NATURAL PRODUCTS

Discovery, engineering, and production of natural products

ISOMERASE

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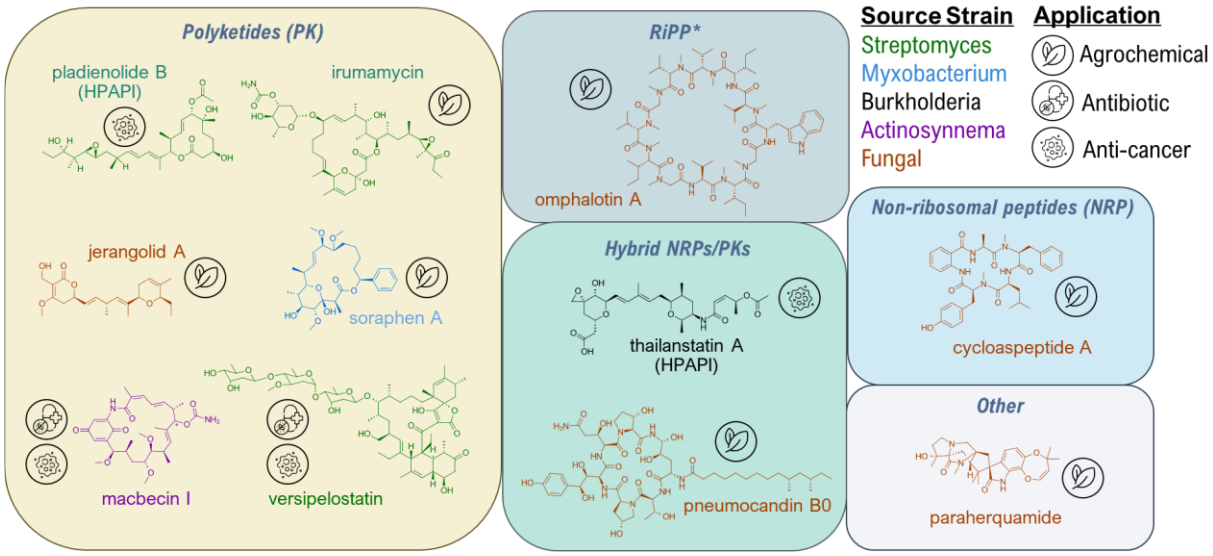
Natural products

Which natural products does Isomerase work on?

Natural product compounds occur in all microbes and are also known Genetically Encoded Molecules (GEMs) or secondary metabolites. Isomerase can and has worked on natural products from a wide range of bacteria, including actinomycetes, and fungi.

Microbial strains produce an impressive array of secondary metabolites, including polyketides, non-ribosomal peptides, terpenes, ribosomally synthesised and post-translationally modified peptides (RiPPs), lantibiotics, glycosylated products, nucleosides, β-lactams, butyrolactones, siderophores and melanins. These not only have a significant structural diversity, but also an enormous range of bioactivities. Illustrative examples of some of the wide variety of microbial natural product biosynthetic pathways Isomerase has worked with are shown below:



*Ribosomally synthesized and post-translationally modified peptide (RiPP)

Each box stands for a chemical class. The colour of each structure shows the source strain. The icon which industry the molecule has been shown to have an application in.

What problems does Isomerase help partners with?

Accessing rare and hard-to-source natural products and producing strains

We can search the Isomerase, patent and public strain databases for strains that produce the desired natural product (e.g. based on literature data), then set up scale appropriate USP, DSP and analytical methods to generate small quantities of the required product.

Supply natural products directly from our compound collection

We have already set up processes and isolated material for several natural products, such as pladienolide B (separate flyer with more information available upon request).

Generating and/or accessing analogues of a target natural product

Where a partner is interested in generating diversity around a target product, for example to generate new IP and/or products with improved properties (such as improved pharmacokinetics, stability or activity), we can use bioengineering and/or semisynthetic chemistry to generate and supply analogues to the partner or testing facility.

Genome or metagenome sequencing and analysis

Where a genome sequence of a microbial strain is not available, we can generate genomic or metagenomic DNA, use preferred suppliers for next-generation sequencing to generate raw data, then conduct assembly, annotation, and analysis in-house. This can be used to assess the biosynthetic pathways in a strain and is a common precursor to genetic manipulation of a strain.

Increasing the productivity of a strain that makes a desired natural product

We can carry out strain improvement using a variety of methods including classical mutagenesis and selection and targeted genetic manipulation with an aim to improve the titre of a producer strain.

