Institution: University of Bristol

Unit of Assessment: 5 – Biological Sciences

Title of case study: Health, agriculture and industry benefit from Bristol's groundbreaking molecular toolkit

1. Summary of the impact

The Basidio Molecular Toolkit developed at the University of Bristol has enabled the pharmaceutical industry to achieve the efficient genetic manipulation of a group of basidiomycete fungi (mushrooms and toadstools) and thereby produce medically important antibiotics and proteins cost-effectively. For example, GlaxoSmithKline's collaboration with the Bristol team saved 70,000 hours of research and development in getting a natural antibiotic called pleuromutilin to market. In China, the system is used to produce medicinal anti-cancer proteins from fungi in commercially viable quantities. In addition, government agricultural research programmes in the US and Ireland have adopted the toolkit to increase the efficiency of their search for disease-resistant crops in the interests of farmers, consumers and economies.

2. Underpinning research

Key researchers and their contributions

Professor Gary Foster (Chair in Molecular Plant Pathology, Bristol 1996-present) and Dr Andy Bailey (Senior Lecturer, Bristol 1999-present), both in the School of Biological Sciences at the University of Bristol, developed the original concepts and experimental design for the project. They obtained funds, coordinated collaborations, authored papers and patents and supervised PhD students, postdocs and technicians. Dr Mike Challen, a researcher at Horticulture Research International (Warwick, UK), was a key collaborator on some aspects of experimental design and co-authored papers and co-supervised some PhD students on this project.

Nature of the research findings

In the late 1990s, few molecular tools were available to help study the fungal group Basidiomycota, despite their importance economically and ecologically. This was largely because conventional approaches did not work due to the complex genetic organisation in these fungi. Most basidiomycetes were difficult to transform genetically and it was almost impossible to achieve efficient expression of foreign genes. In 1998, Foster and his colleagues recognised that basidiomycetes were not being explored to their full potential due to the shortage of molecular manipulation techniques and decided to develop the tools necessary to transform and manipulate this group of fungi.

In 2005, Foster and Bailey published techniques that enabled the genetic manipulation of the commercially important button mushroom (*Agaricus bisporus*) and the model species (*Coprinopsis cinereus*) [1], allowing both the efficient expression of foreign genes and the switching off of native genes through gene-silencing. They produced a toolkit, called the Basidio Molecular Toolkit, which contained the necessary components to facilitate easy genetic modification and study of *A. bisporus* and *C. cinereus*, as well as other basidiomycetes. The breakthrough discoveries that led to the development of the Basidio Molecular Toolkit can be summarised as follows:

- Between 2006 and 2010, the Bristol team made vital advances in identifying, understanding and testing suitable foreign genes that could be deployed to select modified basidiomycetes [2-4]. They also created a range of ready-to-use DNA vehicles, with easily interchangeable components, that could carry the foreign DNA into the fungus, and made them available within the toolkit.

- Gene silencing: Many basidiomycetes have a complex genetic organisation that makes conventional gene-knockout unachievable for the analysis of gene expression and manipulation. Foster and Bailey and their colleagues demonstrated gene-silencing and generated a range of easy-to-apply approaches and vectors for the full spectrum of basidiomycetes, which were then made available within the Basidio Toolkit [3, 4].

- Foreign gene expression: Foreign gene expression or over-expression of native cDNA had been

unachievable in most basidiomycetes, despite significant research input. A detailed analysis of the location of introns within constructs and experimental testing led to the breakthrough discovery that an intron is required near or within the 5' region of any gene for efficient full expression [1-5].

With the toolkit developed, the research team forged collaborations with international partners to explore different species as well as new challenges in genetic manipulation.

3. References to the research

[1] Burns, C., *et al.* (2005) 'Efficient GFP expression in the mushrooms *Agaricus bisporus* and *Coprinus cinereus* requires introns', *Fungal Genetics and Biology* 42: 191-199. DOI: 10.1016/j.fgb.2004.11.005

[2] Burns, C., *et al*. (2006) 'Evaluation of Agrobacterium-mediated transformation of *Agaricus bisporus* using a range of promoters linked to hygromycin resistance', *Molecular Biotechnology*, 32: 129-138. DOI: 10.1385/MB:32:2:129

