proportion invested in Higher Education Institutions and in Research Institutes. There are no accurate records for much of the early research funding and it was not possible to produce a graph showing the long-term funding that *Streptomyces* research has received since the 1960s. The post-1997 funding trends are complicated by the introduction of FEC in 2002/2003. The graph shows that the peak of BBSRC investment was in 2006/2007 at just over £3.5 million, of which about £2.3 million was awarded to Universities and £1.2 million to Research Institutes. Most of the Institute funding was awarded to the John Innes Centre. It was pointed out that the figures overall were likely to be an underestimate. Participants were asked to contribute information on the importance of other non-BBSRC funding streams:

1. The John Innes Institute's *Streptomyces* Club was the first UK organisation to effectively channel industrial funding into *Streptomyces* genetics research. This (relatively informal) network fostered industrial/ academic collaboration and helped to grow the *Streptomyces* community in both fields. The history of this Club has been described in more detail by David Hopwood (Appendix 4). Briefly the John Innes *Streptomyces* Club began in 1968 when scientists from Pfizer UK in Sandwich approached Hopwood for advice. Hopwood followed this up by persuading them to make a grant to support *Streptomyces* work at the John Innes Institute, a grant that became annual. From these small beginnings Hopwood evolved the idea of a '*Streptomyces* Club'. Over many decades other companies were persuaded to contribute to the Club's funds and these were employed to fund *Streptomyces* students and postdocs. The level of contribution requested was tailored to the size of the company involved and was for varying periods of time but typically ranged from £2,000 to £17,000 per year. The Club's peak, in terms of the amount of money going into it, was 1983 to 1994 when nearly all the JII *Streptomyces* students were funded by the Club (freeing up research council funds for other projects in the JII Genetics Department). The Club continues today on a reduced scale. In return for the financial investment 'there was no exclusivity, and no rights were ever transferred to a company. Company members were all entitled to receive "available" strains, plasmids and phages (in practice almost every one of the group's stocks with an occasional exception for IP reasons), and to use them for their own commercial purposes. They could also ask for reasonable amounts of advice from members of the JII group and sometimes they made occasional visits to JII to help solve specific problems' (Appendix 4). Several participants commented on the vital role played by this Club in growing the *Streptomyces* user community, see Section 2 on Networks and Training.

2. A second organisation that had a key influence on *Streptomyces* research funding was the Biotechnology Directorate (BTD). The BTD made a major investment in *Streptomyces* in the mid-1980s and by funding studentships and research facilitated the development of a number of new groups beyond the John Innes Institute (e.g. the Manchester and Southampton groups). David Hodgson reported having seven or eight

students funded under this scheme at Warwick University. The BTD was set up in 1981 under the Biological Sciences Committee of the Science and Engineering Research Council to support strategic research into areas that would underpin the British biotechnology industry. The Directorate worked by holding round table discussions between academics and industry to identify areas where useful work could be started. An innovation included the setting up of a number of 'Club' schemes which consisted of 3 or 4 companies and a similar number of university departments. One Club was set up for recombinant DNA technology and DH reported that was actually funding five *Aspergillus* projects and something like seven or eight *Streptomyces* projects which helped to widen the network of *Streptomyces* research in academia. The John Innes Institute stood outside the BTD scheme because it wasn't eligible for 'University money' but it did participate by providing strains and other research material. The recombinant DNA technology group led on to the 'Secondary metabolites of *Streptomyces*' which was a *Streptomyces* specific group. Ian Hunter was the director of the first group, and there were four companies participating (including Beecham, Glaxo, ICI); the second group was smaller and included Beecham and Glaxo. There was also some DTI money, channelled through industry, to support this industry/academic partnership.

3. Participants commented that they had been able to build on the BBSRC investment in *Streptomyces* sequencing and had subsequently been successful in attracting NERC funding (Mike Goodfellow). Liz Wellington's work at Warwick was cited as another example. It was emphasised that NERC funded graduate and PhD students and this was key to growing new groups of *Streptomyces* scientists and advancing the research at Warwick (David Hodgson). Other funding streams that have been accessed as a consequence of BBSRC investment in the *S. coelicolor* sequence include USDA and NIH (Greg Challis).

4. The *Streptomyces* sequencing project also opened up BBSRC funding for the sequencing of other non-pathogens studied by smaller research communities, beginning with *Rhizobium*. It was reported that these communities benefitted considerably from a change of policy at the BBSRC to a position generally supportive of sequencing non-pathogens in a context where only pathogenic microbes were succeeding in getting funded [David Hodgson].

5. The meeting considered the historical background to BBSRC's decision to sequence *S. coelicolor*. Further background information has been provided by David Hopwood (Appendix 5) It was agreed that it was a courageous decision on the part of the BBSRC leadership to invest £1.5M, a huge sum in 1997. It was pointed out that this pre-dated the Comprehensive Spending Review that identified genomics, broadly speaking, as a key area (Mari Williams). *S. coelicolor* was said to fulfil all the criteria sought by the BBSRC Microbial Genomics Working Group: it had industrial relevance; there was a lot of underpinning fundamental science of great interest, and there was an ordered cosmid library which had just been published or had just been made. Finally, a factor considered to be very important at the time, there was a community to exploit it (Maggie Smith quoting Nigel Brown); already that community was developing the tools to exploit the sequence. Although some opposition to the sequencing project (unspecified) was reported, and other organisms were debated as candidates for sequencing at the time (for example, *S. lividans* and *Rhodococcus*) the overwhelming feeling was in support of the project. Peter Leadlay puts it as follows: 'I don't think that there was really any strong voice raised against sequencing, or that it be *coelicolor*, I think it would have been **perverse** quite frankly for another organism to have been chosen'.

6. The size and integration of the *Streptomyces* community at the time set it apart from other groups and this was emphasised as one of the most important factors in the BBSRC decision to sequence *coelicolor*, the first initiative of its type. The competitor

organism *Rhodococcus* matched all the other criteria but failed as a contender because it did not have an integrated community to exploit the sequence. (Even in the case of *E. coli*, traditionally the 'workhorse' of bacterial genetics, its research community was said to be 'not integrated at all' in the late 1990s).

7. The question of how public funds came to be committed to the sequencing of *S. coelicolor* was considered. From academia the driver was interest in understanding the fundamental science of how *Streptomyces* grows and develops in its environment. Many scientists were interested in Actinomycetes in general and in the prospect of comparing the *coelicolor* genome sequence to the TB sequence underway at the time (published in 1998), looking for Actinomycete *specific* genes. It was suggested that the fact that the organism did not [then] make an industrially relevant compound was significant in that it left the field free from the complication of industrial interests, of appearing to favour one industry over another. On the other hand, the importance of Streptomycetes to the pharmaceutical industry meant that the sequence could provide vital tools for the understanding and manipulation of other Streptomycete species that do produce industrially relevant compounds. The cost of the sequencing under prevailing technologies at the time meant that it would not have been done in the private sector.

8. The BBSRC and its predecessor organisations (ARC, AFRC) had made a significant investment in *S. coelicolor* over the long-term. The advanced state of *S. coelicolor* genetics research was a product of this investment, had given the UK a strategic lead, and meant that a range of genetic tools for post-sequence work on *coelicolor* were available. Further investment in sequencing by BBSRC was helped by the fact that *S. coelicolor* is not a pathogen: although in the world of microbial sequencing the more powerful driver overall was pathogen-focussed, it would have been difficult for the BBSRC to fund the sequencing of a pathogen as this would have been considered to fall under the MRC's domain. (Hence the argument that *Streptomyces* is highly related to the pathogen *Mycobacterium* wasn't used- it wasn't germane in this context).

9. Another factor in the choice of *S. coelicolor* (already mentioned) was that an ordered cosmid library was available (a set of overlapping fragments or cosmids of the *S. coelicolor* chromosome). Sequencing could therefore proceed cosmid by cosmid (i.e. in manageable chunks). Whole shotgun genome sequencing was not a viable option for sequencing *coelicolor* because computationally it was too difficult (it was too big, 8 megabases of high GC content). Although the computational capacity did not exist for whole genome sequencing of *coelicolor* when the sequencing work started, by the time the project was half way through the technology was available to make whole genome sequencing possible (Merv Bibb, Greg Challis). However, the cosmid by cosmid approach turned out to have significant advantages for the *Streptomyces* community (see 10, below).

