**Institution:** University of Nottingham

**Unit of Assessment:** 5 - School of Life Sciences

**Title of case study:**
*Industrial process enhancements: improved efficacy of weak acid anti-fungal preservatives used in foods and beverages, and other manufacturing improvements.*

## 1. Summary of the impact

Weak acids (e.g. sorbic acid) are used by food manufacturers to prevent fungal contamination of food and beverages. Professor Archer in the Molecular Microbiology group determined the fungal species that cause such contamination, and identified fungal genes and enzymes that confer resistance to sorbic acid during initial outgrowth of fungal spores. They characterised the biochemistry of the resistance mechanism, enabling design of improved mould inhibitors. These inhibitors, used at the correct time, have improved manufacturing processes to prevent mould contamination and product wastage. Knowledge of mould genetics has also been applied to other industries to improve food additive and biofuel manufacturing processes.

## 2. Underpinning research

This research is funded, 2005 to present, through two Defra/BBSRC Link projects[i, ii], a Knowledge Transfer Partnership[iii] and a BBSRC Industry Partnering Award[iv] to Professor David Archer. Dr Simon Avery is a co-investigator on some of those awards. The Nottingham staff members employed on the Defra/BBSRC projects are post-doctoral fellows: Drs Malcolm Stratford, Andrew Plumridge and Michaela Novodvorska and, on the KTP, Dr Rachel Osborn and Solomon Agyare.

The theme of the underpinning research is to understand and exploit fungal biology and biochemistry for benefit. There are two principal strands to the research: 1) exploiting fungal biology to suppress undesirable fungal contamination, and 2) to manipulate fungal biosynthesis to produce desirable metabolic products.

<u>Fungal Contamination</u>
Weak acids are used in the food industry as safe, palatable preservatives in foods and beverages. At the outset of this project, there was an established model for the mode of action of weak acid preservatives, derived from studies with bacteria and yeast. It was known that some moulds showed some resistance to sorbic acid but there was no knowledge of the mechanism involved. The most common species of moulds that contaminate foods and beverages were known, but there was very little knowledge of how species varied from factory to factory, how they varied with location or time of year, how their resistance to sorbic acid differed or even how they gained access to the factories. There were no strategies for improving the effectiveness of sorbic acid as a preservative. The research team debunked the classic theory of the mode of action of weak acid preservatives and provided a new mechanism[1]. The team identified the decarboxylation of sorbic acid in moulds as conferring resistance[2] and showed it is not a mechanism of resistance in yeasts[3]. They identified the genes that are necessary for resistance and showed how they are regulated[4]. The team mapped the active site of the encoded decarboxylation enzyme complex[5] and identified non-competitive inhibitors that may be useful in designing new anti-mould strategies. Importantly, they also showed that the resistance mechanism is only active during the very early stages of spore outgrowth[1], providing a new method to improve anti-mould treatments. The team conducted several surveys of moulds in factories and showed how the fungal spores gain access. That knowledge has led to changes in manufacturing practice in the main factory with which the group collaborates (GlaxoSmith-Kline) that are being rolled out worldwide.

<u>Fungal Biosynthesis</u>
Prof Archer's longstanding research, funded largely through the BBSRC, into protein secretion and stress in *Aspergillus* and *Pichia pastoris* resulted in genome annotations for secretion processes of several *Aspergillus* species and led to co-authorship on 3 separate papers in Nature (2005) and one in Nature Biotechnology (2007). The team undertook genetic modification of fungi to produce

essential fatty acids as nutritional additives[6], funded by the Dutch life sciences and material sciences company, DSM. Professor Archer also advises DSM and Adisseo (France) on the development of fungal strains that produce enzymes for use in biofuels processing and other applications. Those areas align directly with the university's BBSRC-funded LACE project, in the area of production of biofuels from lignocellulose, where Professor Archer is a Strand Leader, and he has held BBSRC funding in the REF period through the BioResearch Industry Club for studies of protein secretion and associated stresses. His expertise in fungal genomics and biotechnology has also led to contracts with a variety of other companies (e.g. Genencor, US; Novozymes BioPharma, UK and Roal, Finland) and he is also a member of the Scientific Advisory Board of the Kluyver Centre for Genetics of Industrial Microorganisms. Additional collaborators include Unilever and Mologic (a diagnostic devices company).