Altering the productivity profile of a production strain reducing side products

We can reduce or remove impurities or side products from an existing natural product producer strain or process using targeted genetic manipulation of the producer strain.

Developing novel natural product-inspired warheads for antibody drug conjugates

We have ability in working with a range of natural product-based antibody-drug conjugate warheads. This can involve activities such as generating material for testing, pathway engineering, generating analogues, adding synthetic handles and production processes.

Generating robust processes to enable phase-appropriate or commercial manufacture of a natural product

A decade of expertise is applied to generating robust processes for natural product manufacturing within our specialised, dedicated bioprocess facility. We have tailored methodologies to ensure scalability and product integrity, enabling seamless transition from research to commercial production. With our commitment to quality, we drive innovation and accessibility in the natural product industry.

Technical transfer to scaled manufacturers

During the natural product process development program, we can generate all the technical documentation including technology books, quality reports, risk registers and ideal control strategies for mitigating failure modes and control of critical process parameters (CPPs) and critical material attributes (CMAs) in the process. We then work closely with the manufacturing facility teams to enable efficient process familiarisation and implementation for successful biomanufacturing.

What technologies and capabilities does Isomerase use?

Biology

Isomerase has developed a flexible and powerful platform for the bioengineering and optimisation of biosynthetic processes for production of known and novel microbially produced natural products. This includes:

Natural Product discovery and sourcing: We have accessed microbial natural products for partners from a variety of industrial sectors – including the production of compounds that are difficult to source or the isolation of novel natural products.	**Microbial strain husbandry:** We are skilled at working with both wild-type and genetically modified microbial strains producing a wide range of microbial natural products, including unicellular and filamentous fungi, and a wide variety of Gram-positive and Gram-negative bacteria.	**Pathway engineering:** We can build and optimise biosynthetic pathways in industrial strains to make a new related natural product or more of an existing product of interest. This engineering can increase productivity, remove impurities, or generate novel products.

***De novo* genetic manipulation methods**: Where established methods do not exist. We do this using selective markers, vector backbones, gene editing tools and transformation methods that can be changed and used in various strains.	***In silico* methods for identification and characterisation of biosynthetic pathways:** We have developed processes and software for generation and analysis of microbial genome sequences allowing assessment of the encoded biosynthesis.	**Biosynthetic enzyme engineering:** We have proprietary enzyme engineering technologies, specific for complex modular natural products, such as polyketides, non-ribosomal peptides and glycosylated products allowing us to make novel natural products.
Precursor-based methods for generating diversity: These methods include directed biosynthesis and mutasynthesis, whereby microbes incorporate synthetic small molecule analogues of building blocks with diverse structures into otherwise natural products.	**Strain improvement methods:** We have expertise in methods that improve the titre of natural products including selection and characterisation of high producing strains generated through rational engineering and random mutagenesis.	**Antibiotic activity testing:** We can run a range of antibacterial and antifungal assays using typical BSL1 or 2 indicator strains to support antibiotic discovery or development projects. .

Chemistry

Isomerase specialises in figuring out the chemical consequences of synthetic biology experimentation and developing robust processes to enable manufacture and purification of compounds produced or changed by microorganisms or enzymes.