10. In order to complete the sequence, the BBSRC had to approach the Wellcome Trust for funding (the total cost of the sequence was around £2M). This was an unprecedented step for the Trust and the argument had to be made for the importance of the *S. coelicolor* sequence (based on its potential to reveal the fundamental biology of the anti-pathogen). In autumn 1998, the Wellcome Trust, through the Beowulf Genomics Initiative, an initiative newly established by the Trust to ensure wide distribution to researchers of microbial genome sequences, provided the remaining funding. This partnership had consequences in that BBSRC had to accept the Trust's conditions about the availability of data. The other key partner, the Sanger Institute, who carried out the sequencing work in collaboration with JIC, also imposed conditions about data access. The Sanger's policy had always been that as soon as a sequence came out the information went into the public databases. The meeting was surprised to learn (from

Maggie Smith quoting Nigel Brown) that a major stumbling block to the negotiations at the beginning was the BBSRC's initial desire to keep the sequence within the BBSRC community.

11. Several participants concurred on the importance of open access to sequence information in growing the *Streptomyces* community and advancing the field internationally. Going cosmid by cosmid, sequence data was released on a weekly basis which gave the project an exciting immediacy and allowed the *Streptomyces* community to analyse the information in manageable chunks. It was suggested that the 'whole pond hit' approach would have been much more difficult to handle. The Sanger Institute provided the community with a 'Rolls Royce' service in that the sequence data released was already annotated (additional information was provided, for example, on where genes start and stop, functions of genes or resemblances to other genes). It was unusual to have sequence data annotated in this way. As one participant commented: 'I guess [it was] much more expensive than it would have been just to give you the sequence but again in terms of people on the ground using it was phenomenal. You were waiting for the email to find out what the next interesting things were' (David Hodgson).

12. It was remarked that public funding had benefited the international scientific community enormously because of the openness of the activities and that this benefit was reciprocated in that developments and people came back to further advance the work in Britain (Colin Smith, Brian Rudd).

13. BBSRC was commended for being forward-looking in funding community tools for exploiting genomics both during and after sequencing. Colin Smith: 'One thing that was critically important was the foresight that some people showed like Nigel Brown, Douglas Kell, to start developing the tools just as the sequencing started all the tools that would go with it, microarrays, proteomics, knock outs, and that started in the late 90s, a tool kit for functional genomics, well before exploiting genomics [the Exploiting Genomics Initiative]. And that was almost unique internationally getting things sorted together to produce community tools'. The meeting agreed that strategic funding in this area had kept Britain in the lead of *Streptomyces* genetics, ahead of potential competitors in Japan and the United States.

14. Participants were asked if sufficient BBSRC funding to keep Britain ahead in this area had continued and it was reported that the funding had dried up. The last major funding input into this was the Exploiting Genomics Initiative. Since then EU funding had been picked up by a consortium led by Paul Dyson because British funding was not available. This initiative had also recently ended (December 2009). Dyson stressed that the EU funding was a direct consequence of the investment from BBSRC who did the genome sequencing, and subsequent, IGF, EGI initiatives.

15. Public funding has assumed even greater importance against the background of a 'disenchantment' of the pharmaceutical industry with the natural products sector and in many cases a withdrawal from R&D investment in this area. The number of companies in natural products research drug discovery was significantly lower in the 1980s than in the 1970s and in the future may remain the province of the small biotech companies rather than of big pharma. This phenomenon of declining industrial interest has been almost inversely related to the increase in tools available to molecular microbiologists and therefore provides something of a paradox. One factor cited was the simultaneous rise of a competing discipline and associated technologies- combinatorial chemistry- which proved very attractive to pharmaceutical companies (chemical methods for rapid synthesis of a large number of molecules). Though often blamed for the move away from natural products, combinatorial chemistry was not the only competing technology- there was also a drive towards biologicals (Iain Hunter). Brian Rudd pointed out that natural

products did not fit the modern paradigm of screening in industry whereas combinatorial chemistry fitted very readily into it (natural products would come as crude mixtures most of the time, not the pure compounds in related classes offered by combinatorial chemistry). Although natural product chemistry was improving all the time it was operating against this strong competition from other disciplines and the screening methods (reductionist and based on single protein screens) were not well suited for getting out the value of natural products (Barrie Wilkinson). Another factor is the circumscribed nature of the market for new anti-infectives (a market still provided for by some very old antibiotics) which makes it a less good commercial prospect for drug companies than 'lifestyle' drugs which have to be taken for long periods. The exit of big pharma, however, has provided a market opportunity for small biotech companies; Dick Baltz predicted that in the future a new industry for natural products would be built around genomics, based on thousands of sequences.

16. Commenting on the 'lessons of history' regarding funding, Peter Leadlay argued that the key failure of imagination was that sequencing was believed to be so hard to do it would be a very long time before it would be taken up in a broader sense. 'That is a mistake that has been made ever since Sanger was doing three bases a day. We have consistently underestimated the rate of progress. And so I feel the mistake that collectively if you like, the decision makers and the scientists have made…. was the failure to grasp that things would get cheaper, that a failure of nerve at a critical point means that you are far behind'.

Networks and Training

Interaction between academia and industry in *Streptomyces* research has been driven by a number of formal and informal networks which are listed below. The John Innes *Streptomyces* Club and the clubs established by the Biotechnology Directorate (BTD) have also been discussed under 'Funding' (see above).

1. The John Innes Institute (now Centre) was described in the meeting as the most important of the informal networks. Very few of the participants at the meetings had not spent time working at the John Innes at some stage in their careers. Through the sabbatical system visiting scientists enriched the JI experience, bringing new technologies and widening the scope of the network (for example, Stanley Cohen spent 6 months on sabbatical at JI as early as 1976, a critical period for the development of gene cloning). For many of the industrial scientists, their first point of contact was the *Streptomyces* Club. As part of their firm's membership they were offered space in the lab for two or three days at a time. For others the point of contact was through consultancy work for their firm by David Hopwood and Keith Chater. Consultancy could be the gateway to more involvement with John Innes and to signing up for the Club. The international symposia (see below) also gave industrial scientists opportunities to meet academics from John Innes and again might later lead to membership of the Club. The EMBO (European Molecular Biology Organization) training courses organized by the John Innes helped to grow the *Streptomyces* network and to open it up to new entrants from industry and academia (see n. 7, below).

2. Sabbaticals have supported a two-way movement of scientists between academia and industry (individuals who have moved between the two sectors and back again include Merv Bibb, Iain Hunter, Steve Gould, Bill Strohl). It was suggested that there are limited exchange opportunities for chemists working in this area, but for biologists it is an area where the shifts between academia and industry are more comfortable

as this field of biology is more closely related to industrial application than many. (Greg Challis; Flavia Marinelli). The exchange between academia and industry is affected by the economics of the natural products industry which may be less in the future driven by big pharma (who can afford to fund sabbaticals) and fall more into the province of smaller companies.

3. A key formal network for the *Streptomyces* community is the International Symposia on the Biology of Actinomycetes (ISBA) which began in 1968 as an organisation devoted mainly to taxonomy with very little genetics. David Hopwood joined and gradually there was more and more involvement from the geneticists so that the meetings became dominated by *Streptomyces* genetics. Since then it has broadened out again and is now the chief forum for discussions about Actinomycete biology. One of the reasons for this broadening out is that participants share many of the same techniques now, whether they're ecologists, taxonomists or geneticists. This network was credited with bringing good students from overseas to work in British labs (bringing funding with them) and for opening up the *Streptomyces* community to young entrants to the field. For many years ISBA was driven from the UK but there are now some important focal groups outside the UK which are both a benefit to and in competition with UK *Streptomyces* science (Mike Goodfellow). ISBA is also one of the biggest meetings for the natural products industry who have joined in larger numbers during the last decade (Greg Challis). The forum promotes dialogue between academia and industry: 'The diversity of talks at ISBA meetings is very important so it is an opportunity to disseminate really basic fundamental biology of Actinomycetes amongst industrialists and so on, that people can pick up on and exploit' (Mark Paget).

