

# Economic Impact of *Streptomyces* Genetics Research

BBSRC

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## Appendices

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## 1.0 Introduction

- 1.1 DTZ was commissioned by the Biotechnology and Biological Sciences Research Council (BBSRC) to conduct an economic impact assessment of the *Streptomyces* Genome Project. This was subsequently expanded to include the broader impact of UK-based *Streptomyces* genetics research. The aim was to assess the actual and potential socio-economic value delivered through this investment and to demonstrate the economic case for public sector investment in such research.
- 1.2 This report highlights and quantifies the potential range of economic impacts generated around the UK and beyond through the on-going activity resulting from *Streptomyces* genetics research, and especially the Genome Project. It also describes key landmarks attributable to the research since its inception and identifies a range of non-quantifiable benefits.
- 1.3 This report presents the findings of the work and is structured as follows:
- **Section 2** provides detail on *Streptomyces* and the research undertaken as part of the Genome Project. This includes a time-line of *Streptomyces* genetics leading up to genome sequencing and genetic manipulation of antibiotic biosynthesis
  - **Section 3** outlines the rationale for public sector funding to support *Streptomyces* research, taking into account HM-Treasury guidance on market failure
  - **Section 4** quantifies the economic value of UK-based *Streptomyces* research, particularly the impacts on the antibiotic and pharmaceuticals arena arising from the *Streptomyces* Genome Project
  - **Section 5** summarises the wider social and qualitative impacts of *Streptomyces* research. This includes the training of PhD students and the creation of spin-out companies, both of which are related to activities undertaken at the John Innes Centre (JIC).

## 2.0 *Streptomyces* Research & Key Milestones

### Overview of *Streptomyces*

- 2.1 *Streptomyces* species are filamentous Gram-positive actinomycete bacteria that live in soil and water (as decomposers of organic material). Some actinomycetes form symbioses with plants while a few are pathogenic to plants, humans and animals.
- 2.2 A key feature of actinomycetes, and especially *Streptomyces*, is their ability to produce secondary metabolites such as antibiotics and other useful products. Actinomycetes use these metabolites to compete with fungi and other bacteria for resources. *Streptomyces* products are also used widely in agriculture as herbicides and pesticides, and in veterinary practice.
- 2.3 Many of these secondary metabolites have been isolated and developed as highly successful drugs in the treatment of human diseases. *Streptomyces* species and other closely related actinomycetes are a crucial source of these compounds, many of which have been developed as antibacterials, antifungals, and anti-cancer drugs.
- 2.4 Historically important antibacterial compounds made by actinomycetes include:
- Tetracyclines (longstanding broad spectrum antibiotics made by *Streptomyces rimosus* and *Streptomyces aureofaciens*)
  - Chloramphenicol (made by *Streptomyces venezuelae* and used to treat typhoid and eye infections)
  - Erythromycin (made by *Saccharopolyspora erythraea* and often used as a substitute for penicillin when people are allergic to it, and to treat Legionnaires disease)
  - Vancomycin (made by *Amycolatopsis orientalis*, and the last line of defence against methicillin-resistant *Staphylococcus aureus* (MRSA))
  - Rifamycin (made by *Amycolatopsis mediterranei* and used as a front-line treatment for tuberculosis and leprosy)
  - Gentamicin (made by *Micromonospora purpurea* and used to treat multi-drug resistant infections)
  - Imipenem (a derivative of thienamycin made by *Streptomyces cattleya*, and used to treat infections resistant to penicillin)
  - Clavulanic acid (made by *Streptomyces clavuligerus* and used as a blockbuster drug (Augmentin) in combination with a penicillin derivative)
  - Daptomycin (made by *Streptomyces roseosporus*, and used to treat MRSA and enterococcal infections. One of only three new classes of antibiotics introduced into clinical practice in the past 40 years).
- 2.5 In addition, a number of the antibiotics produced by *Streptomyces* have proven to be too toxic for use as antibiotics in humans, but as a result of their toxicity towards dividing cells they have been deployed as anti-cancer drugs. Doxorubicin, made by *Streptomyces peucetius*, is commonly used in the treatment of many different cancers. *Streptomyces* species are also an important source of clinically important immunosuppressants, including sirolimus (rapamycin) and tacrolimus (FK-506) that are used extensively in transplant operations.

- 2.6 *Streptomyces*-derived antifungals and anti-parasitic agents include:
- Nystatin (the first actinomycete-sourced human antifungal, made by *Streptomyces noursei*)
  - Amphotericin B (made by *Streptomyces nodosus*)
  - Natamycin (made by *Streptomyces natalensis*)
  - Avermectin (made by *Streptomyces avermitilis*, used to treat river-blindness in humans and widely in animal husbandry to treat nematode and arthropod infestations).

### Development of Antibiotics

- 2.7 The majority of antibacterial and antifungal agents in clinical use were discovered during the 'Golden Age' of antibiotics in the 1940s–1960s through isolation and screening of soil actinomycetes and fungi. It has been estimated that over 16,000 secondary metabolites with antibiotic activity have been discovered, with 40% of them produced by the genus *Streptomyces*, 13% by other actinomycetes, 17% by non-filamentous bacteria and 30% by fungi. About 160 of these compounds are used in human and veterinary medicine, and agriculture; of these, about 100 are used in human therapy and are mostly derived from actinomycetes.
- 2.8 Penicillins, cephalosporins, tetracyclines, aminoglycosides, glycopeptides, macrolides and polyenes were all discovered in that period and have been vital in the battle against bacterial and fungal infections over the past 50 years, revolutionizing the treatment of infectious disease. From 1960 to 1980 major pharmaceutical efforts were directed towards the incremental improvement of existing chemical structures, increasing potency, stability, and pharmacokinetics or reducing adverse reactions by medicinal chemistry.
- 2.9 Recognition of the value of *Streptomyces* species and their relatives as versatile producers of secondary metabolites began with the discovery of actinomycin in 1940, followed by streptomycin in 1943, and currently two-thirds of marketed microbial drugs are produced by actinomycetes. Since 1940, *Streptomyces* species in particular have been subjected to extensive isolation and characterisation, and as a result the current chance of discovering a novel *Streptomyces* antibiotic has declined markedly (the law of diminishing returns). Consequently, effort is also being focused on less-easily isolated actinomycetes using technology and expertise first developed with *Streptomyces*.
- 2.10 The genes required to produce any one secondary metabolite are clustered together in the genomes of the producing organisms. For example, the genome of *Streptomyces coelicolor* contains around 20 such clusters, of which only four were known before the genome sequence was available despite extensive prior analysis. Each of the 16 cryptic gene clusters is predicted to encode a different secondary metabolite. Projects that use genome sequence data from actinomycetes to yield new antibiotics are already underway, as well as large-scale genome sequencing of diverse actinomycete species for the purpose of "genome mining" to find further new gene clusters. Many projects focus on activating these cryptic gene clusters while others are using genetic engineering to create entirely new chemicals by splicing together 'machinery' from the different gene clusters.

## Milestones in *Streptomyces* Genetics – the Role of the UK and JIC

- 2.11 Sequencing of the *Streptomyces coelicolor* genome began at the Sanger Institute in 1997 and was completed in 2001, before being written up in Nature in July 2002. It has supported a significant number of discoveries, many of them extending what would have been much narrower generalisations about bacterial biology. It has provided key underpinning for subsequent international efforts to sequence the genomes of other antibiotic-producing actinomycetes. In an applied context, arguably the most important output is ‘*the demonstration that actinomycete genomes are teeming with unsuspected gene clusters for potentially interesting secondary metabolites, setting the stage for new campaigns of natural product discovery via genome mining and development via the engineering of expression hosts.*’<sup>1</sup>
- 2.12 The decision to fund the sequencing of the *Streptomyces coelicolor* genome may be seen as a consequence of the extent and quality of the genetic research that had already taken place within the UK and elsewhere, in particular at the JIC and in JIC-driven collaboration. Amongst other aspects, this research played a vital role through the mapping of the genome sequence. Table 2.1 therefore sets out the key discoveries central to genetic manipulation and the genome sequencing of *Streptomyces coelicolor* (many other important discoveries have been omitted). Where other laboratories are not mentioned, the discoveries were made at JIC.

