Institution: Royal Holloway, University of London

Unit of Assessment: A5 Biological Sciences

Title of case study: The development of foods with enhanced carotenoid contents

1. Summary of the impact

Carotenoids (e.g. β–carotene, provitamin A) are antioxidants which are essential in the human diet and which reduce the onset of chronic diseases. Research in the unit on the carotenoid pathway has provided the tools and strategies to deliver foods with increased levels of nutritional carotenoids. This has led to the production of novel food supplements and to *Golden Rice* (GR), a humanitarian product aimed at alleviating Vitamin A deficiency in the developing world. Field and intervention trials have shown that GR is effective and its production feasible. The research has led to beneficial impacts on **health and welfare**, **international development**, **commerce**, **public understanding** and **education**.

2. Underpinning research

Following the commercialisation of GM crops resistant to herbicides in the 1990s, plant biotechnology companies turned to food crops with enhanced nutrient content, taste and flavour. The integrated crop management system adopted by Zeneca (now Syngenta) included the development of GM tomatoes and other crops with increased carotenoid levels. However, they lacked the in-house expertise to carry out the scientifically challenging research on antioxidants such as carotenoids and began long term collaboration with Prof Bramley, who had worked at Royal Holloway on carotenoid biosynthesis and its regulation since 1972 and produced the first GM tomato line with altered levels of carotenoids [1]. Zeneca was the industrial partner in 6 EU consortia for a decade from 1993, three of which were coordinated by Bramley, and which led to underpinning publications for the development of GR [2-5]. Zeneca funded Fraser (as research assistant since 1992, now a permanent member of staff), a PhD student and technician, to the value of over £550k between 1994 and 2000.

A key breakthrough in this research was the transformation of tomato with a bacterial gene (*crtI*), which produced not only the carotenoid lycopene, as predicted, but also β–carotene, thus minimising the number of transgenes required [3]. This was done by Bramley, together with Fraser and Roemer (PDRA 1993-1996), between 1997 and 2000 at Royal Holloway. This strategy also avoided the detrimental co-suppression of the endogenous tomato genes, a finding that had been reported by Bramley's lab earlier [5]. Their work has progressed throughout the reporting period to develop GM tomato fruit continuing zeaxanthin and ketocarotenoids, important in the alleviation of macular degeneration in the METAPRO programme.

The EU consortia coordinated by Bramley included Prof Beyer of Freiburg University, who aimed to address Vitamin A deficiency (VAD) by elevating the carotenoid levels in rice. VAD is a serious public health problem in many countries, including highly populated regions of Asia and Africa. It is estimated that 250 million pre-school children have VAD, of which an estimated 670,000 children die per year. The elevation, if in a sufficient quantity, would reduce the incidence of night-blindness, weakened immune system, xerophthalmia and bone growth deficiencies, which are typical symptoms of VAD. This can only be achieved by GM technologies, due to the lack of any rice cultivars naturally producing β-carotene in the grain which has no phytoene synthase (*Psy*), the first step of the pathway. It was thought that four different plant genes would be required to enable this pathway, a large genetic load for the plant. To reduce the genetic load, Beyer used the protocol from Bramley and Fraser's work on tomato, transforming rice with *crtI*, to yield *Golden Rice.* The introduction of *crtI* reduced this load significantly, thus avoiding loss of yield. Following the GR prototype version in 2000, newer varieties have been produced in the *indica* variety, which is the type eaten in developing countries. These varieties all contain the transgene *crtI* that was originally shown to be effective in work on GM tomato by the research of Bramley and Fraser and also a *Psy* from *Narcissus* that avoids co-suppression [1,3,6].

Further research, funded by the EU (projects COLORSPORE, METAPRO) identified the carotenoids responsible for the yellow/orange pigmentation present in bacilli. Potential biosynthetic

pathways for the formation of these apocarotenoids in vegetative cells, and spores have been reconstructed from intermediates [6] in 2010 by Fraser and Bramley together with Royal Holloway team members Perez Fons, and Kaneja (Research Assistants) and Cutting (Professor of Molecular Microbiology), all at Royal Holloway. These carotenoids are gastric stable, suitable as food supplements and a patent has been filed for their use as food supplements, probiotics, colourants, and sources of carotenoid and isoprenoid derived metabolites. The Bacillus strains HU58 and HU36 have been licensed, taken to production, and have been brought to the market.