## 3. References to the research

Key Publications (Nottingham authors in bold, key author underlined)


1. **Stratford M**, **Plumridge A**, Nebe-von-Caron G, **Archer DB**. (2009) Inhibition of spoilage mould conidia by acetic acid and sorbic acid involves different modes of action, requiring modification of the classical weak-acid theory. International Journal of Food Microbiology 136, 37-43. doi: 10.1016/j.ijfoodmicro.2009.09.025
2. **Plumridge A**, **Stratford M**, **Lowe K**, **Archer DB**. (2008) The weak-acid preservative, sorbic acid, is decarboxylated and detoxified by a phenylacrylic acid decarboxylase, PadA1, in the spoilage mold *Aspergillus niger*. Applied and Environmental Microbiology 74, 550-552. doi: 10.1128/AEM.02105-07
3. **Stratford M**, **Plumridge A**, **Archer DB** (2007) Decarboxylation of sorbic acid by spoilage yeasts is associated with the PAD1 gene. Applied and Environmental Microbiology 73, 6534-6542. doi: 10.1128/AEM.01246-07
4. **Plumridge A**, **Melin P**, **Stratford M**, **Novodvorska M**, **Shunburne L**, **Dyer PS**, Roubos JA, Menke H, Stark J, Stam H, **Archer DB** (2010). The decarboxylation of the weak-acid preservative, sorbic acid, is encoded by linked genes in *Aspergillus spp*. Fungal Genetics and Biology. 47: 683-692. doi: 10.1016/j.fgb.2010.04.011.
5. MacKenzie DA, Wongwathanarat P, Carter AT, **Archer DB** (2000) Isolation and use of a homologous histone H4 promoter and a ribosomal DNA region in a transformation vector for the oil-producing fungus, *Mortierella alpina*. Applied and Environmental Microbiology 66: 4655-4661. doi: 10.1128/AEM.66.11.4655-4661.2000
6. Pel HJ, de Winde JH, **Archer DB**, **Dyer PS**, et al. (2007) Genome sequencing and analysis of the versatile cell factory *Aspergillus niger* CBS 513.88. Nature Biotechnology 25, 221-231. doi: 10.1038/nbt1282


Grants

i. **Archer DB**: New insights and applications in the prevention of food contamination by fungi. Defra/BBSRC Link (Food Quality and Safety, FQS69); 2005-2008; £605k.
ii. **Archer DB**: Reducing food waste due to contamination by fungi. BBSRC/Defra Link (Food Quality and Innovation, FQI28) BB/G016046/1; 2009-2012; £235k.
iii. **Archer DB**: Knowledge Transfer Partnership No 7681 with GSK; 2010-2012; £122k.
iv. **Archer DB**: Fungal spore germination, the critical stage in infection and food spoilage, and weak spot for new antifungal strategies. BBSRC Industrial Partnership Award BB/K001744/1; 2012-2015; £445k.

Patents

**Archer DB**, **Stratford M** and **Plumridge A**. (2008) Preservative. WO 2008/149102 A2.

## 4. Details of the impact

Impact has been generated as a consequence of the underpinning research in two principal areas:

1) improvements in food and beverage manufacturing processes to reduce product wastage and customer complaints at Glaxo SmithKline (GSK).
2) Improvements in production of nutritional supplements used in food manufacture at DSM.

**Impact 1: Industrial Process Benefits at GSK and beyond**

The underlying research has benefitted the manufacture of foods and beverages at GSK through a LINK project from 2009 – 2012 and a Knowledge Transfer Partnership (KTP) in 2010. The principal outcomes of the LINK and KTP projects were a better understanding of the sources, species and seasonal variations in fungal contamination entering the aseptic manufacturing production lines, methods to reduce fungal contamination, and improved, rational use of sorbic acid as an anti-fungal preservative in manufactured foodstuffs.