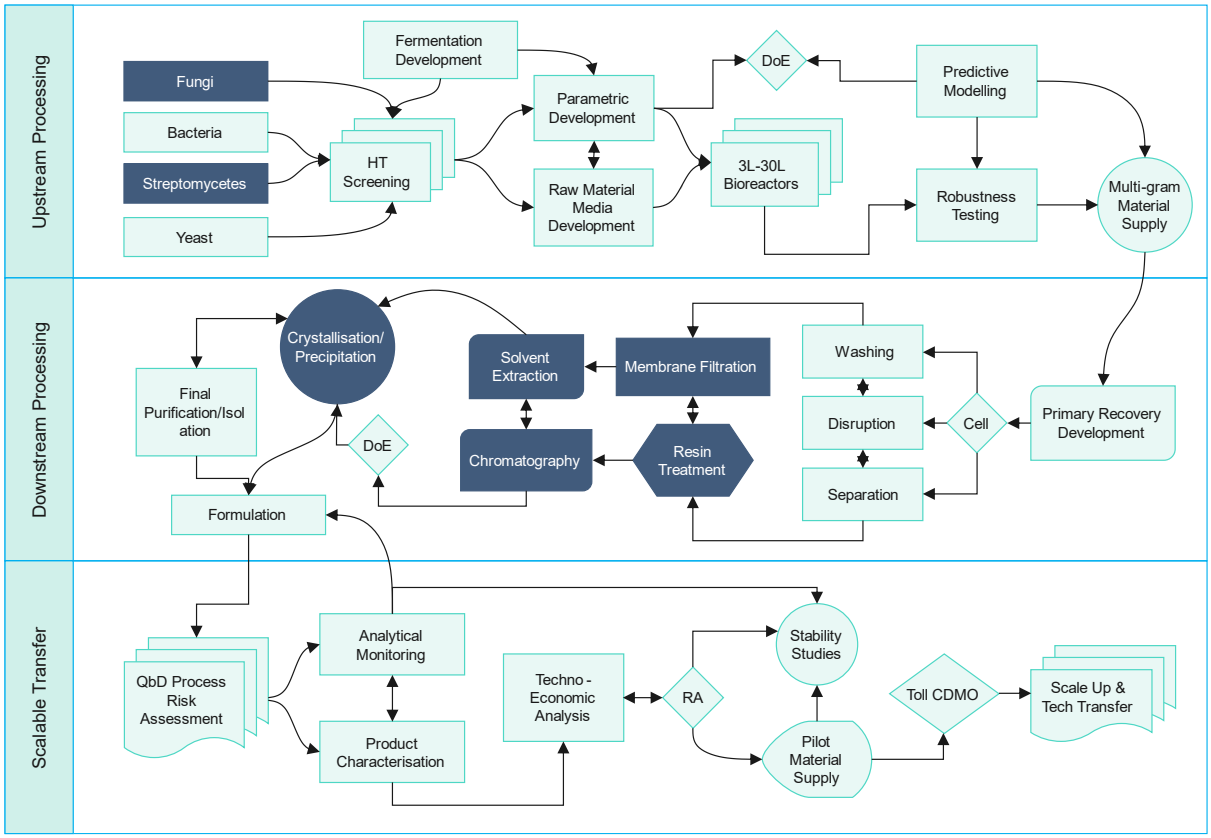
Assay Development: We rapidly devise and validate analytical methods to enable natural product discovery and development projects. These qualitative or quantitative methods could be HPLC, LCMS or spectrophotometric based.	**Preparative Chemistry:** We are skilled at purifying and characterising natural products generated from microbial sources. We adopt a phase appropriate approach to generate materials to enable discovery and support development.	**Organic Chemistry:** We can characterise compounds in great depth. This includes structure elucidation and related compound identification. Our biosynthetic and synthetic know-how enable access to added chemical space including semisynthesis or preparing metabolites for development projects.

Bioprocess

Our bioprocess development group collaborate constructively with the chemistry and synthetic biology groups to develop efficient, cost-effective methods producing biopharmaceuticals and bio-based products in a variety of sectors. It encompasses a wide range of activities, including fermentation optimisation, downstream processing, analytical monitoring, technology transfer, techno-economic modelling and using enhanced experimentation through quality by design principles to apply development tools to the higher risk technology areas, fast tracking progress and ensuring intensified processes are commercially ready. The bioprocess group has development equipment specifically

aligned to natural product fermentation and isolation, to ensure robust and efficient processes are generated.

USP development: We specialise in optimising fermentation methods, ensuring efficient cost-effective scalable growth with high yields. Trust us to innovate and streamline your biotechnological processes for enhanced productivity and success.	**DSP development:** We excel in developing platforms for primary recovery purification and product isolation, and activities are developed with robustness and scalability in mind for producing high-quality bio-products.	**Technology Transfer:** With the product in mind throughout our development platforms we build de-risked informative and detailed technical transfer dossiers to enable process commercialisation



* **CDMO** Contract Development Manufacturing Organisation, **DoE** Design of Experiment, **HT** High Throughput, **MF** Microfiltration, **QbD** Quality by Design, **RA** Risk Assessment, **UF** Ultrafiltration.

What advice can Isomerase provide as consultants?

Isomerase offers a range of consultancy services including the following.

Identifying natural products to pursue and designing research plans

Isomerase advises partners who have a target product profile in mind but want advice on what potential technical solutions are possible with natural products derived from microbes. These solutions can include candidate molecules, an outline plan of research to show suitability and analysis of the patent landscape for freedom to operate and patentability.