4. In the US the Society for Industrial Microbiology (SIM), which held its first annual meeting in 1950, has declined in importance as a network for the *Streptomyces* community. Over the past 15 to 20 years the percentage of the meeting that is devoted to natural products and *Streptomyces* has declined along with the industry. SIM meetings cover the whole field of industrial microbiology and it is estimated that about 80% of the content would now be on other fields (Dick Baltz). For the *Streptomyces* community SIM cannot compete with ISBA where the whole meeting will be focussed on some aspect of Actinomycetes.

5. The Genetics of Industrial Microorganisms (GIM) meetings were also formerly a major focus for the *Streptomyces* networks. The GIM international Symposia, held every four years, began in Prague in 1970. GIM was the leading forum in the 1980s but is not now the main meeting place for Actinomycete scientists - the transition point with ISBA taking on this role occurred around 1990. The GIM symposium at Strasbourg in that year was the first to be dominated more by Bacillus than *Streptomyces* papers. Another related organisation GIMBIM held meetings at 2-year intervals in Bloomington, Indiana, to provide a focus between the GIM meetings and this for a time provided a forum for *Streptomyces* research rather more like ISBA meetings (Dick Baltz).

6. Other formal networks mentioned include an annual German speaking *Streptomyces* meeting, the annual VAAM meeting run by Hildgund Schrempf, Osnabrück University: Vereinigung fur Allgemeine und Angewandte Mikrobiologie (Association for General and Applied Microbiology), and a European Actinomycete group (which has folded). It was pointed out that many of these networks have overlapped and fostered new exchanges between laboratories. VAAM partly exists to promote German interaction with UK groups. The Society for General Microbiology (SGM) is a valuable resource for students: they offer travel grants to go abroad and this can help students attend ISBA meetings and access that network.

7. For training in *Streptomyces* genetics participants identified the EMBO training courses (four were run by the John Innes between 1983 and 1990, with another run by John Innes Scientists in Wuhan, China in 1989, funded by the ICGEB or 'International Centre for Genetic Engineering and Biotechnology', and two courses on targeted mutagenesis) as important for disseminating the new laboratory skills. These were 2-week 'hands on' practical courses for students. These courses could also be a very effective introduction to the *Streptomyces* community (Gilles van Wezel).

8. EU fellowships were instrumental in bringing in scientists to the UK (for 6-month periods) with expertise in different aspects of *Streptomyces* biology. At the JI a very strong Spanish connection developed (Paco Malpartida, Jose Gil, and Juan Suarez, for example), and there were also visiting researchers from Eastern Europe, Russia and Eastern Germany coming under various funding schemes. This helped grow the *Streptomyces* community overseas and facilitated knowledge exchange. It was commented that *Streptomyces* labs are characteristically open places, welcoming visitors for specific short projects or to learn techniques.

9. The BBSRC dissemination meetings for the *Streptomyces coelicolor* sequence were acknowledged as highly significant for establishing peer-to-peer relationships between graduate students. This was a forum where students could feel at ease and begin to identify with each other. Participants emphasised the importance of the inclusiveness of these meetings. They were not restricted to group leaders, but the emphasis was on young researchers and they were encouraged to give presentations. The peer network developed then has been enduring. BBSRC funding of dissemination meetings around genome projects was felt to be critical in that it enabled lots of students and postdocs to go who might have otherwise struggled to find funding from their labs (Greg Challis).

10. For the interface between chemistry and *Streptomyces* an important initiative for graduate students was the polyketides meeting initiated by Jim Staunton at St John's in Cambridge. Originally an informal day- meeting with participants from John Innes, Bristol, Cambridge and 5 or 6 other UK groups (with industry invited as well), organized to provide an opportunity for graduate students to speak, this evolved into firstly, a series of three Royal Society of Chemistry polyketide meetings in Bristol focused on biosynthesis which were attended by American groups who had benefitted from the John Innes, secondly, an annual meeting organized by Russell Cox and Tom Simpson (Bristol) for graduate students, and thirdly, an international meeting run by the Royal Society of Chemistry called 'Directing Biosynthesis' in 2006 (to be repeated in Durham, September 2010).

11. There has been strong networking generally within natural products chemistry groups but the community of chemists that engage is quite small. Antibiotics research has required a strong interaction among a small number of chemistry groups.

12. European Union initiatives under FP6 (6^{th} Framework Programme) for Integrated Projects have been important for establishing new collaborations between European labs (a minimum of three partners from different countries is required). The programme aims to create very large scale pan-European projects and involves putting together consortiums of interested labs. Paul Dyson reported being very pleased with the scientific outcomes and with the interchanges between labs, which he thought would endure longer.

13. The summer schools organised jointly by the John Innes Centre and the Institute Ruđer Bošković, Zagreb, Croatia in 2007 and 2008 (and again this summer 2010: http://www.jic.ac.uk/science/molmicro/summerschool/) build on the momentum produced by the *S. coelicolor* genome sequence and the subsequent work on functional genomics and have attracted participants from around 25 nationalities to train in secondary metabolites and genomics. The summer schools have helped seed new Actinomycete labs in Brazil and China, and have effectively created new international peer networks for students in the field (Flavia Marinelli, Greg Challis). Natural products work in Brazil is being modernised as a result of the School and it is having a huge impact (Greg Challis).

14. The BBSRC-supported functional genomics initiatives, including the 'Investigating Gene Function' research programme for *Streptomyces*, provided microarray practical training at the University of Surrey. These initiatives have been important in attracting people from all over the world to train, and also supported workshops, associated bioinformatics, data analysis, databases. Together these initiatives have generated a community in itself (Colin Smith).

15. Networks established through training initiatives, and through returning PhD students to overseas academic departments, impact on the UK research base in that they may later form the basis of applications for joint international grants, a linkage that can grow and strengthen over the years. Students completing their training have gone into universities, industry, clinical research organisations, and to work as patent agents.

16. Industrial companies are knowledgeable about research networks and will recruit people coming from 'good groups'. An example was given of a company expansion into Europe that has used networks such as this to recruit in France, Germany and Spain (Barrie Wilkinson).

17. The meeting gave some general consideration to the question of what made the *Streptomyces* community unique and how it is likely to develop in the future. It was agreed that the personalities of the first *Streptomyces* group at the John Innes were a key factor. David Hopwood's laboratory assistant Helen Kieser was referred to as 'mother Streptomyces'- someone who in particular had helped to nurture and cement the network at an interpersonal level. The lack of hierarchy in the way Keith Chater and David Hopwood interacted with students was also felt to be significant. It was suggested that in the future the *Streptomyces* community was likely to change and became less collaborative, more competitive, and perhaps more like the *Bacillus* community. There will be more researchers in the field who have never had a connection with the John Innes. However, participants were very clear that the success of the sequencing project was based on the cohesiveness and strength of the *Streptomyces* community. Succession planning will be important to maintain the critical momentum, and consideration has been given to the next generation who will run ISBA. It was stressed that networks, even strong ones, can become fragile and disappear without the right leadership.

18. In addition to the networks discussed at the meeting there is also the Society for Actinomycetes Japan (http://www.nih.go.jp/saj/index-e.html). SAJ was founded in 1955; the Society for Applied Genetics of Actinomycetes (which was established in 1972) was merged into it in 1990. SAJ provides a focus for the big Japanese interest in Actinomycete biology in all its aspects - taxonomy, ecology, natural products and genetics. The Society holds an annual meeting and has published the journal *Actinomycetologica* twice a year since 1987. *Actinomycetologica* is both a newsletter for SAJ, promoting social and scientific exchanges in Japan and internationally, and

a scientific journal carrying Japanese and English-language contributions to Actinomycete science. SAJ's other activities include holding regular scientific meetings and workshops, producing laboratory manuals and books, and contributing to ISBA.

Impacts

The economic impact of public investment in the sequencing of the *Streptomyces coelicolor* genome has been separately considered by the DTZ report (Appendix 2). This section provides a more qualitative assessment of impacts from the viewpoint of academic and industrial scientists working in related fields. Participants were asked to consider what the sequencing of the *Streptomyces coelicolor* genome had achieved so far and what it might achieve in the future.