**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 <i>Streptomyces</i> by six groups across the globe. Development of chromosomal linkage mapping in <i>S. coelicolor</i> 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 <i>Streptomyces</i> group.	-
1970s	Efficient chemical mutagenesis in <i>Streptomyces</i> 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 <i>Streptomyces</i> by protoplast transformation – a crucial step in development of genetic engineering.	5

<sup>1</sup> Bérédy J. Bioactive microbial metabolites. [J Antibiot](#) 2005 58:1-26.

Period	Overview of Achievements	References (see Appendix A)
	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 <i>Streptomyces</i> 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 “ <i>Genetic Manipulation of Streptomyces: a Laboratory Manual</i> ” – Approximately 3,000 copies distributed, with expanded edition (“ <i>Practical Streptomyces Genetics</i> ”) 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 <i>E. coli</i> to <i>Streptomyces</i> 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
	Development of combinatorial biosynthesis of unnatural natural products.	14
	Construction of a combined genetic and physical map of the <i>S. coelicolor</i> chromosome. A critical step in the genome sequencing project.	15
	Discovery that <i>Streptomyces</i> chromosomes are linear (Taiwan and JIC), leading to an understanding of the novel architecture and replication of <i>Streptomyces</i> chromosomes, including mechanisms of evolution and loss of antibiotic production genes.	16
	Construction of a set of ordered clones covering the <i>S. coelicolor</i> chromosome (JIC, German and Japanese researchers), opening the way for the genome sequencing project.	17
	Sequencing of the <i>S. coelicolor</i> sequence begun at the Sanger Institute in August 1997, completed in July 2001, and published in <i>Nature</i> in July 2002.	18

**Source:** JIC adapted by DTZ

- 2.13 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 <i>S. coelicolor</i> 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 <i>S. avermitilis</i> 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 <i>Streptomyces</i> , 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 <i>Streptomyces</i> , leading to the founding of Novacta Biosystems and may result in the introduction of a completely novel class of clinically useful antibiotics.	23
	Establishment of ScoDB, then StrepDB, the major database for <i>Streptomyces</i> 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 <i>S. coelicolor</i> 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 <i>S. coelicolor</i> (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 <i>Streptomyces avermitilis</i> (collaboration between Pfizer, USA, and Codexis,	29

Period	Overview of Achievements	References (see Appendix A)
	a spin-out from Maxygen, the inventors of gene shuffling).	
	Use of <i>S. coelicolor</i> phage to engineer mammalian genomes.	30
2006	Discovery that the TAT pathway is major route for protein export in <i>Streptomyces</i> .	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 <i>S. coelicolor</i> (University of Surrey).	35
	Generation of novel lantibiotics by genetic engineering (Novacta Biosystems, UK).	36

Source: JIC adapted by DTZ

## 3.0 Rationale for Public Sector Funding

3.1 The *Streptomyces* Genome Project has the potential to underpin significant further discoveries in the antibiotic and pharmaceuticals arena. Such discoveries could result in global impacts. However, market failures prevent the private sector from investing in such projects as outcomes are uncertain, costs can be substantial and there are many risks attached to them.

3.2 The HM-Treasury Green Book guidance states that there must be clear rationale for public sector interventions, and that:

*“This underlying rationale is usually founded either in market failure or where there are clear government distributional objectives that need to be met.”<sup>2</sup>*

### Addressing Market Failure

3.3 Market failure is when the market, by itself, has not and cannot be expected to deliver an efficient outcome. Thus, any research intervention must seek to redress this failure in the market. Market failures can be qualitatively applied to *Streptomyces* research, indicating the requirement for research regardless of the financial value of impacts.

3.4 There are a number of factors to consider in setting out the market failure relevant to government intervention in antibiotic/pharmaceutical research (and BBSRC funding of research) and these are discussed below under the following headings:

- Public goods
- Externality
- Information asymmetry.

#### Public goods

*“The market may have difficulty supplying and allocating certain types of products and services, such as ‘public goods’. Public goods are those that are ‘non-rival’ or ‘non-excludable’ when used or consumed. ‘Non-rival’ means that consumption of the good by one person does not prevent someone else using or consuming that good. ‘Non-excludable’ means that if a public good is made available to one consumer, it is effectively made available to everyone. Non-excludability can give rise to a problem known as ‘free-riding’.”<sup>3</sup>*

3.5 Public goods are those that provide social benefits that are large in comparison to their private benefits. Government intervention is necessary to achieve public good investment in antibiotic and pharmaceutical research that may benefit all of industry through reduced disease, improved understanding of utilising organic products to develop antibiotics or improved efficiency in terms of producing antibiotics.

<sup>2</sup> HM-Treasury, Green Book: Appraisal and Evaluation in Central Government, p.11

<sup>3</sup> *The Green Book – Appraisal and Evaluation in Central Government* HM Treasury, Crown copyright

- 3.6 The research undertaken in relation to the genetic manipulation and genome sequencing of *Streptomyces* involves scientific expertise and equipment investment. There is little incentive for any individual company in the industry to bear the full investment, particularly when success is not guaranteed, unless they can secure exclusive use of any new products/innovations. Moreover, companies also face the added risk of other organisations having the same genome sequence but being further ahead in terms of the research – which could potentially reduce further the chances of success. This means that a large proportion of the industry would be excluded from advantageous innovation, resulting in reduced social benefits as the monopoly supplier seeks to make a financial return on its investment. In addition, as a result of the speed at which antibiotics work, in most cases they do not have the same market size as drugs that treat chronic, long-term conditions or lifestyle issues<sup>4</sup>. To achieve the desirable social outcomes of new antibiotics and reduced disease, the government must therefore enhance access to any such beneficial research.

### Externalities

*“Externalities’ result when a particular activity produces benefits or costs for other activities that are not directly priced into the market. Externalities are associated with, for example, research and development spill-overs, and environmental impacts, such as pollution.”<sup>5</sup>*

- 3.7 As this report demonstrates, there are many positive externalities from this research as knowledge has been disseminated to labs around the world and supported many other discoveries. Similarly, the pharmaceutical industry has benefited and the genome sequence has sped up progress in new drug discovery and allowed the benefits to be shared widely, rather than being held in only one company. Private companies would be unable to capture these benefits in market prices, thus highlighting the need for government intervention.
- 3.8 Furthermore, there are externalities related to research and development (R&D), particularly fundamental research such as the Genome Project – where ideas are difficult to patent so the financial return on research is lower than the total social return, and the scale of potential knowledge vastly exceeds what a company could do. The reverse of this would also lead to market failure, with too much knowledge leading to significant competition between companies if the research was undertaken by the private sector – which may again lead to lower levels of financial return.