[3] Heneghan, M.N., *et al.* (2007) 'A comparison of methods for successful triggering of gene silencing in *Coprinus cinereus', Molecular Biotechnology* 35: 283-296. DOI: 10.1007/BF02686014

[4] Kilaru, S., Collins, C.M., Hartley, A.J., Bailey, A.M., Foster, G.D. (2009) 'Establishing molecular tools for genetic manipulation of the pleuromutilin producing fungus *Clitopilus passeckerianus', Applied and Environmental Microbiology,* 75 (22):7196-7204. DOI: 10.1128/AEM.01151-09

[5] Heneghan, M.N., *et al.* (2009) 'Characterisation of serine proteinase expression in *Agaricus bisporus* and *Coprinopsis cinerea* using GFP and the *A. bisporus* SPR1 promoter', *Applied and Environmental Microbiology,* 75 (3): 792 – 801. DOI: 10.1128/AEM.01897-08

[6] Baumgartner, K., Fujiyoshi, P., Foster, G.D., Bailey, A.M. (2010) '*Agrobacterium tumefaciens*-mediated transformation for investigation of somatic recombination in the fungal pathogen *Armillaria mellea', Applied and Environmental Microbiology,* 76 (24): 7990-7996. DOI: 10.1128/AEM.01049-10

[7] Morin, E., *et al*. (2012) 'Genome sequence of the button mushroom *Agaricus bisporus* reveals mechanisms governing adaptation to a humic-rich ecological niche', *PNAS,* 109 (43): 17501-17506. DOI: 10.1073/pnas.1206847109

Funding:

[8] Foster, Mills (1996-1999) *Development of transient expression vectors and viral resistance within the cultivated mushroom Agaricus bisporus,* BBSRC, £209,508.

[9] Foster, Mills (1997-1998) *Development of transient expression vectors in A. bisporus*, BBSRC, £3,200.

[10] Foster, Mills, Challen (1999-2002) *Transformation of homobasidiomycete fungi*, HRI-Ltd Industrial Support, £12,000.

[11] Foster, Bailey, Mills, Challen (2003-2006) *Agaricus-Verticillium interactions*, Defra, £450,000.

[12] Foster, Bailey (2003-2007) *Gene silencing and gene knockout in Agaricus bisporus,* BBSRC, £175,332.

[13] Foster, Bailey (2005-2009) *Manipulation of basidio metabolic pathways,* GSK, £638,283.

[14] Bailey, Foster (2007-2010) *Investigating the growth characteristics of fungi during large-scale growth for pharmaceutical production using an EST-Microarray approach,* GSK, £49,500.

[15] Foster, Bailey (2008-2009) *Manipulation of metabolic pathways,* Wellcome VIP, £18, 921.

[16] Foster, Bailey (2008-2009) *Manipulation of basidio metabolic pathways*, GSK Incentive Scheme, £35,000.

4. Details of the impact

Many compounds produced by basidiomycetes have already been recognised for their medical importance, but they are synthesised by the organisms in very small quantities. Previously, the inability to alter genetically and manipulate this important group in order to increase expression of desirable compounds made commercial exploitation economically unviable. However, the Basidio Molecular Toolkit, along with the underpinning research, has made the production of valuable pharmaceuticals practicable and opened up industry and government research areas that were previously too costly to develop.

Commercial benefits for the pharmaceutical industry

Between 2005 and 2012, Foster and Bailey and their team worked with the pharmaceutical company, GlaxoSmithKline (GSK), to exploit the molecular toolkit to work with the basidiomycete *Clitopilus passeckerianus.* This species produces a natural antibiotic called pleuromutilin, which is effective against multi-drug-resistant *Staphylococcus aureus* (MRSA). The research team successfully manipulated the genome of *C. passeckerianus* using two different techniques (Agrobacterium-mediated and PEG-mediated transformation) [4]. Using these methods, the partnership between Bristol and GSK, which established techniques for manipulating and increasing the expression of the natural antibiotic pleuromutilin in *Clitopilus passeckerianus*, resulted in a patent, filed internationally with GSK, in 2012 [a]. The collaboration with Bristol and the implementation of the Basidio Toolkit directly saved GSK over 70,000 hours of research and development [b]. GSK estimated that it "would have taken at least twelve full-time equivalents three years" and there would have been the additional "risk of developing tools that were not effective" [b]. The research made it possible to genetically manipulate production of pleuromutilin to generate derivatives and led to increased titres, which reduced production costs.