8. Dick Baltz opened the discussion by drawing attention to the development of phage integration systems, a key technology that has been developed on the back of investment in *Streptomyces* genetics (i.e. a wider investment than that involved solely in the sequencing project). Phage integration systems have had enormous impact not only within the field of *Streptomyces* but in the much bigger arena of engineering cells- mammalian, human, *Drosophila melanogaster*- 'the whole gamut of organisms'. This is the best technology for engineering and started with the work of Natasha Lomovskaya and Keith Chater, Nagaraja Rao's group at pharmaceutical company Eli Lilly, and then Maggie Smith. Maggie Smith explained that her group's work came out of a study of the biochemical mechanism of phiC31 integrase. They found that the properties of this recombinant were such that it made an ideal integrating system that was totally portable into any other system. Her lab wasn't the first who did the movement into mammalian cells but this work has had a huge impact on genome engineering model organisms. The long term aim is to engineer these integrators so that they will target a completely benign sequence in the human genome, so that you can target the pieces of DNA, and correct genes as part of cell regeneration gene therapies.

9. A key impact of the *Streptomyces coelicolor* sequence is that it has revolutionised the way geneticists do their experiments and organise their research. Methodologies now start with an examination of this sequence and a comparison of its features with other sequences. Annotations of the *coelicolor* sequence provide valuable information on what the genes are. A new genome will be compared against *coelicolor* using BLAST technology which helps the investigator annotate the new genome properly. It provides the scaffolding and the reference point for new investigations.

10. A less tangible impact of the *coelicolor* sequence was that it heightened scientific awareness of the possibilities of the new technologies: 'it has a sort of "yes we can" effect on everybody else showing what is possible' (Brian Rudd). The sequencing of the complete genomes of (now) several streptomycete species has revealed the presence of a large number of "cryptic" secondary metabolic gene clusters, and led to the realisation that these organisms have the ability to produce many more natural products than had previously been recognised. This has stimulated research worldwide to identify the physiological signals and regulatory mechanisms responsible for the activation of these "cryptic" pathways, with the hope of unleashing the full biosynthetic potential of these prodigious producers of valuable natural products. These international studies have extended in recent years to include other "rare" actinomycete species.

11. Participants considered how the increase in sequencing power that has occurred since the *Streptomyces coelicolor* sequence was published has affected the value of this original sequence. There was agreement that *coelicolor* would always represent the 'gold standard' because of the investment in annotation of the sequence. When today's sequences are published they are not accompanied by the same level of annotation, ScoDB-like databases that were created to add value for users of the *coelicolor* sequence are not being generated for all of these new genomes. The consequence is that *coelicolor* remains the key starting point. Continuing to focus on this single model system will enable researchers to really start dissecting the function of the individual components (Colin Smith).

12. *Streptomyces coelicolor* continues to have a key advantage as an experimental tool, the same that launched it on its career as a model organism, in that it makes coloured secondary products. This makes *coelicolor* much easier to study because the analytical chemistry is based on looking at colour changes without having to use a machine.

13. The sequencing of *coelicolor* has had a major impact on research into Mycobacteria (important for understanding the behaviour of the disease-causing bacteria *Mycobacterium tuberculosis* and *M. leprae),* providing insights into the function of mycobacterial genes in pathogenesis, maintenance within macrophages. Many academics were interested in comparing the *coelicolor* genome sequence with the already published TB sequence; many of the genes that are currently 'hot topics' in research on Mycobacteria were all described, discovered, and functionally characterised in *Streptomyces* first. For example, they include heat-shock regulators, *Whi* proteins, *Wbl* proteins and sigma factors (Paul Dyson).

14. The creation of a 'superhost' was a tangible output from *Streptomyces* genomics (under the ActinoGEN initiative). Mervyn Bibb's group exploited the *Streptomyces coelicolor* sequence to create a streptomycete that has been used to express heterologous secondary metabolic gene clusters. Employing microarray and global proteome analyses, the strain was improved as an expression host for the production of small molecules (a 'superhost'). This is already being used in small molecule discovery programmes – many people are expressing clusters in it and it has potential for further development.

15. Turning to what the *Streptomyces coelicolor* genome project might achieve in future, participants commented that some of the early approaches to using a genomics-driven way of searching for novel natural products foundered on a lack of understanding of the mechanisms of expression. Using the then available methods (traditional approaches involving experiments with different growth media) it proved possible to convert only a small percentage of the novelty encoded in the genome into compounds. The way forward will be through a better understanding of gene regulation and expression (something that has been progressed by the investment in research on the *Streptomyces coelicolor* genome and other streptomycete genomes).

16. Bioinformatics analysis (another area to have benefitted from the *Streptomyces coelicolor* genome project) has now advanced to the stage that you can get a good indication as to whether a cluster is likely to encode an interesting compound. 'As we go from the genomics era into the metagenomics era [where genetic material is recovered direct from environmental samples rather than clonal cultures in the laboratory], the potential to discover novelty is extremely high' using rational genetic information (Greg Challis). In lots of different countries (for example, in the US,

Switzerland, Netherlands, Spain) there are initiatives now to try and deploy these new approaches to exploiting Actinomycete genomes. There is new investment in sequencing additional Actinomycete genomes from environmental samples with the express aim of discovering new products (Greg Challis).

17. In addition to the promise of new anti-infectives, there are potentially other novel natural products (also secondary metabolites) that can be exploited from Actinomycete genomes that will interest the drugs market. Statins (from fungi) and the immuno-suppressant rapamycin were introduced as examples of important natural products in this category (Flavia Marinelli). It was pointed out that *Streptomyces* may in future also have something to offer companies interested in enzymes. *Streptomyces* is rich in enzymes- cellulase enzymes, proteases, lipases, chitinases and when the future potential of *Streptomyces* research is reviewed we should not only concentrate on secondary metabolites and 'cryptic products' (David Hodgson).

18. Participants stressed that ever more powerful machines for sequencing have been developed since *coelicolor* was sequenced and anticipated that in a couple of years it will be possible to sequence an entire company strain collection (we might look forward to perhaps a 10-fold increase in sequencing capacity). It was agreed that the phenomenal increase in sequencing power will bring a data explosion (and a corresponding need for investment in data storage and bioinformatics), and problems of selection, how to choose candidates for sequencing. In future it will be possible to sequence biomes (geographical communities of organisms) and to explore their genetic complexity. For research on Streptomycete species it was the consensus that the *coelicolor* genome will still be providing key information on what the genes are in the future.

19. There was some discussion on whether there had been too much concentration on model systems in Actinomycete research (and in particular, *Streptomyces coelicolor*). It was pointed out that because of their industrial interest, many Actinomycetes had been sequenced that were not pathogens. The emphasis on sequencing pathogens in molecular microbiology as a whole has narrowed the spectrum of organisms studied. This field is fortunate both in having a wider diversity of organisms sequenced (there are currently 15 *Streptomyces* sequences available on the database) and in having a model organism that gives a much more realistic overview of what is going on in that family, a complex organism that has evolved to live in a very complex and challenging environment. The diversity of Actinomycete sequences available is set to expand very soon under the US Department of Energy's Joint Genome Institute (JGI) project: GEBA or Genomic Encyclopedia of Bacteria and Archaea, a project which aims to sequence genomes of bacteria and Archaea from across the tree of life (sequencing organisms that are distantly related from any that have been sequenced so far). This will help with the annotation of existing Streptomycete genomes and may in future stimulate the development of new model systems.

20. New technologies will also bring new model systems to the forefront of molecular microbiology. Maggie Smith predicted that *Streptomyces venezuelae* will become an incredibly powerful alternative model system in the future (developed out of work by Mervyn Bibb and biotechnology company Diversa). It has advantages over *coelicolor* in that it enables a lot of 'omic techniques to be used in a way where you can synchronise the developmental cycle with antibiotic biosynthesis because sporulation is synchronous in *venezuelae* and occurs in liquid culture.

21. There will be an increasing need for biologists to interpret the ever-expanding data from sequencing. An expert knowledge of the biology and chemistry of the organisms will have to be applied to the indications given by automated annotations before sense can be made of the data. One of the really interesting biological puzzles for the future will be the question of what secondary metabolites are actually doing in the soil. The trend to sequencing organisms that are actually in the soil (see n. 9, above) will produce fascinating information on this question; information that can be used translationally to express compounds themselves. It is very likely that studies of the ecology of Actinomycetes in soil (for example, Liz Wellington's studies which aim to unravel the mechanisms controlling responses in bacteria to key environmental signals) will be a key growth area in the future (David Hodgson, Maggie Smith).