### Information Asymmetry

*“Information is needed for a market to operate efficiently. Buyers need to know the quality of the good or service to judge the value of the benefit it can provide. Sellers, lenders and investors need to know the reliability of a buyer, borrower or entrepreneur. This information must be available fully to both sides of the market, and where it is not, market failure may result. This is known as ‘asymmetry of information’.”<sup>6</sup>*

- 3.9 The private sector would be unlikely to invest in this type of research given the lack of information available on the certainty of a successful outcome. This relates to the overall level of information available in the market. In terms of the production of information, there are often high costs of production (i.e. research costs) but low costs of subsequently transmitting

<sup>4</sup> Infectious Diseases Society of America – “Facts About the Antibiotic R&D Pipeline: Why Antibiotics Require Special Treatment”. Available at: <http://www.idsociety.org/Content.aspx?id=5652>

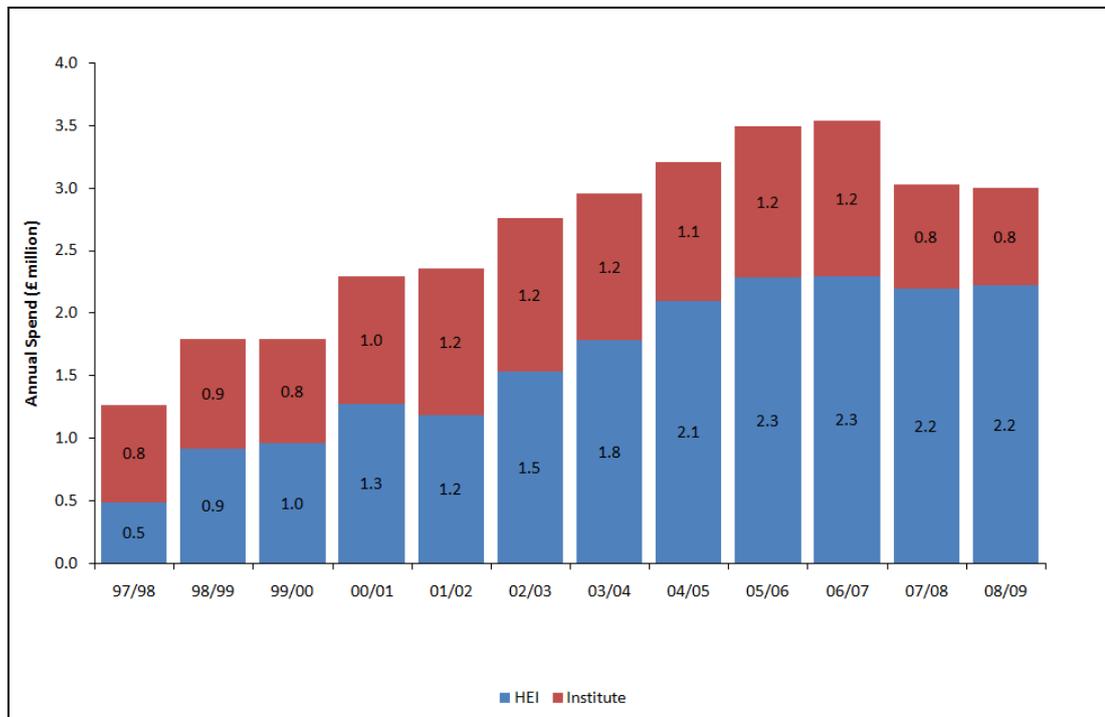
<sup>5</sup> *The Green Book – Appraisal and Evaluation in Central Government* HM Treasury, Crown copyright. p.51

<sup>6</sup> *ibid*, p. 52

information to others given the difficulties of controlling its usage by other parties (limited excludability). This can ultimately lead to sub-optimal levels of market information unless the government intervenes.

- 3.10 The development of *Streptomyces* molecular genetics has addressed this failure through highly cited peer-reviewed research publications, a strong focus on sharing information, the development of spin-out companies to continue the research and by supporting a large number of scientists from around the world – many of whom have gone on to lead their own laboratories. The research has also helped to support a number of courses and workshops in *Streptomyces* genetics. These initiatives are discussed in further detail in section five.
- 3.11 *Streptomyces* research has received long term UK funding since the 1960s. However, there are no accurate records for many of the early research grants. Accurate information is available from 1997 onwards.
- 3.12 The BBSRC funding for *Streptomyces* research is shown in Figure 3.1 for the period 1997-2009, broken down by Higher Education Institutions and Research Institutes. Total funding peaked in 2006/2007 (at just over £3.5 million) and the majority of funding awarded to Institutes was mainly to the John Innes Centre.

**Figure 3.1: Estimated annual BBSRC expenditure on *Streptomyces* research, 1997 onwards**



Source: BBSRC

## 4.0 Economic Impact of *Streptomyces* Genetic Research

- 4.1 The key quantifiable development to result from *Streptomyces* research is the discovery that actinomycetes have huge and untapped potential as antibiotic producers and that this potential can only be harnessed through a deep understanding and exploitation of their genetics. In addition, the research will also help to support efforts towards increasing efficiency within antibiotic production – by influencing industrial approaches to yield optimisation. The *Streptomyces* Genome Project was completed in 2001. It generally takes around 15 years to bring new drugs to market so it is likely that the bulk of impacts from this work will happen in the future.
- 4.2 The importance of *Streptomyces* cannot be overestimated. By identifying the potential of actinomycetes to develop new antibiotics, the Genome Project overturned the widely held belief prevalent in many of the larger pharmaceutical companies that all the useful natural products had been discovered before 1990 – which resulted in the widespread closure of many natural products programmes and a switch to purely chemical approaches in an unsuccessful attempt to fill their antibiotic discovery pipelines.

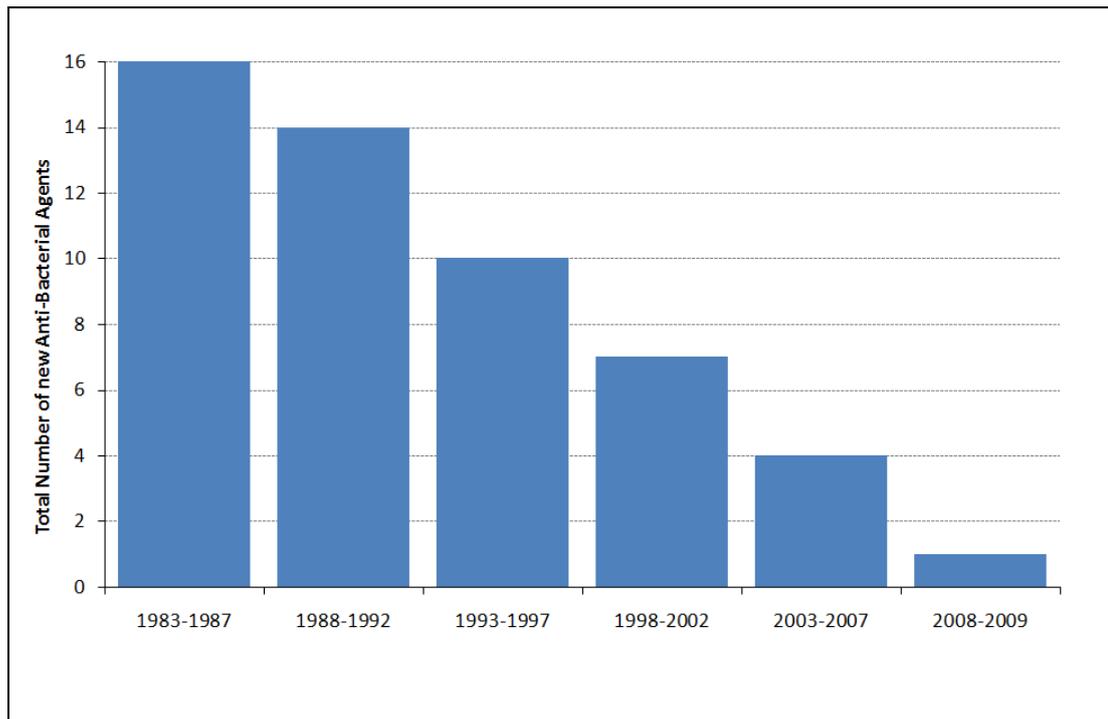
### Development of new Antibiotics

- 4.3 As already noted, one of the key impacts of the Genome Project is the discovery of the potential to develop new antibiotics from actinomycetes. This is a critical issue because the pipeline of new antibiotics is declining (see Figure 4.1) and the Infectious Diseases Society of America<sup>7</sup> (IDSA) has highlighted several challenges facing the market:
- As a result of the speed at which antibiotics work, in most cases they do not have the same market size as drugs that treat chronic, long-term conditions or lifestyle issues
  - The development of resistant strains of bacteria limits the long-term market potential for an antibiotic
  - Infectious disease experts often suggest restrictions on the use of new antibiotics in order to preserve the effectiveness of these drugs for those patients who need them most. Such restrictions can reduce the market potential and the incentive for companies to develop new antibiotics
  - Drug R&D is expensive, risky, and time-consuming. The IDSA has estimated that an aggressive R&D program initiated today would require ten or more years and an investment of \$800 million to \$1.7 billion to bring a new drug to market
  - Side effect issue – antibiotics are now required to be free of any side-effects, an almost impossible expectation, which would have prevented the clinical introduction of many of the “classical” antibiotics.

