BIOCATALYTIC ENZYMES

Discovery of biocatalytic enzymes and their production in E.coli and Pichia

ISOMERASE

Biocatalytic enzymes

Which biocatalytic enzymes does Isomerase work on?

Isomerase supports the discovery and production of biocatalytic enzymes from a wide variety of sources in microbes. All living things use enzymes to catalyse specific biochemical processes linked to their metabolism. Enzymes can have high specificity and activity, whilst being relatively easy to remove and having a low environmental impact through biodegradability. Enzymes are also enantioselective catalysts, which can either separate enantiomers or diastereoisomers from complex chiral mixtures or synthesise chiral compounds from achiral substrates. These properties allow commercial enzyme-assisted processes to run under milder reaction conditions, with improved yields and reduced waste generation compared to processes reliant on other types of catalysts.

Isomerase is expert in "omics" technologies (e.g. genomics, metagenomics, and proteomics), efficient expression systems, and emerging recombinant DNA techniques that together facilitate the discovery of new microbial enzymes either from nature or by creating, engineering (or evolving) enzymes with improved catalytic properties. Due to the wide range of environments microbes can survive in, some microbial enzymes evolved unusual thermotolerance, and tolerance to other environment extremes such as solvent or a wide range of pH and other harsh reaction conditions compared to enzymes from other sources. Isomerase uses technologies like EvoSelect® to help design enzymes that benefit from this hardiness.

Microorganisms are the primary source of enzyme manufacture because they can be cultured in massive quantities in a brief time, on relatively inexpensive media. Isomerase has bioengineered both *E. coli* and *Pichi*a as platforms for enzyme production. We also have in-house cell free protein synthesis (CFPS) methods we can use for rapid or high-throughput protein synthesis.

Characteristics	*E. coli*	*Pichia*	CHO cell
Offered by Isomerase	Yes	Yes	No
Doubling time	30 min	60-120 min	24 hr
Cost of growth medium	Low	Low	High
Complexity of growth medium	Minimum	Minimum	Complex
Expression level	High	Low to high	Low to moderate
Extracellular expression	Secretion to periplasm	Secretion to medium	Secretion to medium
Protein folding	Refolding usually needed	Refolding may be needed	Proper folding
N-linked glycosylation	None	High mannose	Complex
O-linked glycosylation	No	Yes	Yes
Phosphorylation & acetylation	No	Yes	Yes
Drawback	Accumulation of LPS	Codon bias	Contamination with animal viruses

Adapted from Karbalaei *et al.* (2019) J Cell Physiol. 2020; 235: 5867-5881

What problems do we help partners with?

Identification of genes encoding enzymes from microbes with enzymatic activity

We can use existing or generate new genome sequences of microbes expressing an activity of interest, then use bioinformatic analysis to predict gene sequences which may code for this activity. These target enzymes can then be expressed production strains (e.g. in *E. coli* or *Pichia*) and assessed for the desired activity.

Producing and characterising enzymes designed *in silico* or reported in scientific literature

We can generate expression strains for a target enzyme, develop initial USP and DSP processes and assays to assess activity, or replicate existing assays and conduct initial enzyme characterisation.

Finding candidate enzymes to catalyse a given reaction

Based on a target biocatalytic reaction, we can conduct literature assessment of potential reactions and screen enzyme collections to find candidate enzymes.

Using rational design to develop novel enzymes

We can use rational design, for example based on existing crystal structures, to propose targeted enzyme changes leading to improved or altered properties then test these proposals by generating and assessing the enzyme at lab scale.

Use directed evolution to alter the properties of enzymes

We can use proprietary *in silico* methods, such as EvoSelect® to support directed evolution studies aimed at altering properties of an enzyme, including improving thermal stability or changing the substrate or product of a biocatalysis.

Generate IP-free or proprietary enzymes to avoid existing patents or commercial licenses

In tandem with thorough freedom to operate (FTO) searches, we can use EvoSelect® and other methods to support generation of novel enzyme sequences with similar or improved activity to a known enzyme sequence where there are limitations for a partner due to patent claims or commercial licenses.

Transferring flask production of a partner's enzyme to fermenters and material supply

We can develop methods to transfer flask production of a partner's enzyme into bioreactors. We have multiple vessels up to 30L, suitable for method development and material of gram to kilogram scale for partner's use.

Generating robust processes to enable phase-appropriate or commercial manufacture of a biocatalytic enzyme

To ensure we generate the right production process of a chemical or enzymatic reactive property, we employ an enhanced process development paradigm. This integrates know-how, prioritising the 'end goal in mind', quality, high-throughput screening, design of experiment and statistical control, to ensure we are developing grams to kilograms of catalyst, which is highly active, stable, and economic in its application. We can produce kilogram quantities of enzyme in-house or larger quantities at our partner enzyme manufacturers.

What technologies and capabilities do we use?

Biology

***Pichia* bioengineering tools**: Fast and robust engineering tools for *Komagataella phaffii* ("*Pichia*") including rapid engineering tools, secretion and purification tags, vector systems for rapid high-level production.	**Proprietary *Pichia* strains:** We have developed proprietary *Pichia* strains, including: (1) Mut^+ and Mut^S - useful for tuning expression in methanol-induced systems (2) Auxotrophic mutants - enabling increased antibiotic-free plasmid retention and (3) Protease knockouts - reducing breakdown of desired protein products).	**Proprietary *E. coli* strains:** Isomerase has invested in the development of proprietary strains, vectors and processes based on industrial strain BL21.

Rational design:
Rational methods relying on the availability of structural information and can involve targeted changes to the enzyme sequence based on analysis of this data and potentially computational modelling.