22. The meeting closed with some general reflections on the lessons learnt from the investment in *Streptomyces* genetics and genomics. Returning to the networks that had been built it was stressed that a lot of the work underpinning them was essentially 'blue sky' and not applied research. Networks were critical to the successes achieved, and will need maintaining in the future. Small amounts of money to allow people to participate in meetings can be a very effective way of sustaining the community. The long-term nature of the investment in *Streptomyces* genetics was stressed and the timeframe for future advances in this field will also be lengthy. It was suggested that from the sequencing of *coelicolor* to bioactive molecule it had taken perhaps another 10 years and that it would take probably another 20 years to get a product (a new antibiotic, for example) to the clinic. It doesn't just take time in academia but there is a long lead-in time in industrial research and development before you get to the point of marketing a new antibiotic.

Appendix 1: List of participants

Dr Richard Baltz
Cubist Pharmaceuticals Inc

Professor Mervyn Bibb
Department of Molecular Microbiology, John Innes Centre

Professor Gregory Challis
Department of Chemistry, University of Warwick

Professor Paul Dyson
School of Medicine, Swansea University

Professor Michael Goodfellow
School of Biology, University of Newcastle upon Tyne

Professor David Hodgson
Department of Biological Sciences, University of Warwick

Dr John Hodgson

Professor Iain Hunter
Dean of Faculty of Science, University of Strathclyde

Professor Peter Leadlay
Department of Biochemistry, University of Cambridge

Professor Flavia Marinelli
Dipartimento di Biotecnologie e Scienze Molecolari, Università degli Studi dell'Insubria

Dr Mark Paget
School of Life Sciences, University of Sussex

Dr Brian Rudd
Novacta Biosystems Ltd

Professor Colin Smith
Faculty of Health and Medical Sciences, University of Surrey

Professor Maggie Smith
Institute of Medical Sciences, University of Aberdeen

Dr Gilles van Wezel
Leiden Institute of Chemistry, Leiden University

Dr Barrie Wilkinson
Biotica

BBSRC

Dr Celia Caulcott
Director, Innovation and Skills Group

Dr Mari Williams
Deputy Director, Corporate Policy and Strategy Group

Dr Sarah Wilmot
Outreach Curator, John Innes Centre

Mrs Carol Milner
Assessment Manager, Corporate Policy and Strategy Group

Appendix 2: Timeline from DTZ Report

Table 2.1: Time-line of *Streptomyces* genetics leading up to genome sequencing and genetic manipulation of antibiotic biosynthesis

Period	Overview of Achievements	References (see Appendix A)
1950s	Discovery of genetic recombination in *Streptomyces* by six groups across the globe. Development of chromosomal linkage mapping in *S. coelicolor* by David Hopwood in Cambridge.	1
1968	David Hopwood appointed Head of Genetics Department at the John Innes Institute and John Innes Professor of Genetics at UEA – forming the Norwich *Streptomyces* group.	-
1970s	Efficient chemical mutagenesis in *Streptomyces* by NTG (a nitrosoguanidine). Efficient natural mating system developed (plasmid-determined).	2 3 4
	Genetic recombination through protoplast fusion found independently at JIC and Eli Lilly (USA) – an important tool for strain improvement for antibiotics.	3 4
	Plasmid DNA introduced into *Streptomyces* by protoplast transformation – a crucial step in development of genetic engineering.	5
	Discovery that genes for antibiotic production are clustered, greatly facilitating their manipulation.	6
1980s	Joint work between JIC and USSR on bacteriophage genetics, laying the foundation for their use in genetic manipulation.	7
	Development of gene cloning in *Streptomyces* by groups at Stanford University and JIC, leading to the later cloning of complete sets of antibiotic production genes.	8
	Discovery at the University of Tokyo that bacterial hormones control antibiotic production.	9
	Publication of *"Genetic Manipulation of Streptomyces: a Laboratory Manual"* – Approximately 3,000 copies distributed, with expanded edition (*"Practical Streptomyces Genetics"*) published in 2000.	10
	First model hybrid antibiotics produced by genetic engineering, in a joint project between JIC, Japanese and USA researchers.	11
1990-2002	Transfer of DNA from *E. coli* to *Streptomyces* by conjugation (Pasteur Institute, Paris).	12
	Discovery of 'assembly line' mechanisms controlling biosynthesis of polyketide natural products by scientists in Cambridge UK and Chicago, USA, opening up great potential for genetic engineering.	13

Period	Overview of Achievements	References (see Appendix A)
	Development of combinatorial biosynthesis of unnatural natural products.	14
	Construction of a combined genetic and physical map of the *S. coelicolor* chromosome. A critical step in the genome sequencing project.	15
	Discovery that *Streptomyces* chromosomes are linear (Taiwan and JIC), leading to an understanding of the novel architecture and replication of *Streptomyces* chromosomes, including mechanisms of evolution and loss of antibiotic production genes.	16
	Construction of a set of ordered clones covering the *S. coelicolor* chromosome (JIC, German and Japanese researchers), opening the way for the genome sequencing project.	17
	Sequencing of the *S. coelicolor* sequence begun at the Sanger Institute in August 1997, completed in July 2001, and published in *Nature* in July 2002.	18

Source: JIC adapted by DTZ

Since the completion of the *S. coelicolor* genome sequence many technological advances have been made and resources developed to exploit the genomes and genome sequences of *Streptomyces* species and other actinomycetes. Some of these are highlighted in Table 2.2.

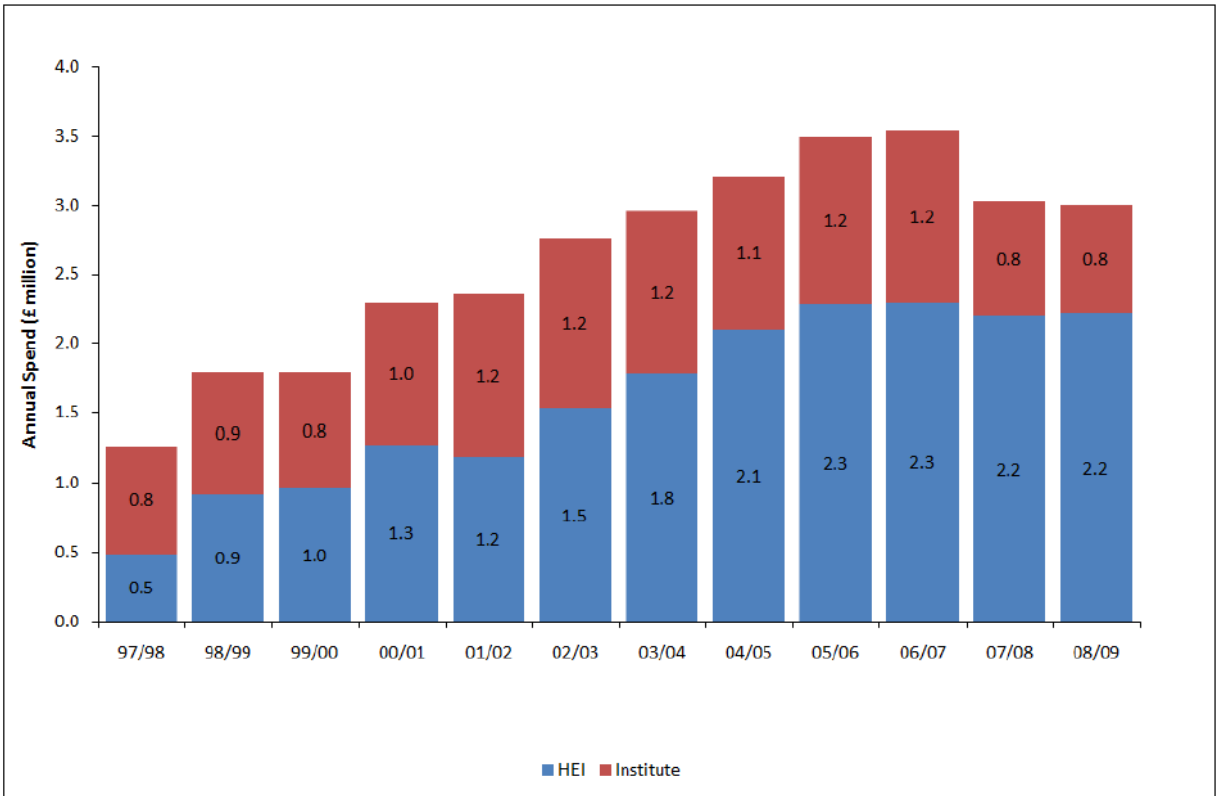
Table 2.2: Enabling technologies and resources for exploitation of *Streptomyces* genomes and their sequences