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<sup>7</sup> Infectious Diseases Society of America – “Facts About the Antibiotic R&D Pipeline: Why Antibiotics Require Special Treatment”. Available at: <http://www.idsociety.org/Content.aspx?id=5652>

**Figure 4.1: Anti-Bacterial Agents Approved, 1983-2009**

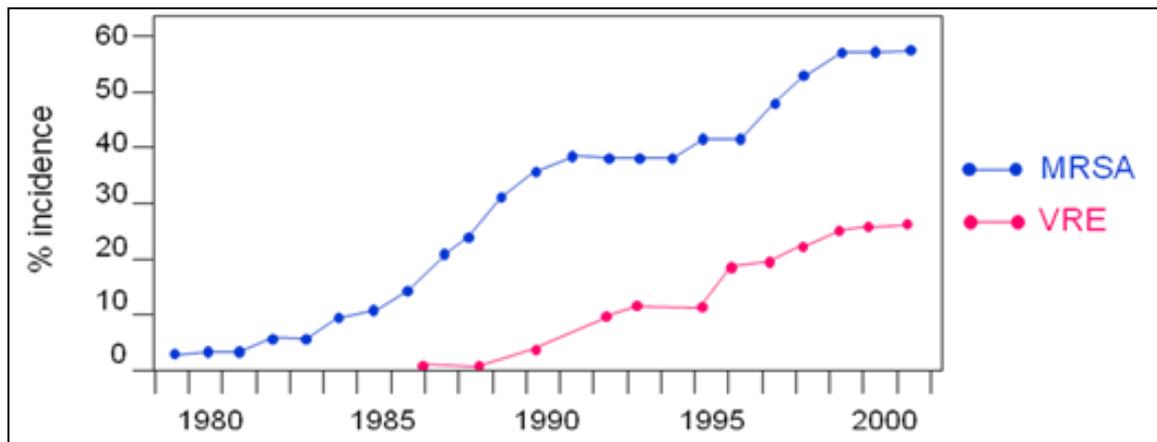


**Source:** Spellberg et al., *Clinical Infectious Diseases*, May 1, 2004 (modified)<sup>8</sup>

- 4.4 At the same time as the number of new antibiotics has declined, the IDSA has highlighted the enormous challenge posed by increasing resistance of bacteria to antibiotics. Figure 4.2 shows the increasing occurrence of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Taking the United States as an example from the developed world, in 1974 MRSA resistance was 2.0% in US hospitals, yet by 2002 the figure had risen to more than 57.0%. In some US hospitals, it is now 100%, and many of these isolates are also resistant to many other antibiotics. Worryingly, similar trends towards the acquisition of antibiotic and multi-drug resistance are observed with almost all human pathogens, with the seemingly inevitable conclusion that eventually most pathogens will become resistant to most currently available clinically used antibiotics.

<sup>8</sup> Spellberg B, et al. Trends in antimicrobial drug development. *Clinical Infectious Diseases*. 2004; 31: 1279-1286.

Figure 4.2: Spread of Resistant Strains of MRSA and VRE in the United States



Source: John Innes Centre

- 4.5 Of the 13 commonly used classes of clinically useful antibiotics, seven are derived from actinomycetes and *Streptomyces* research offers the chance to increase this – helping to address the problems of a slowing development pipeline and growing resistance outlined in Figures 4.1 and 4.2 respectively.
- 4.6 In 2005, actinomycete-derived antibiotics constituted around 30.0% of total antibiotic sales. In a report published in December 2009<sup>9</sup>, the total annual sales value of the global anti-infectives market was estimated at \$79 billion. Of this total, the anti-bacterial market accounted for \$37 billion. Applying the 30.0% market share, actinomycete-derived antibiotics would account for revenues of approximately \$11 billion. **If *Streptomyces* research supports the development of a substantial number of new anti-bacterial products, new revenue streams will be created in this market. Even a very conservative increase of 1% would lead to additional sales revenue potential of \$370 million (£247 million<sup>10</sup>) per year as a result of *Streptomyces* research.**
- 4.7 The hugely important beta-lactam class of clinically useful antibiotics has also benefitted from *Streptomyces* research. Dutch States Mines (DSM), one of the world's major producers of beta-lactam antibiotics, is making their cephalosporins (a class of beta-lactam antibiotics) using a strain of the fungus *Penicillium* genetically engineered to contain a gene from *Streptomyces clavuligerus*. The knowledge required for the new process development was based on both academic and industrial research programmes undertaken in the mid-1980 and 1990s.
- 4.8 Information on the market share and value of this process is commercially sensitive and cannot be disclosed. However, it is possible to look at potential impacts across the whole cephalosporins market. Total sales of cephalosporins in 2005 were around \$7.0 billion (£4.6 billion<sup>11</sup>). **If *Streptomyces* research leads to sales increases of 1% per year, this**

<sup>9</sup> Vision Grain (December 2009) – The World Antibacterial Treatments Market 2010-2024.

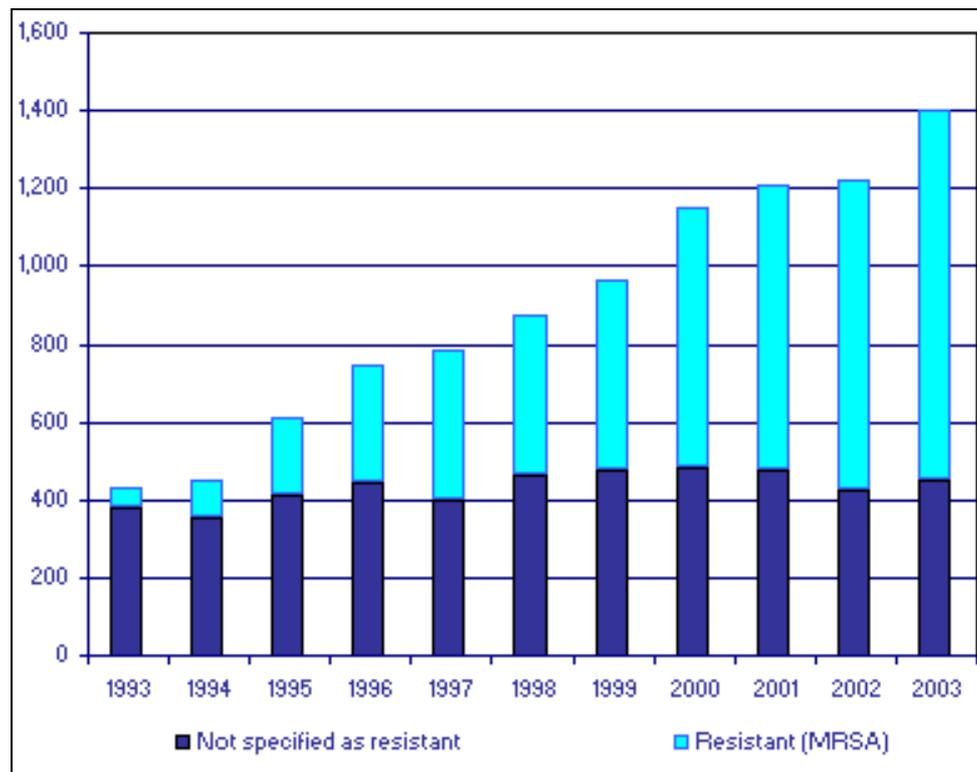
<sup>10</sup> Figure converted from US dollars to UK sterling using an exchange rate of \$1 = £0.668049 on 24<sup>th</sup> March 2010

<sup>11</sup> Figure sourced from Datamonitor, MIDAS Sales Data, IMS Health (November 2006) and converted from US dollars to UK sterling using an exchange rate of \$1 = £0.668049 on 24<sup>th</sup> March 2010

amounts to \$70 million (£46.4 million<sup>12</sup>) of additional revenue.