3. References to the research

Peer reviewed papers

1. Fray, R.G., Wallace, W., Fraser, P.D., Valero, D., Hedden, P, Bramley, P.M. and Grierson, D. (1995) Constitutive expression of a fruit specific phytoene synthase gene in transgenic tomatoes causes dwarfism by redirecting metabolites from the gibberellin pathway. *Plant J*. 8, 693-701. doi: 10.1046/j.1365-313X.1995.08050693.x
2. Bramley, P.M. (1997) The regulation and genetic manipulation of carotenoid biosynthesis in tomato fruit. *Pure Appl. Chem*. 69, 2159-2162 doi: 10.1093/jxb/erf059
3. Romer, S., Fraser, P.D., Kiano, J.W., Shipton, C.A., Misawa, N, Schuch, W. and Bramley, P.M. (2000) Elevation of provitamin A content of transgenic tomato plants. *Nature Biotech*. 18, 666-669. doi:10.1038/76523
4. Fraser, P.D., Romer, S., Shipton, C.A., Mills, P.B., Kiano, J.W., Misawa, N., Drake, R.G., Schuch, W. and Bramley, P.M. (2002) Evaluation of transgenic tomato plants expressing an additional phytoene synthase in a fruit-specific manner. *Proc. Natl. Acad. Sci, USA* **99**, 1092-1097. doi: 10.1073/pnas.241374598
5. Fraser, P.D., Romer, S., Kiano, J.W., Shipton, C.A., Mills, P.B., Drake, R., Schuch, W. and Bramley, P M. (2001) Elevation of carotenoids in tomato by genetic manipulation. *J. Agr. Fd. Chem*. 81, 822-827. doi: 10.1002/jsfa.908
6. Perez-Fons, L; Steiger, S; Khaneja, R; Bramley, P.M; Cutting, S.M; Sandmann, G.; Fraser, P. D. (2011) Identification and the developmental formation of carotenoid pigments in the yellow/orange Bacillus spore-formers. *Biochimica et Biophysica Acta Molecular and Cell Biology of Lipids*, 1811, 177-185. doi: 10.1016/j.bbalip.2010.12.009

Patents filed

- Bacterial production of carotenoids, Inventors: Paul Fraser, Simon Cutting (2005, application nr GB0524873.7)

Grants awarded to Bramley for metabolic engineering of the carotenoid pathway

- Zeneca Agrochemicals/Syngenta, 1994-2000 £550k plus in kind benefits (equipment, glasshouse facilities).
- EU: 1993-7, B102-CT-93040, £ 200k
- EU: Project of technological priority, 1993-7, 200k euro
- EU: Carotene Plus CT96 1633; Mechanisms for the regulation of carotenoids in plants, 1994-8, 205k euro.
- EU: Functional engineering of plant carotenoids for added nutritional value, 1994-8, 250k euro
- EU: ProVitA, 2000-4, 250k euro.

Grants coordinated in the unit for metabolic engineering and production of secondary metabolites

- EU: METAPRO.The development of tools and effective strategies for the optimisation of useful secondary METAbolite PROduction in planta. 2010 -2013. £425,547 (http://www.isoprenoid.com/). Awarded to Fraser. EU consortium coordinated by Fraser
- EU: COLORSPORE. Utilisation of Bacillus spore formers for the production of probiotic natural colorants 2008 -2011 £827,489. (http://www4.inra.fr/cepia-eng/You-are-looking-

for/Projects/Europe/Colorspore). Awarded to Cutting and Fraser. EU consortium coordinated by Cutting

4. Details of the impact

The work of the Bramley and Fraser group and their *crtI* protocol provided an enabling technology and pivotal step in the development of *Golden Rice*, which was then used by Beyer and Potrykus in further development [7]. **Beneficiaries** of this research are those with Vitamin A deficient diets (globally approx. 200 million people), rice research institutes, notably the International Rice Research Institute (IRRI) and their end users, and the companies Syngenta, Viridis Biopharma and Anabio. Per year in excess of 20,000 'A' level students are taught about *Golden Rice* as part of their studies, and it has informed the general public in the debate about genetically modified organisms.

Impact on health and welfare: *Golden Rice* is a rice variety with enhanced provitamin A content that can improve the quality of life of hundreds of million people with a VAD, preventing hundreds of thousands of deaths per year. It is estimated that 190 million pre-school children and 19 million pregnant women are Vitamin A deficient. Field trials, in 2004 and 2005, yielded rice with 6µg β-carotene/g, which provides the normal daily amount needed in 70g of rice, while the typical intake is 100-200g/day [8]. Since 2008 bioavailability and bioconversion to retinol (Vitamin A) has been trialled. These trials demonstrated that β-carotene can be taken up from *Golden Rice* in a highly efficient manner [9,10].

Impact on production and international development: In 2008, the Rockefeller Foundation provided funding to a value of $4M to the IRRI for *Golden Rice* to be submitted to regulatory authorities in Bangladesh, Indonesia and the Philippines. Field testing has taken place in the Philippines and Bangladesh. In the Philippines the field trials have taken place to assess the performance and biosafety of Golden Rice. This assessment is part of the process to obtain regulatory approval for Golden Rice [11]. It is expected to be grown commercially in the Philippines in 2014 or 2015 and then introduced into the diet. The Bill and Melinda Gates Foundation have provided funding ($10.5M) for the IRRI and its partners (Philippine Rice Research Institute, Bangladesh Rice Research Institute) to further develop and evaluate *Golden Rice* varieties [12].