Period	Overview of Achievements	References (see Appendix A)
2001	First microarray experiments based on *S. coelicolor* genome sequence, leading to discovery of cross-regulation between antibiotic pathways (Stanford University, USA).	19
2002	Application, in a recursive fashion, of protoplast fusion for rapid strain improvement and increases in antibiotic production (Codexis and Eli Lilly, USA).	20
2003	Complete sequence of the *S. avermitilis* genome (Kitasato University, Japan).	21
2002 & 2003	Discovery from the genome sequences of many “sleeping” gene clusters for the production of secondary metabolites, ushering in a new era of drug discovery with decades of potential.	18, 21
2003	Development of genome-based targeted mutagenesis for *Streptomyces*, dramatically increasing the ease of genetic manipulation of the genome and of cloned antibiotic pathway genes – now used throughout the world.	22
	Discovery and manipulation of lantibiotic gene clusters in	23

Period	Overview of Achievements	References (see Appendix A)
	Streptomyces, leading to the founding of Novacta Biosystems and may result in the introduction of a completely novel class of clinically useful antibiotics.	
	Establishment of ScoDB, then StrepDB, the major database for *Streptomyces* genomics, currently getting 500-600 viewings per day by people looking at the annotation of individual features such as genes, RNAs etc., and about 200-300 more hits per day to search forms and blast forms, about 54% from outside the BBSRC domain.	24
2003 & 2008	Application of proteomics based on the *S. coelicolor* genome sequence leading, among other discoveries, to new insights into the importance of extracellular biology for antibiotic production and development.	25, 26
2004	Development of an efficient transposon mutagenesis system for *S. coelicolor* (Swansea University).	27
2005	Development of efficient general methods for introducing "foreign" antibiotic gene clusters into a convenient host for analysis and manipulation (Tübingen University, Germany).	28
	Use of "gene shuffling" to improve biosynthesis of the antiparasitic agent Doramectin made by *Streptomyces avermitilis* (collaboration between Pfizer, USA, and Codexis, a spin-out from Maxygen, the inventors of gene shuffling).	29
	Use of *S. coelicolor* phage to engineer mammalian genomes.	30
2006	Discovery that the TAT pathway is major route for protein export in *Streptomyces.*	31
	Production of novel lipopeptide antibiotics by genetic engineering (Cubist, USA).	32
2008	Production of new aminocoumarin antibiotics by pathway engineering (Tübingen University, Germany).	33
	Development of decoy oligonucleotides, subsequently applied to counter antibiotic resistance and leading to founding of Procarta Biosystems.	34
2009	Development and application of versatile high density microarrays for genome-wide analysis of *S. coelicolor* (University of Surrey).	35
	Generation of novel lantibiotics by genetic engineering (Novacta Biosystems, UK).	36

Source: JIC adapted by DTZ

Appendix 3: BBSRC expenditure on *Streptomyces* research (graph)



Appendix 4:

Industrial interactions between the JIC *Streptomyces* group and commercial companies, 1968-1998 – David Hopwood

During my time as head of the Genetics Department the *Streptomyces* group had dealings with a very large number of companies from many different countries. These ranged from simple enquiries for advice, through the purchase of Streptomyces strains, plasmids and phages, to ongoing financial support of the group. The last led to the *Streptomyces* Industrial Fund and took various forms. It began in 1968 when scientists in the strain development department of Pfizer UK in Sandwich approached me for advice. Following this up I persuaded them to make a grant to support *Streptomyces* work at JIC. This funding was renewed annually, with inflation-proofing, for decades, later being taken over by the Development group at Pfizer in Groton, USA. Meanwhile other companies were persuaded to provide funding. Some did so *via* tailored arrangements, but eventually I came up with the concept of a "Club", and many companies contributed financial support, at various levels and for varying periods of time, as Club members. Irrespective of the paperwork, the conditions were always the same. There was no exclusivity, and no rights were ever transferred to a company. They were all entitled to receive "available" strains, plasmids and phages (in practice almost every one of the group's stocks with an occasional exception for IP reasons), and to use them for their own commercial purposes. They could also ask for reasonable amounts of advice from members of the JIC group and sometimes they made occasional visits to JIC to help solve specific problems. Outside of this arrangement, several group members – certainly including Keith Chater, Mervyn Bibb and myself – also entered into personal consulting agreements with companies. Under these we signed confidentiality agreements and made and received visits to and from the companies to discuss their research programmes in detail and give advice, in exchange for personal payment.

Doubtless we could have secured one or two larger grants in exchange for exclusivity and a direct involvement with the companies' research, perhaps even doing contract research. However, in my view this would have been incompatible with the remit of a non-profit organisation such as JIC. It would almost certainly have meant interacting only with one or a very limited number of companies and would have been at the whim of a board of directors whose priorities could change suddenly over time.

The JIC group benefited enormously over decades from the funding provided by the Industrial Fund, which was quite significant: see the summary table below for the period 1982-1994, during which the cash balance topped £570,000. Most of the expenditure was on the stipends of graduate students, post-doctoral fellows and visitors. For many years, all the PhD students in the *Streptomyces* group were funded from the Industrial Fund, leaving studentships from the Research Council and the John Innes Trustees for the other two groups in the Genetics Department, working on *Rhizobium* and *Antirrhinum*. This was a significant factor in the development of the Department, which was judged to be extraordinarily strong by Research Council visiting groups during that period. For a number of years there was an informal right for any of the PhD students to be funded for a year as a post-doc after writing their thesis, which was mutually beneficial, one advantage being that it made the writing of papers based on the thesis work much more efficient, while it also gave them a base from which to apply for positions elsewhere. Also very importantly, when we received requests from scientists wishing to work in the *Streptomyces* group, we could very often respond immediately with funding, thereby attracting some very good post-docs. Sometimes they were later able to cover their own costs, but being able to use the Industrial fund to underwrite them was enormously valuable.

Year	£ Balance at 1 April
1982	28,028
1983	99,378
1984	148,880
1985	232,994
1986	325,800
1987	394,742
1988	425,262
1989	473,483
1990	503,050
1991	524,598
1992	572,077
1993	536,302
1994	535,018

Appendix 5:

Chronology of the *Streptomyces coelicolor* Genome Sequencing Project

1. 19 December 1995	John Cullum co-ordinates EU bid to sequence and analyse 50 cosmids in 13 laboratories - application *just* fails
*1a 26-27 June 1996	BBSRC Chemicals and Pharmaceuticals Directorate: *Streptomyces* Grant-Holders' Meeting at the Grand Hotel, Birmingham
2. Summer 1996...	Various attempts to raise funding, following publication of Redenbach *et al.* on the ordered cosmid library for the *S. coelicolor* genome
3. 26 September 1996	BBSRC review of Genomics - microbial genome sequencing strongly supported
*4. 29 October 1996	Brainstorming session on "The future of *Streptomyces* research in the UK" at the Royal Society
5. Winter 1996/97	BBSRC allocates £1.5M over 3 years for microbial genome sequencing
*6. 27 February 1997	NERC review of Envirogenomics
7. 7 March 1997	Microbial Genomics working party decides on *S. coelicolor*
8. 20 May 1997	Meeting at Sanger Centre to discuss a contract - pilot funding for 10 cosmids recommended
9. 16 June 1997	Pilot funding announced (£60k)
10. 1 August 1997	Pilot project begins
11. Summer 1997...	Various attempts to raise further (non-BBSRC) funding
12. Autumn/winter 97/98	BBSRC discusses funding for main project
13. 10 March 1998	Main grant announced - £1554.3k to sequence 70% of the genome, and to construct a database. A condition is to set up a Streptomyces User Liaison Group (SULG) – see 16.
14. Spring/summer 1998	Wellcome Trust approached
15. 24 September 1998	Beowulf Genomics awards £875K to finish the sequence

16. SULG meetings:

    i. 8 September 1998 at JIC
    ii. 11 February 1999 at MRC
    iii. 29 September 1999 at MRC
    iv. 16 March 2000
    v. 13 November 2000 at MRC

17. 20-21 May 1999	Dissemination meeting at Hinxton Hall
18. 28 Feb-1 Mar 2001	Dissemination meeting at Scarman House, University of Warwick
19. 21 July 2001	Sequence completed
20. 8 May 2002	Press conference at the Royal Institution to announce the Nature publication

Post-sequence dissemination meetings:

21. 22-23 July 2003	*Streptomyces* dissemination meeting, University of Surrey
22. 11-12 January 2005	*Streptomyces* dissemination meeting, University of Surrey
23. 10-11 January 2006	*Streptomyces* dissemination meeting, John Innes Centre

*These meetings were peripheral to the main chronology towards the genome sequence.