- 4.9 Antibiotic resistance causes a significant number of deaths, with MRSA alone contributing to nearly 1,000 deaths in the UK in 2003 (Figure 4.3). MRSA has a significant cost burden upon health systems around the world. In the UK the 2007 cost can be estimated to be in the region of £45 million<sup>13</sup>. UK hospital costs associated with MRSA are estimated to be in the region of £9,000 per patient, a figure that was published at a conference at the Royal College of Physicians in London in 2008.<sup>14</sup>
- 4.10 Within the USA, MRSA currently costs £2.1 billion per annum<sup>15</sup>. Finding new antibiotics that can fight MRSA will significantly reduce hospital costs around the world. The MRSA antibiotics market alone was estimated at \$2 billion in 2006<sup>16</sup>.

**Figure 4.3: Deaths due to MRSA resistance in the UK**



Source: Health Protection Agency

- 4.11 Taking the value of the 1,000 deaths caused by MRSA in the UK in 2003 and using standard valuation methods, **even a saving of 10% in the lives lost (100 lives) through better**

<sup>12</sup> Figure converted from US dollars to UK sterling using an exchange rate of \$1 = £0.668049 on 24<sup>th</sup> March 2010

<sup>13</sup> Based on statistics published at a conference at the Royal College of Physicians. Overview of the key statistics available at: <http://www.telegraph.co.uk/news/uknews/2194132/Every-MRSA-case-costs-NHS-an-extra-9000.html>

<sup>14</sup> <http://www.parliament.the-stationery-office.co.uk/pa/cm200708/cmselect/cmhealth/1137/1137we38.htm#n205>

<sup>15</sup> Costs in the UK and US are not directly comparable. Health care costs are higher in the US and in 2006, health expenditure as a percentage of GDP in the US stood at 15.3%. In the UK it was 8.2%. See: World Health Statistics 2009 – World Health Organisation. Available at: <http://www.who.int/whosis/whostat/2009/en/index.html>

<sup>16</sup> [http://www.researchandmarkets.com/reportinfo.asp?report\\_id=338074](http://www.researchandmarkets.com/reportinfo.asp?report_id=338074)

antibiotics as a result of *Streptomyces* research would be worth £100 million per year<sup>17</sup> and this is just for MRSA.

### Development of Anti-Cancer Drugs and Immunosuppressants

- 4.12 As well as antibiotics, *Streptomyces* produce compounds used in other medical treatments including anti-cancer drugs and immunosuppressants for a range of conditions. Examples include:
- Actinomycin and Anthracyclines – Both of which are anti-cancer agents. For example anthracyclines are used to treat a wide range of cancers, including leukemias, lymphomas and breast, uterine, ovarian and lung cancers.
  - Sirolimus and tacrolimus – Sirolimus (also known as rapamycin) and tacrolimus (also known as FK-506) are immunosuppressant drugs used to prevent rejection in organ transplantation.
- 4.13 An example of an anthracycline is Doxorubicin, whose origins can be traced back to identification of a new strain of *Streptomyces peucetius* in the 1950s by scientists in Italy and France. The drug was developed in the 1960s<sup>18</sup> and treats cancers including: bladder, breast, head and neck, leukemia (some types), liver, lung, lymphomas, mesothelioma, multiple myeloma, neuroblastoma, ovary, pancreas, prostate, sarcomas, stomach, testis (germ cell), thyroid and uterus. Doxorubicin is administered via intravenous injection and in the United States alone, sales of this *Streptomyces*-derived drug were estimated to be in excess \$30 million (£20 million) in 2006<sup>19</sup>. The work funded by BBSRC has created the potential for significant further discoveries which could well exceed the value of existing *Streptomyces*-derived anti-cancer drugs. Taking Doxorubicin, if the US is assumed to represent a third of global usage, global sales could easily be £60 million per annum. Immunosuppressants offer just as much potential for new discoveries. **It is likely that global pharmaceutical firms will take forward *Streptomyces* discoveries to develop new products for both anti-cancer and immunosuppressant drugs. On the basis of Doxorubicin, these annual sales are likely to exceed £120 million per annum in the long term.**

<sup>17</sup> Work undertaken by the Department for Transport has estimated that the average cost of one human fatality is £1 million. Multiplying this by the 1,000 MRSE deaths gives a value of £1 billion. 10% of this gives £100 million. See: Department for Transport – Transport Analysis Guidance. TAG Unit 3.4.1, April 2009. Available at: <http://www.dft.gov.uk/webtag/documents/expert/unit3.4.php>

<sup>18</sup> Weiss RB (December 1992). "The anthracyclines: will we ever find a better doxorubicin?". *Seminars in Oncology* 19 (6): 670–86.

<sup>19</sup> IMS Health - Figure converted from US dollars to UK sterling using an exchange rate of \$1 = £0.668049 on 24<sup>th</sup> March 2010

## Increased Productivity in Pharmaceutical Companies

- 4.14 Research on *Streptomyces* genetics has contributed, and continues to contribute to marked improvements in antibiotic productivity, providing significant cost reductions to pharmaceutical companies. The discovery of efficient mutagenesis in *Streptomyces* by nitrosoguanidine under conditions very different from those in *E. coli* (Ref. 2 to Table 2.1) had a very significant impact on such programmes. Protoplast fusion to combine desirable mutations from divergent selection lines (Ref. 4 in Table 2.1) has also been very beneficial, even if there is little mention of this in the published literature. Application, in a recursive fashion, of protoplast fusion technology has been shown to allow improvements in antibiotic yield within a year that would have taken 20 years to accomplish using classical approaches (Ref. 19 to Table 2.2). Selection of deoxyglucose-resistant lines has also been used very effectively to increase antibiotic production by removing inhibition caused by glucose present in fermentation media. Fundamental insights into the regulation and physiology of antibiotic biosynthesis will increasingly allow knowledge-based approaches to strain improvement.
- 4.15 The pharmaceutical sector employs nearly 70,000 staff in the UK and in 2007 had one of the highest trade balances at £4.28 billion. Annually it spends £4.47 billion on research and development in the UK<sup>20</sup>. The sector faces strong competition from generic products plus NHS price sensitivity for drugs. Even a small increase in process productivity is important and could have a significant economic impact within the UK. For example, **a 1% efficiency saving through better antibiotic productivity could save £44.7 million of the industry's annual R&D spend.**

## Supporting the Animal Health Industry<sup>21</sup>

- 4.16 As well as human drug discoveries, *Streptomyces* research has led to one of the most widely employed anti-parasitic drugs within the animal health industry – Ivermectin. The drug was developed in the early 1970s by the Kitasato Institute in Tokyo as part of a collaborative research programme with Merck, Sharp and Dohme (MSD).
- 4.17 By researching the organism *Streptomyces avermitilis*, scientists were able to isolate a new class of anthelmintic compound, the avermectins, which showed an ability to kill living organisms such as nematodes, insects and arachnids. In total, *Streptomyces avermitilis* makes eight avermectins, one of which was developed through the research programme under the name Ivermectin. In testing, the drug proved to be effective against mite, tick and botfly ectoparasites, which can all inflict substantial damage on the livestock industry. It was also effective in killing endo and ecto-parasites in horses, cattle, sheeps and pigs, in addition to killing larval worms in dogs.
- 4.18 As a result of *Streptomyces* research, Ivermectin was subsequently launched as a commercial animal health product in 1981 by MSD and generates annual revenues of approximately \$1 billion. **The sustained BBSRC *Streptomyces* research programme will potentially lead to further animal health drug discoveries by pharmaceutical and**

<sup>20</sup> The Association of British Pharmaceuticals Industry

<sup>21</sup> Information sourced from: "Ivermectin: one of Nature's healthiest gifts", *Appropriate Technology* – 2005, Volume 32, Issue 2

agrochemical companies. Achieving even 10% of the success of Ivermectin could generate additional annual sales of £60 million.