Impact on commerce. The on-going collaboration throughput the reporting period with Syngenta (formerly Zeneca) since the mid 1990s, with contract income and funded posts (amounting to an direct investment in excess of £300K), was instrumental in developing a strategy focusing on output rather than input characteristics, for example, nutritional characteristics of food crops. The expertise and technical skills on carotenoids provided by Bramley and Fraser, were essential for this aspect of Zeneca's plant biotechnology business. The expertise and skills were discussed and transferred in closed meetings of the EU consortia listed above, in which Syngenta was the industrial partner.

The discovery of and expertise on the biosynthetic pathways for the formation of bacterial apocarotenoids in vegetative and bacterial spores of *Bacillus* spp. has been commercialized through SporeGen, a commercial service governed by Royal Holloway Enterprise Ltd. SporeGen has a collection of carotenoid-producing *Bacilli* and most of these have been typed for safety and virulence markers. These can be developed as food and feed additives, as dietary supplements, or as food colorants [13]. Since 2011 two international companies, Viridis Biopharma (India) [14] and Anabio (Vietnam)[15], have acquired licences for the production of the carotenoid producing bacillus strains HU36 and HU58 as food supplements. These producers have commercialized these products, and they have invested in and developed production facilities and have started to market these. No sales figures have been provided as yet; the licence income to date is £30k.

Impact on education. GM crops and GM technology, including tomato and *Golden Rice* are included in the compulsory learning for students studying for both GCSE and A level OCR examinations meaning, for example, at A Level approximately 33% of UK A level students (June

2012 OCR figures indicate that this was approximately 20,500 students, [16]) learn about *Golden Rice* as a good example of basic science linked to recombinant DNA technologies to produce worthwhile, high value crops.

Impact on society. The development of *Golden Rice* has been an important component of the on-going global public debate around genetically modified crops [17], as regularly summarised by frequent articles in the press and news outlets from the 1990s to date. *Golden Rice* was heralded to be the first genetically modified crop with clear beneficial effects for farmers as well as consumers. Although the development of *Golden Rice* was covered by intellectual property rights, the developed varieties are largely covered by Humanitarian Use Licences, which allows distribution for free to those in need who cannot afford it, such as subsistence farmers in Asia or Africa. This has generated much publicity for *Golden Rice*, but also informed the debate for the use of GMO in general. Although the use of GMOs is far from being widely accepted, and has sparked off intense debate, the overall response to *Golden Rice* has been positive. This has had further impact on the use of GMO technology in the agricultural industry, in that it provided an example of a successful and acceptable application of a GMO crop (e.g. [18]).

5. Sources to corroborate the impact

7. *The contribution of research in the unit to the development of Golden Rice is corroborated by:* http://www.goldenrice.org/Content2-How/how1_sci.php
8. *Golden Rice Project website* (http://www.goldenrice.org/Content1-Who/who2_history.php), *corroborates the carotene yield of Golden Rice in field trials.*
9. Tang, G., Qin, J., Dolnikowski, G.D., Russell, R.M. and Grusak, M.A. (2009) Golden Rice is an effective source of vitamin A. American Journal of Clinical Nutrition 89:1776-1783. doi: 10.3945/ajcn.2008.27119. *This source reports the outcome of the clinical trial NCT00680355 (2008) "Bioavailability of Golden Rice carotenoids in humans".*
10. Tang, G., Hu, Y., Yin, W., Dallal, G.E., Grusak, M.A. and Russell, R.M. (2012) β-Carotene in Golden Rice is as good as β-carotene in oil at providing vitamin A to children. American Journal of Clinical Nutrition 96:658-664. doi: 10.3945/ajcn.111.030775. *Reports the outcome of the clinical trial NCT00680212 (2008) "Vitamin A equivalence of Plant Carotenoids in Children".*
11. http://www.irri.org/index.php?option=com_k2&view=item&id=12466:two-seasons-of-golden-rice-trials-in-camarines-sur-concluded&lang=en *corroborates the efforts at the International Rice Research Institute to develop and evaluate Golden Rice as a new rice variety.*
12. http://www.gatesfoundation.org/Media-Center/Press-Releases/2011/04/Nutritious-Rice-and-Cassava-Aim-to-Help-Millions-Fight-Malnutrition *corroborates the investment of the Bill and Melinda Gates foundation in Golden Rice development.*
13. http://www.sporegen.com/ *shows the commercial services and products offered by Sporegen*
14. http://viridisbiopharma.com/active_ingredients.htm#Probiotics *the section on "Probiotics" shows the licensed strains HU58 and HU36 that Viridis has brought to the market.*
15. http://www.blazelead.com/Anabio-Rd-Jsc/aboutus/ *shows that the strains HU58 and HU36 have been licensed to Anabio and brought to the market.*
16. http://www.ocr.org.uk/Images/16477-provisional-exam-statistics-june-2012.pdf *corroborates the claim that approximately 20500 A level students were taught about GM technology relating to Golden Rice and GM tomato.*
17. http://goldenrice2.weebly.com/main-actors.html *corroborates the extent of the arguments participants and arguments in the debate around Golden Rice.*
18. http://www.bbc.co.uk/news/science-environment-22967571 *corroborates the use of Golden Rice in the GM debate. The number of comments on this article given an indication of the extent of this debate.*