Notes

David Hopwood,
13 September 2010

1. In the summer of 1994 we learned that, whereas the vast majority of the EU funds allocated to genome sequencing under Framework 4 would be reserved for *Bacillus*, yeast and *Arabidopsis*, 6-8% might be available for other organisms where a pressing case could be made. The European *Streptomyces* community started to discuss the possibility of a bid and John Cullum at the University of Kaiserslautern took the initiative to coordinate one.

There was correspondence with the various labs to be included in the bid over the summer of 1995, leading to submission in December 1995. The proposal was to sequence a contig of 50 overlapping cosmids, representing about 1.1 Mb of unique sequence, from the so-called “silent region” of the *S. coelicolor* genome in order to establish the best methods, and to carry out some functional genomics studies.

In March-April 1996 we learned that the bid had been graded 4 in the priority listing and that competition would be intense. Attempts were made to lobby Brussels via JIC and BBSRC representatives, but there was no change in the ranking and the bid just failed.

In retrospect this was certainly a good thing. If the work had been done in many different laboratories, some inexperienced in significant sequencing, progress would have been slow and variable, and annotation of the sequence would have been patchy at best. In any case, the bid was intended to cover only about one eighth of the *S. coelicolor* genome. It soon became apparent that the best way forward was to try to raise enough funding to commission sequencing of the whole genome by a single professional organisation. We approached Lark, probably the only commercial contract sequencing company at the time, for a quotation but came to the conclusion that they could not cope with such a complex project.

1a. This grant holders’ meeting was peripheral to the main pathway to the sequencing project but helped to demonstrate the strength of the UK *Streptomyces* community,

especially to Celia Caulcott, who had been appointed as consultant to the BBSRC Microbial Cell Technology area.

2. In December 1995 I wrote to Craig Venter at TIGR to see if he was interested in sequencing the *S. coelicolor* genome, in whole or in part, and suggested a meeting with him. (This was before the outcome of the EU application was known, but I was concerned to get the whole genome sequenced, and I was also not happy with the piecemeal project.)

I visited TIGR on 13 June 1996 and spoke with Craig Venter and, especially, Brian Dougherty. Craig was interested in doing the whole sequence, not part of it. They would shotgun sequence from genomic DNA. A rough estimate of costs would be $2.4 million direct costs, plus indirect, making $4-5 million total.

On 10 July 1996 Brian provided a draft worksheet for 8-fold coverage and confirmed direct costs of $2.3 million, $4.15 million total. Craig would definitely do it if we could raise the money. They would use the cosmid contig as a valuable scaffold for assembling the sequence, but not as a sequencing template.

On 26 July 1996 I informed John Cullum of my thoughts.

On 2 August I wrote to Bridget Ogilvie, chief executive of the Wellcome Trust, asking if Wellcome might be interested in adding *S. coelicolor* to their list of microbial sequencing candidates. On 9 August J. R. Stephenson replied on behalf of the Trust saying that they would consider in principle doing one *Streptomyces* strain.

3. On 15 August 1996 I was invited to chair a BBSRC Review of Genomics to be held on 26 September 1996. The remit was to analyse genomics research funded by the BBSRC in the context of research funded by other bodies, primarily by carrying out a SWOT analysis, and to identify opportunities to carry forward over the next 5-10 years. The field was to be considered under the headings of plants, animals, and microbes. Members of the panel were asked to send their preliminary SWOT analyses in advance of the meeting. I sent one for microbes, which formed the basis of the combined document presented at the meeting.

It was evident that there was a major difference between the needs of genomic research for plants and animals on the one hand and microbes on the other. For the former, complete genome sequencing was not yet feasible, so emphasis was laid on mapping at a grosser scale, identifying breeding trait determinants, etc, whereas for microbes complete genome sequencing was the priority. I was strongly supported in the latter by Mark Bailey of IVEM (Institute of Virology and Environmental Microbiology) Oxford, who promoted Environmental Genomics [see 6], and Michael Morgan of the Wellcome Trust, who emphasised the need for the sequencing of pathogenic microorganisms. He outlined the Trust's plan to create a funding consortium, including industrial partners, to sequence pathogen genomes.

The conclusions of the meeting were: that a further round of funding for plant and animal genomics (extending the GAIT initiative) should be supported; that there should be an initiative in microbial genome sequencing; and that bioinformatics was in need of strong support. I wrote up a case for microbial sequencing, followed by some remarks about "beyond sequencing" (material that was later to be called functional genomics).

In retrospect, this meeting was a very significant step in the development of support for genomics by BBSRC, extending beyond sequencing to the various "post-genomics" initiatives that followed.

4. On 26-27 June 1996 there had been a BBSRC grant holders meeting concentrating on UK *Streptomyces* research held at the Grand Hotel, Birmingham. During a discussion

session there was a recommendation to hold a brainstorming follow-up meeting specifically to develop the idea of a *Streptomyces* "super-host" suitable for the heterologous expression of antibiotic biosynthetic pathways.

The meeting took place on 29 October 1996 at the Royal Society, London, attended by most of the UK PIs and some working on other microbes, as well as industrial scientists working with *Streptomyces*, with a few from continental Europe. A professional facilitator attended to catalyze the deliberations. Celia Caulcott also attended.

The meeting was positive and a detailed report was produced summarising the conclusions. I then brought together the John Innes PIs to produce a document building on the report and presenting arguments for developing *S. coelicolor* as the super-host.

The idea of support for work on the super-host fell foul of reorganisations of the committees and directorates of the BBSRC, in particular the dissolution of the Chemicals and Pharmaceuticals Directorate in March 1997. Nevertheless the meeting at the Royal Society and the discussions following it almost certainly helped to raise the profile of *Streptomyces* in general, and *S. coelicolor* in particular, in the BBSRC. I was able to refer to it in the crucial meeting on 7 March at which *S. coelicolor* was accepted for sequencing [see 7].

6. On 27 February 1997 NERC held a discussion meeting on Envirogenomics at the Royal Society, largely promoted by Mark Bailey of IVEM, Oxford. I particularly promoted the idea of shotgun sequencing – influenced by my discussions with TIGR. Interestingly, in view of the fact that the *S. coelicolor* sequencing project subsequently used clones, Colin Harwood (Newcastle University) pointed out some advantages of using a clone-by-clone approach, as had been used for *Bacillus subtilis*, which had allowed the sequence to be put together from work done in an international consortium of laboratories, mostly in the EU. One of the arguments was that the user community was involved right from the start, and the clones formed the basis of future functional genomics work. This turned out to be a very prescient view.

7. On 26 February Debbie Poole of BBSRC wrote to me about a Microbial Genomics Working Group to meet on 7 March at the Research Councils' London pied-a-terre in Henrietta Street to establish a strategy for both the acquisition and exploitation of microbial genomics. She asked Nigel Brown of Birmingham University to chair the meeting. I presented the case for sequencing the *S. coelicolor* genome and, in the absence of any well-developed cases for other microbes, the meeting decided to recommend devoting the whole of the £1.5M budget allocated by the BBSRC Council, over 3 years, to *S. coelicolor*.

At the meeting, Michael Morgan of the Wellcome Trust very helpfully suggested that BBSRC might contract the Sanger Centre to do the work, outside the constraints of the Pathogenomics consortium which was still under discussion (later it morphed into the Beowulf initiative of Wellcome alone).

This was a seminal meeting with an almost surreal outcome – suddenly, after years of pushing, the door seemed to swing open!

8-9. On 20 May 1997 there was a meeting at the Sanger Centre, under the auspices of the Acquisition of Microbial Genomics initiative of the BBSRC, and attended by Debbie Poole, who had moved to the Wellcome Trust.