### Summary of Economic Impact

4.19 This section has identified the economic impacts arising from *Streptomyces* research. The estimates are based on very conservative assumptions, however the impacts are significant and can be summarised as follows:

- Supporting the development of new anti-bacterial products:
  - Additional global sales revenue potential of £247 million per year for actinomycete-derived antibiotics
  - A further £46 million of additional revenue potential for global sales of cephalosporins (a class of beta-lactam antibiotics)
  - Reducing the number of deaths in the UK from MRSA through better antibiotics could be worth £100 million per year
- Supporting the development of new products for both anti-cancer and immunosuppressant drugs. On the basis of Doxorubicin, these annual sales are likely to exceed £120 million per annum in the long term
- Increasing productivity – efficiency savings through better antibiotic productivity could save £44.7 million of the UK pharmaceutical sector’s annual R&D spend
- Potential animal health drug discoveries, which could generate additional annual sales of £60 million.

## 5.0 Qualitative Impact

5.1 As well as quantitative impacts, a wide range of qualitative benefits have resulted from *Streptomyces* research. Looking specifically at benefits associated with JIC, this includes:

- The achievements of JIC alumni
- Training and exchange of scientists between laboratories
- Development of spin-out companies from JIC

### Achievements of Alumni

5.2 In total, more than 100 JIC alumni have gone on to work in senior positions in other academic institutions or in the private sector, with many leading their own laboratories across the globe. Among the most prominent positions held by the JIC *Streptomyces* alumni (PhD students, post-docs, visiting workers and sabbatical visitors) are:

- Head, Unit of Biotechnologies, European Commission
- Head of Microarray Facility, University of Cambridge
- Professor of Chemical Engineering, Stanford University (co-founder of Kosan Biosciences)
- Professor of Microbiology, University of British Columbia
- Professor, National Centre for Biotechnology, Madrid
- Professor of Pharmaceutical Sciences, University of Michigan (co-founder of Acera Biosciences)
- Professor, University of Leiden (co-founder of Mycobics)
- Senior Research Director, Diversa Corporation
- Senior Scientist, Cubist Pharmaceuticals
- Senior Scientist, Novacta Biosystems
- Senior Scientist, GenWay Biotech
- Head of Infectious Disease, Merck
- Professor and Head of Department of Life Sciences and Biotechnology, Shanghai Jiaotong University.
- Professor and Deputy Institute Head, Chinese National Academy of Science Institute of Microbiology, Beijing
- Professor of Functional Genomics, University of Surrey
- Professor of Microbiology, University of Warwick.

5.3 In addition, a further 32 JIC *Streptomyces* alumni hold Professorial positions at Universities worldwide.

5.4 One of the impacts of these alumni has been to carry the benefits of *Streptomyces* research across the globe. Some of their most notable achievements in *Streptomyces* research since leaving JIC are listed below:

- *Streptomyces* genome sequencing and its exploitation (Japan, UK)
- Development of genome mining (France, Netherlands, UK)
- Many examples of the production of new antibiotics by genetic engineering (China, Germany, USA, Spain, UK)
- Commercialisation of actinomycete antibiotics (USA, UK)
- Understanding antibiotic biosynthesis and its regulation (China, Japan, Netherlands, Spain, Italy, Canada, USA, UK)
- Improvement of fermentation characteristics by manipulating cell division (Netherlands)
- Understanding the biochemistry of terpene biosynthesis (USA)
- Understanding the interplay of primary and secondary metabolism (Netherlands, Germany, UK)
- Discovery of a novel covalent sulphur modification of DNA (China)
- Dissection of morphological differentiation in *Streptomyces* (Netherlands, USA, Canada, UK)
- Understanding fundamental aspects of *Streptomyces* physiology (France, Italy, USA, UK)
- Chromosome replication in *Streptomyces* (Taiwan, Poland)
- The role of non-coding RNAs in *Streptomyces* biology (Netherlands, Canada).

### **Training of Scientists and Developing Linkages Between Laboratories**

5.5 The John Innes Centre has organised five two-week practical courses (between 1983 and 1990) to train more than 100 scientists in *Streptomyces* genetics, and two courses on targeted mutagenesis. In addition to this, the *S.coelicolor* genome sequence and subsequent functional genomics research it has stimulated were a major catalyst in setting up a series of summer schools, organised jointly by the John Innes Centre and the Institute Ruđer Bošković, Zagreb, Croatia. These have focused on training researchers on the topic of secondary metabolites and genomics. The first summer school was held at the Mediterranean Institute of Life Sciences in Split in 2007 and had 38 participants from 20 nationalities. The second was held in 2008 at the Inter-University Centre (IUC) in Dubrovnik and had 44 attendees from 25 nationalities. The third summer school is also scheduled to be held at the IUC in 2010, with a similar mix of attendees. Individual attendees on the summer schools have gone on to work in commercial companies.

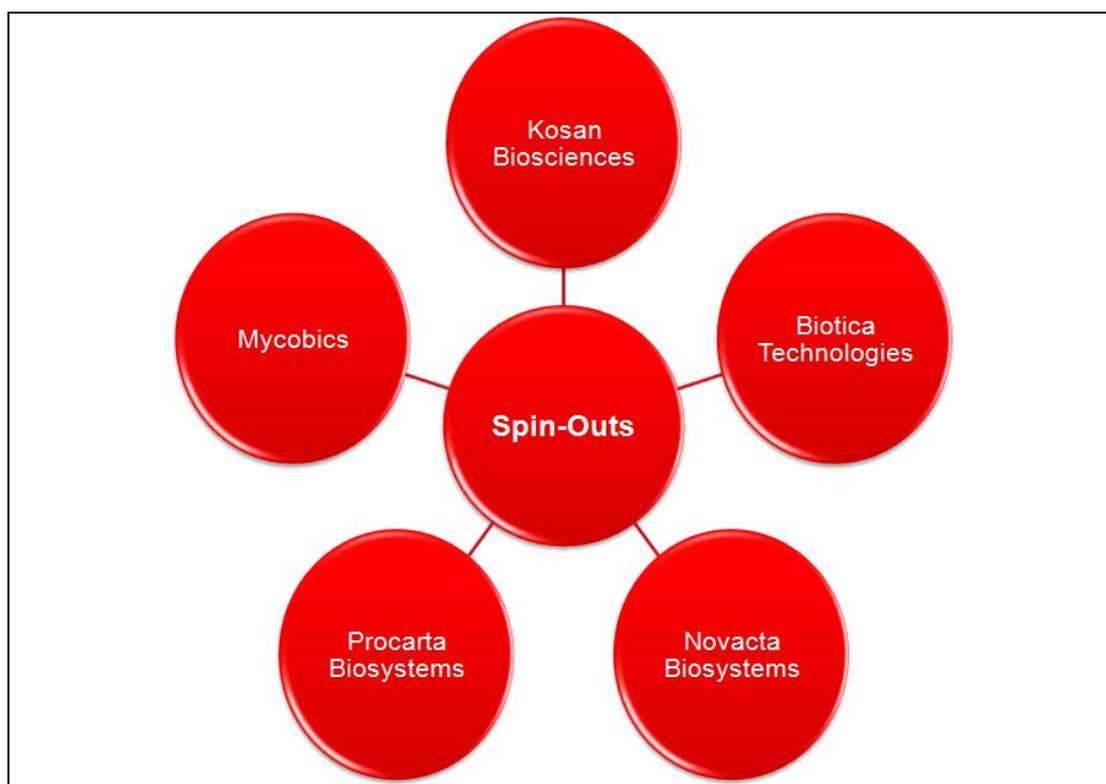
5.6 Moreover, at least two international practical courses have been run by the Functional Genomics Laboratory at the University of Surrey on the use of high-density *Streptomyces* microarrays for gene expression profiling and on the application of ChIP-on-Chip technology. Both technologies provide genome-wide analysis of gene expression and were made

possible by the determination of the *S. coelicolor* genome sequence.