The LINK project at the Coleford site generated a large quantity of data on mould resistance to preservatives and an extensive photographic catalogue of mould isolates from ecological surveys[A], and was very influential in the way the KTP project was used to undertake ecological surveys of the aseptic manufacturing production lines.

The KTP project led to development of a growth medium for isolation of preservative resistant moulds. Initial screening using an antibiotic-based agar has been replaced by an acidified and sorbic acid supplemented agar that isolates higher numbers of preservative resistant species from the manufacturing environment[B]. The greater sensitivity of this improved monitoring method has been particularly useful in assessing the efficacy of the bottle cap treatment system[C].

Furthermore, passive air sampling using exposed agar plates identified problems with recovery of fungal isolates due to desiccation of the medium. A new filter paper capture technique was developed to use in aseptic areas with laminar air flow. This has led to changes in air sampling practices applied in manufacturing sites globally.

As a consequence of the LINK and KTP projects, a program of regular environmental monitoring was implemented at Coleford to take weekly samples across all aseptic manufacturing areas, accompanied by more localised daily screens. The mould isolates catalogue generated by the LINK project has been used as a valuable tool to aid in the identification of fungal species in these ongoing ecological screens at the manufacturing sites. Another consequence of this regular monitoring is that seasonal variation in contamination frequency has been identified, that also correlates with the number of samples returned by customers registering product complaints. The overall consequence of improvements in contamination monitoring during manufacture has been a reduction in customer complaints and product recalls[C].

Beyond GSK, the third party supplier of analytical services (Mologic, used to assess consistency of product formulation and resistance to fungal contamination[D]), has improved their challenge test design to detect the fungal species isolated and identified at GSK[C]. Furthermore, Mologic have investigated the impact of sorbic acid as a preservative on its interaction with [text removed from publication][D]. This identified why [text removed from publication] food products demonstrate spoilage with otherwise preservative-sensitive mould strains. Overall, this has improved the safety assessment of current products, and will help GSK to develop future, more robust, improved product formulations.

The outputs of these projects contributed significantly to change processes for environmental monitoring to reduce fungal contamination in the manufacturing facilities, for identifying fungal contamination in product samples returned by customers (and hence help identify the source of the contamination), and for quality control testing of materials obtained from third party suppliers. We anticipate further progress with the recently-awarded Industrial Partnership Award (2012 – 2015), funded through the BBSRC with input from GSK and DSM. Undoubtedly, the manufacturing improvements arising from this work and instigated at production sites worldwide, have contributed to the value of the £1.35billion sale of GSK's Ribena and Lucozade manufacturing business to Japanese company, Suntory, owners of the globally-recognised Orangina-Schwepps brands[E].

**Impact 2: Additional Industrial Benefits**

Prof Archer has also achieved significant impacts on other industrial processes utilising fungal genetics and biology. His group was the first to describe a method for genetic manipulation in the filamentous fungus, *Mortierella alpina*. This species is in industrial use to produce the essential fatty acid derivative, arachidonic acid, which is added as a nutritional supplement to baby milk to aid nervous system development and function. DSM is a world leader in the production of arachidonic acid[F]. In addition to the partnership with DSM through the LINK project[A], Prof Archer has acted as a scientific advisor to DSM to optimise their arachidonic acid production process and he advises DSM on enzyme production from fungi in relation to the generation of biofuels.

**5. Sources to corroborate the impact**

A. LINK project completion report.
B. KTP project final report.
C. Corroborative statement from a Senior Microbiologist, GlaxoSmithKline, Royal Forest Factory, Rock Lane, Coleford.
D. Corroborative statement from the CEO, Mologic Ltd, Bedford Technology Park.
E. http://www.bbc.co.uk/news/business-24013720
F. http://www.dsm.com/markets/foodandbeverages/en_US/products/nutritional-lipids/life-ara.html

Corroborative documents and copies of webpages are held on file and are available on request.