Impact Objectives

- Further develop the use of biocatalysts or enzymes derived from living organisms in the production of pharmaceutical drugs
- Reduce the cost of drug design and development (DDD) by streamlining the production of active pharmaceutical ingredients
- Ultimately streamline drug synthesis and development for the benefit of a range of diseases, using scalable, green biocatalytic processes

Enzymes from the salt of the Earth

__Professor Brendan Gilmore__ (Chair of Pharmaceutical Microbiology), __Stephen Kelly__ (BBSRC ICASE PhD researcher) at __Queen's University Belfast's__ School of Pharmacy, and __Professor Tom Moody__ of __Almac Sciences__, explain the need for their current research into novel enzymes from halophilic microorganisms and what catalyses their own successful collaboration


Prof Brendan Gilmore


Stephen Kelly


Prof Tom Moody

What are the main applications for transaminase biocatalysts?

SK: Transaminase enzymes are useful for the synthesis of chiral amine moieties, which are present in many small molecule active pharmaceutical ingredients (API) today.

Traditionally, these chiral amines have been synthesised using a myriad of technologies, including chemocatalysis and organocatalysis. More recently however, biocatalysis using transaminase enzymes has become a common process in the chemists' toolbox for undertaking chiral chemistry and the technology has been recently showcased in the Merck drug Sitagliptin.

Enzyme processes are now at the forefront of process design, scale-up and economics in this specific industry sector. In general, enzymes are finding more applications in the production of the intermediates that are needed in the production of final APIs.

However, transaminase use has been limited by drawbacks, including being able to thrive in a relatively small range of substrates, some of which are bulky, and their inability to function under certain challenging industrial conditions. This has driven the search for improved enzymes, either by protein engineering of existing catalysts or through novel enzyme discovery. The latter is the path on which we have embarked.

What prompted your interest in halophilic microorganisms as a source of useful transaminase enzymes?

BG: Our Queen's University Belfast research group had been part of a marine biodiscovery consortium, which originally sparked our interest in deriving bioactives from halotolerant microorganisms, including those living in deep-sea sediments. This field of enquiry facilitated a natural progression toward extreme halophiles and the isolation of organisms from Ireland's only functional salt mine at Kilroot.

The abundant and untapped potential of the Kilroot salt mine, and the ability of the organisms thus far sequenced to thrive in a wide range of conditions, has ensured that our focus remains firmly on halophilic microorganisms for the foreseeable future.

TM: Almac is interested in accessing novel transaminase enzymes with the potential to function under extreme conditions. The harsh environment of the Kilroot salt mine seemed a perfect place to look for microbe-derived enzymes with these abilities.

What types of drugs could contain APIs made with the use of your newly discovered enzymes?

SK: Up to 40 per cent of APIs contain an amine moiety. Thus far, transaminases have been utilised in the synthesis of many different intermediates that are necessary in the production of APIs.

One of the most successful uses of transaminases to date was in the production of the anti-diabetic drug Sitagliptin. In this case, a transaminase was used to remove nine synthetic chemical steps, as well as the need for high-pressure specialised hydrogenation equipment.

In what way will your biocatalysts perform better than existing catalysts?

BG: Enzymes have all the attributes to produce better processes – their use does away with the need for toxic metal catalysts and they are renewable and environmentally friendly. In addition to the carbon footprint reduction, the reduced expense associated with using biocatalysts also makes them desirable from an economic standpoint.

TM: These enzymes have the ability to carry out complex chemical conversions in a single step, circumventing the need for multiple reactions associated with conventional chemistry. Streamlining the manufacturing process has a favourable impact on the final yield obtained, thereby lowering the volume of materials that needs to be processed to deliver the final product.

Deriving durable biocatalysts from haloarchaea

*A four-year project entitled **Discovery of Transaminase Enzymes from Halophilic Microorganisms**, has the potential to streamline the synthesis of intermediates for the production of active pharmaceutical ingredients for a range of diseases*

A collaborative research project between Queen's University Belfast (QUB) and Almac Sciences, is aimed at reducing the cost of drug development. Professor Tom Moody of Almac, notes that: 'The main driver for the Discovery of Transaminase Enzymes from Halophilic Microorganisms project is to enable more economically viable processes for the production of chiral amines,' which are: 'valuable building blocks for the pharmaceutical industry.'

Professor Brendan Gilmore and Stephen Kelly from the School of Pharmacy at QUB, are building on a long-standing and productive association with Almac, which is a major player in the development and production of pharmaceutical ingredients and is also located in Northern Ireland. The four-year project, which began in late 2014, is funded by the UK's Biotechnology and Biological Sciences Research Council, and focuses on producing and testing enzymes derived from the ancient salt-loving microorganisms living deep underground in Ireland's only active salt mine in Kilroot.

CHIRAL AMINES

Many of the catalysts used to synthesise active ingredients – or their precursors – in medical pharmaceuticals have drawbacks, such as toxicity or a requirement for expensive equipment. On the other hand, biocatalysts, which are enzymes derived from living organisms, are endlessly renewable and are already helping to streamline the processes used in drug development.