### Development of Spin Out Companies

- 5.7 Importantly, *Streptomyces* research has directly facilitated the development of five spin-out companies since 1995 (see Figure 5.1), one of which was bought by Bristol Myers Squibb for \$190 million while another has secured a licensing agreement with Wyeth worth up to \$195 million.

**Figure 5.1: Spin-Out Companies arising from *Streptomyces* research**



Source: John Innes Centre

### Kosan Biosciences

- 5.8 Kosan Biosciences was co-founded in 1995 by a former post-doc at the John Innes Centre and a professor at the University of California, San Francisco, to exploit a series of patents arising from work on polyketide biosynthesis started at JIC and continued at Stanford, and co-owned by JIC and Stanford. The aim of the company was to exploit the newly invented field of combinatorial biosynthesis to engineer novel antimicrobials and anti-cancer drugs, with annual license fees payable to JIC: royalties could accrue if successful drugs emerge that depend on the JI-Stanford IP. At its peak as a public company in 2006, Kosan had 130 employees in Hayward, California. Having several anti-cancer candidates in mid-late stage clinical trials and a need for major funding, Kosan was bought by Bristol Myers Squibb (BMS) for \$190 million in May 2008. In acquiring Kosan, BMS recognised the opportunity to enhance its pipeline in creating anti-cancer agents.

### Biotica Technologies

- 5.9 Biotica Technologies was co-founded in 1996 by two academics at the University of Cambridge (who had received substantial BBSRC funding for their work on natural product biosynthesis) as a privately held spin-out from the University. Currently located on the Chesterford Research Park, the company focuses on the discovery and development of novel therapeutics and aims to discover drugs which address serious unmet medical needs, including cancer and inflammatory diseases.
- 5.10 The core specialism of Biotica is in natural product chemistry, molecular biology and fermentation microbiology. This is complemented by a network of academic collaborations and Contract Research Organisations (CROs) in pharmacology and oncology. The company also establishes preclinical partnerships and licensing agreements to accelerate the development and commercialisation of its products – for example, in 2006 it signed a licensing deal with Wyeth worth up to \$195 million focussing on the discovery, development and commercialization of novel rapamycin analogs that target diseases in multiple therapeutic areas.

### Novacta Biosystems

- 5.11 Novacta Biosystems was founded as a JIC spin-out in 2003 based on a series of JIC patents, and it is engaged in the discovery and development of potential treatments for infectious diseases focussed currently on lantibiotics. Based on the Welwyn Garden City Biopark, the company operates in two divisions:
- **Novacta Therapeutics:** Engaged in the discovery and development of anti-bacterial and anti-viral agents for the treatment of bacterial and viral infections
  - **Novacta Biosystems:** Providing solutions for chemical challenges in blue chip drug development and biofuel companies.
- 5.12 In July 2009, Novacta was successful in securing £13.1 million in funding as part of the second Celtic Pharma Fund<sup>22</sup> to help drive the novel antibiotics platform to treat *Clostridium difficile* and potentially MRSA. The company currently employs around 30 people.

### Procarta Biosystems

- 5.13 Procarta is a 2007 JIC spin-out based on the Norwich Research Park. Its main focus is on the development of a novel approach to combating antibiotic resistant pathogens called transcription factor decoys which were first developed using *S. coelicolor* as a model system. By using nucleic-acid based therapies, Procarta aims to restore the efficacy of the current armoury of antibiotics. For his role in developing the Procarta technologies, Dr Michael McArthur was made the BBSRC Most Promising Innovator of the Year for 2010.

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<sup>22</sup> Celtic Pharma Holdings Advisors LLP is a private equity growth capital advisor and manager that makes selected investments in biotechnology and other healthcare companies.



## **Mycobics**

- 5.14 Mycobics is a Dutch company founded in 2006 and developed by former members of the JIC *Streptomyces* group. The two objectives of the company are to discover novel antibiotics through genomics approaches, and to use genetic engineering to modify the growth habit of filamentous microorganisms such as actinomycetes to improve fermentation parameters.



**Appendix A**  
**Publications in Tables 2.1 and 2.2 by Reference Number**

Reference Number	Publication
1	Hopwood, D.A. (1959) Linkage and the mechanism of recombination in <i>Streptomyces coelicolor</i> . Ann NY Acad Sci 81, 887-898.
2	Delic, V., Hopwood, D.A., Friend, E.J. (1970) Mutagenesis by N-methyl-N-nitro-N-nitrosoguanidine (NTG) in <i>Streptomyces coelicolor</i> . Mutation Res 9, 167-182.
3	Hopwood, D.A., Chater, K.F., Dowding, J.E., Vivian, A. (1973) Advances in <i>Streptomyces coelicolor</i> genetics. Bacteriol Rev 37, 371-405.
4	Hopwood, D.A., Wright, H.M., Bibb, M.J., Cohen, S.N. (1977) Genetic recombination through protoplast fusion in <i>Streptomyces</i> . Nature 268, 171-174; Baltz, R.H (1978). Genetic recombination in <i>Streptomyces fradiae</i> by protoplast fusion and regeneration. J Gen Microbiol. 107, 93-102.
5	Bibb, M.J., Ward, J.M., Hopwood, D.A. (1979) Transformation of plasmid DNA into <i>Streptomyces</i> at high frequency. Nature 274, 398-400.
6	Rudd, B.A.M., Hopwood, D.A. (1979) Genetics of actinorhodin biosynthesis by <i>Streptomyces coelicolor</i> A3(2) J Gen Microbiol 114, 35-43.
7	Lomovskaya N.D., Chater K.F., Mkrtumian N.M. (1980) Genetics and molecular biology of <i>Streptomyces</i> bacteriophages. Microbiol Rev. 44, 206-229.
8	Bibb M., Schottel J.L., Cohen S.N. (1980) A DNA cloning system for interspecies gene transfer in antibiotic-producing <i>Streptomyces</i> . Nature 284, 526-531; Thompson, C.J., Ward, J.M., Hopwood, D.A. (1980) DNA cloning in <i>Streptomyces</i> : resistance genes from antibiotic-producing species. Nature 286, 525-527; Suarez J. E., Chater K.F. (1980) DNA cloning in <i>Streptomyces</i> by a bifunctional replicon comprising pBR322 inserted into a <i>Streptomyces</i> bacteriophage. Nature 286, 527-529.
9	Hara O., Horinouchi S., Uozumi T., Beppu T. (1983) Genetic analysis of A-factor synthesis in <i>Streptomyces coelicolor</i> A3(2) and <i>Streptomyces griseus</i> . J Gen Microbiol 129, 2939-2944.
10	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 <i>Streptomyces</i> : A Laboratory Manual. Norwich, John Innes Foundation; Kieser, T., Bibb, M.J., Buttner, M.J., Chater, K.F., Hopwood, D.A. (2000) Practical <i>Streptomyces</i> Genetics. Norwich: John Innes Foundation.
11	Hopwood, D.A., Malpartida, F., Kieser, H.M., Ikeda, H., Duncan, J., Fujii, I., Rudd, B.A.M., Floss, H.G., Ōmura, S. (1985) Production of 'hybrid' antibiotics by genetic engineering. Nature 314, 642-644.
12	Mazodier P., Petter R., Thompson C. (1989) Intergeneric conjugation between <i>Escherichia coli</i> and <i>Streptomyces</i> species. J Bacteriol 171, 3583-3585.
13	Cortes J., Haydock S.F., Roberts G.A., Bevitt D.J., Leadlay P.F. (1990) An unusually large multifunctional polypeptide in the erythromycin-producing polyketide synthase of <i>Saccharopolyspora erythraea</i> . Nature 348, 176-178; Donadio S., Staver M.J., McAlpine J.B., Swanson S.J., Katz L. (1991) Modular organization of genes required for complex polyketide biosynthesis. Science 252, 675-679.
14	McDaniel R, Ebert-Khosla S, Hopwood DA, Khosla C. (1995) Rational design of aromatic polyketide natural products by recombinant assembly of enzymatic subunits. Nature 375, 549-554; Cortes J, Weissmann KE, Roberts GA, Brown MJ, Staunton J, Leadlay PF. (1995) Repositioning of a domain in a modular polyketide synthase to promote specific chain cleavage. Science 268, 1487-1489.
15	Redenbach, M., Kieser, H. M., Denapaite, D., Eichner, A., Cullum, J., Kinashi, H., Hopwood, D. A. (1996). A set of ordered cosmids and a detailed genetic and physical map for the 8 Mb