Techno-economic analysis (TEA)

Isomerase specialises in supplying comprehensive techno-economic analysis (TEA) for natural product manufacturing. Our knowledge of the specific process from the cell energetics to industrial production systems enables us to accurately model using commercial software ensuring optimal decision making

compliance needs. We excel in advising on CAPEX/OPEX needs or sourcing CDMOs that are tailored to our partners' specific processes.

Process Risk Assessment

Isomerase uses proprietary systems to apply a risk profile to ‘as is’ innovator processes and targeting the ‘to be’ process. Using quality by design (QbD) tools we define and characterise the product critical quality attributes (CQAs), perform extensive risk assessment and map all process parameters and materials to the quality specifications. We define what is critical to quality (CTQ) and generate bespoke control strategies to remove and reduce risk for our partner processes.

What proprietary innovations can Isomerase add to projects?

Methods and constructs for producing hybrid polyketides

Polyketide synthases (PKSs), the enzymes that assemble polyketides, can be thought of as being made up from an assembly line of modules, each extending the growing ketide chain with a specific monomer. The use of genetic hot spots for recombination between genes encoding PKS modules allows for replacement of one module with another with a high chance of successfully producing a functional analogue, opening the door to polyketide molecules with novel chemistries.

HiMASS

HiMASS is Isomerase’s **Hi**gh-throughput **M**icrotiter **A**nalytical **S**creening **S**ystem. This proprietary screening platform enables cultures at microscale to achieve consistent active fermentation conditions with high oxygen transfer and controlled fed batch fermentation. Using this platform, it is possible to screen hundreds of conditions producing milligram to gram per litre of product in liquid chemistry that correlates closely to stirred tank reactor environments. Our system can control the rate of microbial growth and find the ideal conditions for high titre and productivity.

What are representative examples of past projects?

CASE STUDY

PRODUCTION AND STRUCTURAL MODIFICATION OF NATURAL PRODUCTS THROUGH BIOLOGY

Request

To generate analogues of a known natural product polyketide, produced by a filamentous *Streptomyces* bacterium.

Our approach

Strain improvement and fermentation optimisation, including DSP development, boosted initial titres from 0.01 mg/L to partner’s target 1 g/L. The biosynthetic pathway was modified by targeted bioengineering, allowing production of related compounds by feeding various synthetic precursors. This process facilitated the integration of synthetic handles into the natural product, overcoming limitations of semisynthetic methods.

Value added

Starting with a native strain and an unoptimised process, Isomerase was able to improve both to sufficient productivity for initial development. Genetic manipulation tools were designed to generate isolates of novel analogues by direct fermentation, enabling biological and other assessment by the partner.

CASE STUDY

ENABLING THE MANUFACTURE FOR FIRST-IN-MAN STUDIES

Request

Partner discovered a novel microbial - derived compound for clinical studies, sourced from an atypical microbe. Faced challenges in scalability using stirred tank reactors and complex downstream purification.

Our approach

Isomerase created a risk register to prioritise challenges, leading to streamlined production optimisations in seed train and DSP, and effectively tackling fermentation challenges. Advanced to scale-up, identifying ideal CDMOs for GMP production. Led tech transfer, acting as key client liaison, ensuring seamless process flow.

Value added

The original request was to enable GMP production of a preclinical asset. Isomerase triaged the most critical issues to detail a phase appropriate solution for partners to enable them to take their project forwards.

CASE STUDY

SOURCING MICROBIAL STRAINS AND USING THEM TO PRODUCE ANTIBIOTICS

Request

Leading biotech requested 1-5 mg of 10-20 different microbial antibiotics, previously documented but not commercially available.

Our approach

Source microbial strains from internal and external collections and develop methods for their fermentation, analysis, and isolation within a 2-month timeframe.

Value added

Leverage our expertise in cultivating challenging bacteria to rapidly develop effective production and isolation protocols, enhancing process's efficiency.

CASE STUDY

RAPID INACTIVATION OF MULTIPLE BIOSYNTHETIC CLUSTERS

Request

Multiple deletions of various different types of secondary metabolite clusters from an actinomycete strain was required by Partner.

Our approach

We were rapidly able to develop a transformation protocol and customise in-house bioengineering tools to be able to successfully manipulate the strain and perform the required knockouts. The manipulated strains were analysed for their new production profiles by chemical analysis, and further confirmed by genomic sequencing.

Value added

Executing the desired knockouts swiftly, with both chemical and bioinformatic analysis of the resulting strains. Flexibility throughout project to change targets when required, as driven by new data.

Ways to contact Isomerase

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