A significant proportion of the active ingredients in pharmaceuticals and their precursor intermediaries, use precise asymmetric molecules called chiral amines. Stable chirality is important in pharmaceuticals since the mirror-image counterparts to symmetrical amines can perform quite differently in the human body. This was first highlighted in the case of the drug thalidomide, in which the amine counterpart caused major foetal abnormalities.

Chiral amines are traditionally synthesised using chemocatalysts and organocatalysts, however biocatalytic transaminase enzymes, which facilitate the transfer of an amino group to an acceptor ketone or aldehyde, are now being commonly used. At Almac, Moody explains that often: 'the resulting ketones and aldehydes require further functional group interchange,' making these catalysts most useful for the development of intermediate chemicals as an important step towards the final production of active pharmaceutical ingredients.

According to Moody: 'As well as being low cost, transaminase enzymes have the ability to carry out complex chemical conversions in a single step.' In addition, the resulting amines exhibit a high level of chirality, leading to a better yield of the required molecules when isolated.

Despite the increasing reliance on biocatalysts, there is a pressing need to extend the range of operating conditions in which the use of transaminase enzymes are viable. One option is to improve enzymes through protein engineering, however a potentially more promising route is to search for new enzymes in the realm of extremophiles. Extremophiles are a range of organisms that are able to thrive in extreme conditions, such as very cold or hot temperatures, or – in the case of halophilic organisms – in high salt concentrations.

HALOPHILIC MICROORGANISMS

The research team at QUB were first drawn to the pharmaceutical potential of halophilic organisms when taking part in a marine biodiscovery consortium. This included isolating bioactive components from organisms found living in deep-sea sediments. The nearest available source of similar organisms proved to be the active Kilroot salt mine, which represented a previously untapped source of halophilic extremophiles.

Halophilic microorganisms are a type of archaea, which are the oldest organisms ever found and which share many similarities with bacteria. However, there are a number of important differences between the two groups, which lend archaea their own unique biochemistry. Haloarchaea are often characterised by a red or purple pigment, which can sometimes colour shallow salt lagoons owing to their density. They can thrive in environments of between 10 and 37 per cent salt concentration, conditions that few other organisms could tolerate.

Gilmore is enthusiastic about the potential of these ancient haloarchaea to yield: 'biocatalysts capable of activities unlike anything else currently available.' Halophiles have adapted over millennia to live in high salt conditions and as Gilmore notes: 'This has caused them to develop many features which could make them suitable for industrial processes in which high concentrations of organic solvents are used.' This would be the case where substrates display poor aqueous solubility. He also notes that: 'Extreme halophiles can be adapted to more than one extreme condition at a time, often showing thermo-tolerant and alkali-tolerant behaviour, as well as their salt-loving characteristics.'

The research team has grown halophiles on a range of culture media, and used standard and enhanced isolation techniques to begin building a comprehensive catalogue of the various haloarchaea found in Kilroot. Once different organisms were isolated, their whole genomes were sequenced in order to develop a DNA database of halophilic genomes. Already a vast array of data has been assembled, such that metagenomic profiling has enabled the characterisation of the diversity of organisms present in the salt mine. These resources now represent an important data repository on which analysis and searches can be undertaken by researchers seeking specific biocatalyst functionality.

BIOCATALYTIC ENZYMES

The project has not been without its challenges and setbacks. Halophilic organisms have their own peculiar set of problems, including a lack of reference material on which to base desk research. As Kelly explains: 'The amount of available resources for halocarchaeal genetics is still somewhat lacking in comparison to its bacterial counterparts.' In addition, halophiles are slow growing in comparison to other organisms, particularly during initial isolation. Also, their need for salt conditions can inhibit the use of experimental protocols that have been designed for use with non-extremophiles.

"Enzymes have all the attributes to produce better processes – their use does away with the need for toxic metal catalysts and they are renewable and environmentally-friendly"

Despite these challenges, Gilmore says the team has now: 'Cloned and expressed a number of active halotolerant transaminase enzymes, which thrive on a broad diversity of substrates and are able to function well in a number of difficult conditions.' Normal mesophilic enzymes – those that tolerate moderate conditions – have been outperformed by their halophilic counterparts. Two major peer-reviewed papers have now been submitted for publication, which describe the team's findings.

The recent success of the anti-diabetic drug Sitagliptin has amply demonstrated the potential of transaminase enzyme catalysts to transform drug research and development. In this case, a transaminase was used to replace nine synthetic chemical processes and it removed the need for expensive hydrogenation equipment. In the future, enzymes isolated and tested by QUB and Almac could be used to speed up drug development for a whole range of debilitating diseases.

Transaminase enzymes are derived from the salt-loving microorganisms located deep underground in active salt mines, such as those in Kilroot, Northern Ireland

Project Insights

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Brendan Gilmore is Professor of Pharmaceutical Microbiology at Queen's University Belfast, School of Pharmacy, where he leads the Biofilm Research Group. His research is focused on the control and exploitation of microbial biofilms, including extremely halophilic microorganisms for the discovery of novel antibiotics, antimicrobials and biocatalytic enzymes.

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