In addition, research conducted by Bristol [3, a] fed directly into information prepared for the regulatory approval phase of one of GSK's pleuromutilin derivatives, known as retapamulin. Armed with this information, GSK was able to submit it with confidence as a new class of fermented antibiotic [b]. This led to the development and commercialisation of the topical antibiotic, Altargo/Altabax, which was approved for use in the EU in 2007. This was the first new class of topical antibiotics approved by the US Food and Drug Administration in nearly two decades and is one of four anti-bacterials in GSK's product line, which had a combined turnover of £1.2 billion in 2012 [c].

In a similar case, in 2010, use of the Basidio Molecular Toolkit allowed production of Fungal Immuno-modulatory Proteins (FIPs) at commercially viable levels [d]. *Ganoderma lucidum* is a popular medicinal mushroom in traditional Chinese medicine. The anti-cancer effects of this and similar species have been well documented [e, for review] and are associated with FIPs, which are normally produced in very low quantities in the fungus. The use of FIPs could result in improved outcomes for cancer patients. The Basidio MolecularToolkit enabled the Institute of Bioresource and Bioactive Substance in China to "get maximum expression of the FIP", which will "allow full scaling up to industrial production" [d].

Government agricultural research adopts new processes

From 2007 to the present, Foster and Bailey used the Basidio Molecular Toolkit in their work with the US Department of Agriculture (USDA) to develop a genetic transformation system for the pathogenic basidiomycete fungus *Armillaria mellea*, commonly known as honey fungus [6]. This genetic transformation system has become part of the screening approach used by the USDA in developing crops that are resistant to root disease [e]. *Armillaria* is responsible for root disease in almond and walnut trees in California – two very significant crops for the state. California produces almost all the almonds and walnuts sold in the US and approximately 70% of these crops worldwide [e]. Root disease caused by *Armillaria* reduces the average life expectancy of these tree crops from 30 years to 10 years.

Ireland's Agriculture and Food Development Authority, Teagasc, established and adopted an Agrobacterium-mediated transformation of *Clitopilus passeckarianus.* It now serves as the "prerequisite control to assess the effectiveness" of all their gene-transfer technology, such as the development of disease-resistant crops [f]. "This simply would not [have been] possible without the Kilaru *et al.* [4] publication" [f].

Other commercial areas significantly advanced by the Basidio Molecular Toolkit

The tools and techniques developed by Foster and Bailey and their colleagues at Bristol have enabled full gene analysis of a number of basidiomycete species. From 2008 to 2012, Foster and Bailey collaborated with a group of international researchers to publish the full genome of the common button mushroom *Agaricus bisporus* [7]. This species is cultivated in more than 100 countries with an annual global production of over two million metric tons. The tools developed at Bristol helped demonstrate the functionality of enzymes important in the adaptation of *Agaricus* to

grow in a humic-rich leaf-litter environment [5], which significantly advanced the research programme [g]. It was estimated that the toolkit saved the equivalent of six scientists up to three years in research and development [g].

Sylvan Biosciences, the global leader in fungal technology that provides spawn and related products to the mushroom industry, was also part of the consortium that generated the full genomic sequence of *A. bisporus.* The tools developed at Bristol have had a "significant impact" on the "exploitation of this important group of organisms" [h].

5. Sources to corroborate the impact

[a] Bailey, A.M., *et al.* (inventors) '*Method of increasing yields of Pleuromutilins*', Patent: International Publication Number WO 2011/051820 A2. 5 May 2011 <http://patentscope.wipo.int/search/en/detail.jsf?docId=WO2011051820&recNum=54&docAn=IB2010003289&queryString=(PA/Glaxo)&maxRec=2191>

[b] Manager Biotechnology Development, GlaxoSmithKline

[c] GSK (2013) *Product portfolio* <http://www.gsk.com/investors/product-portfolio-pipeline.html>. Evidence of annual financial value of GSK's anti-bacterial product line.

[d] Director, Institute of Bioresource and Bioactive Substance

[e] USDA-ARS Research Plant Pathology, Department of Plant Pathology, UC Davis

[f] Senior Research Officer, Teagasc

[g] Head of the Lab of Excellence ARBRE and Research Director, French National Institute for Agricultural Research (INRA)

[h] Director of Research, Sylvan Biosciences, USA