Reference Number	Publication
	<i>Streptomyces coelicolor</i> A3(2) chromosome. Mol Microbiol 21, 77-96.
16	Lin, Y-S., Kieser, H.M., Hopwood, D.A., Chen, C.W. (1993) The chromosomal DNA of <i>Streptomyces lividans</i> 66 is linear. Mol Microbiol 10, 923-933.
17	Redenbach, M., Kieser, H. M., Denapaite, D., Eichner, A., Cullum, J., Kinashi, H., Hopwood, D. A. (1996) A set of ordered cosmids and a detailed genetic and physical map for the 8 Mb <i>Streptomyces coelicolor</i> A3(2) chromosome. Mol Microbiol 21, 77-96.
18	Bentley, S.D. et al. (2002) Complete genome sequence of the model actinomycete <i>Streptomyces coelicolor</i> A3(2). Nature 417, 141-147.
19	Huang J, Lih CJ, Pan KH, Cohen SN. <i>Global analysis of growth phase responsive gene expression and regulation of antibiotic biosynthetic pathways in Streptomyces coelicolor using DNA microarrays</i> . Genes Dev. 2001 15: 3183-3192.
20	Zhang YX, Perry K, Vinci VA, Powell K, Stemmer WP, del Cardayré SB. <i>Genome shuffling leads to rapid phenotypic improvement in bacteria</i> . Nature. 2002 415: 644-646.
21	Keda H, Ishikawa J, Hanamoto A, Shinose M, Kikuchi H, Shiba T, Sakaki Y, Hattori M, Ōmura S. Nat Biotechnol. Complete genome sequence and comparative analysis of the industrial microorganism <i>Streptomyces avermitilis</i> . I 2003 21: 526-531.
22	PCR-targeted <i>Streptomyces</i> gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. Gust B, Challis GL, Fowler K, Kieser T, Chater KF. Proc Natl Acad Sci U S A. 2003 100: 1541-1546.
23	Widdick DA, Dodd HM, Barraille P, White J, Stein TH, Chater KF, Gasson MJ, Bibb MJ . <i>Cloning and engineering of the cinnamycin biosynthetic gene cluster from Streptomyces cinnamoneus</i> DSM 40005. Proc Natl Acad Sci U S A. 2003 100: 4316-4321.
24	<a href="http://strepdb.Streptomyces.org.uk">http://strepdb.Streptomyces.org.uk</a>
25	Hesketh A, Chater KF. J Ind. <i>Evidence from proteomics that some of the enzymes of actinorhodin biosynthesis have more than one form and may occupy distinctive cellular locations</i> . Microbiol Biotechnol. 2003 30: 523-529.
26	Kim DW, Hesketh A, Kim ES, Song JY, Lee DH, Kim IS, Chater KF, Lee KJ. <i>Complex extracellular interactions of proteases and a protease inhibitor influence multicellular development of Streptomyces coelicolor</i> . Mol Microbiol. 2008 70: 1180-1193.
27	Herron PR, Hughes G, Chandra G, Fielding S, Dyson PJ . <i>Transposon Express, a software application to report the identity of insertions obtained by comprehensive transposon mutagenesis of sequenced genomes: analysis of the preference for in vitro Tn5 transposition into GC-rich DNA..</i> Nucleic Acids Res. 2004 32: e113.
28	Eustáquio AS, Gust B, Galm U, Li SM, Chater KF, Heide L. <i>Heterologous expression of novobiocin and clorobiocin biosynthetic gene clusters</i> . Appl Environ Microbiol. 2005 71:2452-2459.
29	Stutzman-Engwall K, Conlon S, Fedechko R, McArthur H, Pekrun K, Chen Y, Jenne S, La C, Trinh N, Kim S, Zhang YX, Fox R, Gustafsson C, Krebber A. <i>Semi-synthetic DNA shuffling of aveC leads to improved industrial scale production of doramectin by Streptomyces avermitilis</i> . Metab Eng. 2005 7: 27-37.
30	Dafnis-Calas F, Xu Z, Haines S, Malla SK, Smith MC, Brown WR. <i>Iterative in vivo assembly of large and complex transgenes by combining the activities of phiC31 integrase and Cre recombinase</i> . Nucleic Acids Res. 2005 33:189.
31	Widdick DA, Dilks K, Chandra G, Bottrill A, Naldrett M, Pohlschröder M, Palmer T. (2006) The twin-arginine translocation pathway is a major route of protein export in <i>Streptomyces coelicolor</i> . Proc Natl Acad Sci U S A. 103: 17927-17932.
32	Miao V, Coëffet-Le Gal MF, Nguyen K, Brian P, Penn J, Whiting A, Steele J, Kau D, Martin S,

Reference Number	Publication
	Ford R, Gibson T, Bouchard M, Wrigley SK, Baltz RH. <i>Genetic engineering in Streptomyces roseosporus to produce hybrid lipopeptide antibiotics</i> . Chem Biol. 2006 13: 269-76.
33	Heide L, Gust B, Anderle C, Li SM. <i>Combinatorial biosynthesis, metabolic engineering and mutasynthesis for the generation of new aminocoumarin antibiotics</i> . Curr Top Med Chem. 2008 8: 667-679.
34	Manipulating and understanding antibiotic production in <i>Streptomyces coelicolor</i> A3(2) with decoy oligonucleotides. McArthur M, Bibb MJ. Proc Natl Acad Sci U S A. 2008 105: 1020-1025.
35	Bucca G, Laing E, Mersinias V, Allenby N, Hurd D, Holdstock J, Brenner V, Harrison M, Smith CP. <i>Development and application of versatile high density microarrays for genome-wide analysis of Streptomyces coelicolor: characterization of the HspR regulon</i> . Genome Biol. 2009: R5.
36	Boakes S, Cortés J, Appleyard AN, Rudd BA, Dawson MJ Mol Microbiol. <i>Organization of the genes encoding the biosynthesis of actagardine and engineering of a variant generation system</i> . 2009 72:1126-36.