The main business was to discuss and implement the sequencing of the *S. coelicolor* genome. Mervyn Bibb and I presented the background. John Sulston, Director of the Sanger Centre, expressed concern that the Redenbach ordered cosmid library had been sub-cultured after the original clones were made, raising the possibility of deletions and

rearrangements, so that the DNA would not constitute a perfect template for sequencing. His concern stemmed from their earlier experience with *Mycobacterium* clones, which had turned out to be a disaster. (This preceded the later very successful sequencing of the *M. tuberculosis* and *M. leprae* genomes using other material.) In view of these concerns, and the need to establish a real cost for sequencing high G + C DNA, it was proposed to begin with a pilot project to sequence 10 contiguous cosmids. On 16 June 1997 a contract letter for £60K was sent by BBSRC to John Sulston.

It was decided to choose a contig including the *red* antibiotic gene cluster. In July 1997 Helen and Tobias Kieser and I drove over to the Sanger Centre to deliver the 10 cosmids, which Helen had prepared. We met with Bart Barrell, head of Pathogen Sequencing, Julian Parkhill, currently annotating *Mycobacterium* DNA but who would become the first annotator for *S. coelicolor*, and Mike Quail, in charge of clones. There followed the first of many email exchanges between Helen and Mike about the nuts and bolts of the project, and with Marie-Adele Rajandream, Bart's programmer, who would be handling the web site for the project.

11. Attempts to raise further funding included correspondence with German laboratories, Koreans, Teruhiko Beppu (Japan), Stefano Donadio (Italy), Charles Thompson (Switzerland), Julian Davies (Canada), Rich Losick and Stephen Gould (USA), and Chris Farnet (Ecopia, Montreal). The Germans were interested in collaboration, but this foundered on IP issues. Only Chris Farnet came up with a definite offer – to sequence one cosmid on a trial basis.

12. There was a meeting at the Henrietta Street office on 5 December 1997 at which progress of the pilot project was reviewed. (There was also consideration of other microbes, with a detailed case for *Aspergillus nidulans*, but nothing concrete was concluded for these.) The main event was the raising of the issue of IP on the sequence information. This led to a temporary embargo on the release of any more sequencing data, but soon the issue went away.

The main grant was approved by Council on 10 February 1997. Funding up to £1.5M was approved, at a spend rate of £0.5M per year over three years. The shortfall of a nominal £0.5M (the total estimate was £2M) was to be made up by obtaining international and/or industrial collaborations.

Council stipulated that a user group be set up, consisting of the main PIs from UK universities and companies interested in *Streptomyces* genetics.

13. The main grant was to the Sanger Centre, for £1,435,932, with a grant of £118,396 to employ a bioinformaticist to construct an ACeDB-type database (A *C*oenorhabditis *e*legans Data Base) for the *S. coelicolor* sequence. This format was stipulated by John Sulston at the Sanger Centre. (Later it proved poorly adapted for the purpose, largely because the data and web interface are intimately connected, making it difficult to update, and in any case, being devised for a complex eukaryote, most of it was inapplicable to a prokaryotic genome. It was eventually replaced by Govind Chandra with a relational database and separate web interface.) Andreas Wietzorrek was appointed on the grant; he made good progress with constructing SCoDB, but left before the end of his three-year appointment.

14. I had a lot of discussion with Bart Barrell at the meeting on Microbial Genomes II at Hilton Head Island, 31 March-3 February 1998, about approaching the Wellcome Trust for the remainder of the projected funding. This was followed by correspondence with Celia Caulcott and Pat Goodwin at the Trust which led up to the actual application by the Sanger Centre.

15. The grant to the Sanger Centre of £875,540 to complete the sequence was made on 24 September 1998. The grant provided a big psychological boost to the project, since we now knew that it could be completed.

Appendix 6: Select bibliography

Books:

Hopwood, D. A. (2007). *Streptomyces in Nature and Medicine: The Antibiotic Makers.* New York, Oxford University Press.

Hopwood, D.A., Bibb, M.J., Chater, K.F. Kieser, T., Bruton, C.J., Kieser, H.M., Lydiate, D.J., Smith, C.P., Ward, J.M., Schrempf, H. (1985) *Genetic Manipulation of Streptomyces. A Laboratory Manual*. Norwich, The John Innes Foundation.

Kieser, T., Bibb, M.J., Buttner, M.J., Chater, K.F., Hopwood, D.A. (2000). *Practical Streptomyces Genetics*. Norwich, The John Innes Foundation.

International Streptomyces Project / Shirling and Gottlieb (1966):

Int J Syst Bacteriol **16** (1966), 313-340; DOI 10.1099/00207713-16-3-313
Methods for characterization of *Streptomyces* species[1]
Shirling, E. B. and Gottlieb, D.
Department of Botany and Bacteriology Ohio Wesleyan University, Delaware, Ohio
Department of Plant Pathology University of Illinois, Urbana, Illinois
ABSTRACT
The methods used by collaborators in the International *Streptomyces* Project (ISP) for emendation of descriptions of type and neotype strains of the genus *Streptomyces* (*Actinomycetales*) are presented.

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[1] This project is supported in part by a research grant from the National Science Foundation, U.S.A. The Subcommittee on Actinomycetes of the Committee on Taxonomy, A.S.M. and the Subcommittee on Taxonomy of Actinomycetes of the International Committee on Bacteriological Nomenclature are co-sponsoring advisors.

Frameplot:

Gene. 1984 Oct; **30** (1-3):157-66.
The relationship between base composition and codon usage in bacterial genes and its use for the simple and reliable identification of protein-coding sequences.
Bibb M.J., Findlay P.R., Johnson M.W.

A-factor: (this is covered in the DTZ report)

J Antibiot (Tokyo). 1982 Mar; **35** (3):349-58.
Mutants blocked in streptomycin production in *Streptomyces griseus* - the role of A-factor.
Hara O, Beppu T.
Abstract
Ninety-five streptomycin-nonproducing mutants derived from *Streptomyces griseus* FT-1 by UV-irradiation could be classified into major two classes by cosynthesis tests. Class I mutants (42 strains) were mutants blocked in the pathway of streptomycin biosynthesis while class II mutants (49 strains) required a factor for streptomycin biosynthesis which was excreted by the parental or class I mutant strains. The factor could be replaced by synthetic A-factor (2S-isocapryloyl-3-S-hydroxymethyl-gamma-butyrolactone) which restored both streptomycin biosynthesis and spore formation in the class II mutants. A-Factor deficient mutants were obtained from several strains of *S. griseus* and S. *bikiniensis* at high frequency by treatment with acridine orange or incubation at high temperature. A mutant whose streptomycin biosynthesis was independent of A-factor deficiency was found. The production of A-factor was distributed among various species of actinomycetes.

Linear plasmids:
Nature. 1987 Jul 30-Aug 5; **328** (6129):454-6.
Giant linear plasmids in *Streptomyces* which code for antibiotic biosynthesis genes.
Kinashi H, Shimaji M, Sakai A.
Abstract
A number of examples of circular plasmids with specific functions are known in both prokaryotes and eukaryotes. Several linear plasmids have also been identified, but these are all relatively small: large linear plasmids cannot be separated from chromosomal DNA by conventional techniques. There are several cases where the genetic evidence suggests that a character is encoded by a plasmid but no plasmid can be physically detected. This has been the case for antibiotic synthesis genes in *Streptomyces*; in particular a plasmid SCP1 in *Streptomyces coelicolor* has been shown to be involved in methylenomycin production by genetic evidence. We report here the application of orthogonal-field-alternation gel electrophoresis to the isolation of linear plasmids from *Streptomyces*. We have discovered a large linear plasmid of around 520 kilobases in *Streptomyces lasaliensis* and subsequently similar giant linear plasmids in other *Streptomyces* strains. We have confirmed that genes for methylenomycin biosynthesis are located on a series of giant linear plasmids in S. coelicolor. These observations may bear on the genetic variability and unstable genetic character of *Streptomyces* species.

pSET152:
Gene. 1992 Jul 1; **116** (1):43-9.
Plasmid cloning vectors for the conjugal transfer of DNA from *Escherichia coli* to spp.
Bierman M., Logan R., O'Brien K., Seno E.T., Rao R.N., Schoner B.E.