***In silico*-assisted directed evolution:**
These methods require iterative generation of libraries of variant enzymes followed by assessment of the desired properties. The selection of the enzyme sequences in these libraries can be guided by EvoSelect®.

Cell-free protein synthesis
Rapid cell-free production of proteins using a range of different in-house CFPS systems.

Chemistry

Assay Development:
We rapidly devise and confirm analytical methods to enable biocatalysis discovery and applied biocatalysis development projects. These qualitative or quantitative methods could be HPLC, LCMS or spectrophotometric based.

Preparative Biocatalysis:
We are skilled at generating and characterising compounds via biocatalysis. For instance, generating drug metabolites for assay development.

Applied Biocatalysis:
We can readily optimise chemical transformations with a variety of different biocatalysts. This includes enzyme & substrate loading, solvent & temperature selection, and product purification studies.

Bioprocess

USP development:
We have robust drop in solutions for enzyme manufacture. The processes are fine tuned for your biocatalyst of choice. The fermentation methods ensure efficient cost-effective scalable growth with high yields.

DSP development:
We have drop in robust DSP platforms. The processes are fine tuned for your biocatalyst of choice. Isomerase excels in developing platforms for primary recovery purification and product isolation, and activities are developed with robustness and scalability in mind for producing high-quality biocatalysts.

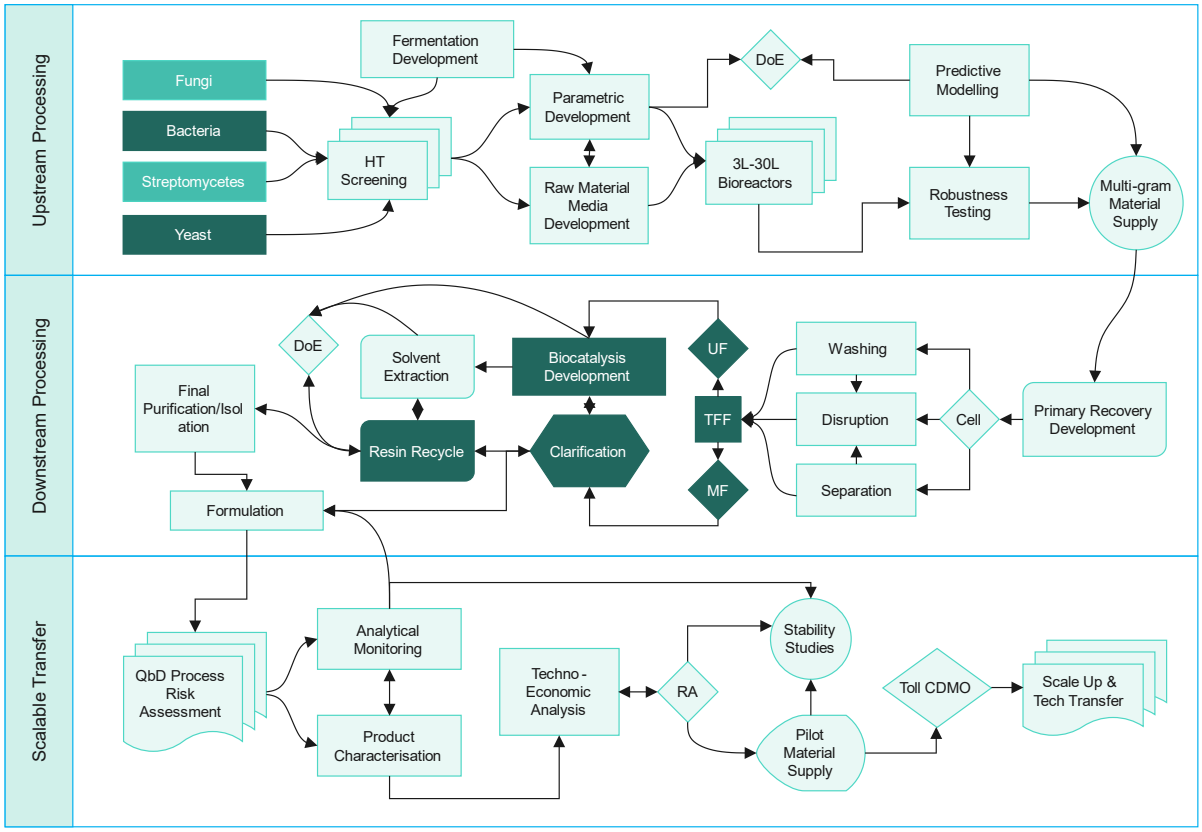
Material supply: Supply of milligram, gram, or kilogram quantities of lyophilised enzyme from existing or newly developed bioprocesses using lab-scale fermentation up to 30L fermenters and DSP we can take your process through pilot scale to get the quantities you need.

Optimise strain performance:
For any given strain we can screen hundreds of conditions representing stirred tank reactor environments to find the ideal conditions for high titre and activity. Then monitor the impact of strain improvement.

Cell banks: Having optimised strains we can assist with, produce, and qualify master cell banks (MCBs) and working cell banks (WCBs) for manufacturing campaigns.

Scalable Transfer:
With the product specifications and commercial constraints in mind throughout our development platforms we build de-risked informative and detailed technical transfer dossiers to enable ensure process commercialisation.

Isomerase has an experienced bioprocess development team who collaborate constructively with the chemistry and synthetic biology teams 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 uses 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. We have innovative techniques like our HiMASS (High-throughput Microtiter Analytical Screening System) platform which generates representative predictive models for screening enzyme technologies quickly and efficiently. We can supply material at gram to kilogram scale for supporting research programs.



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

What advice can Isomerase provide as consultants?

Isomerase offers a range of consultancy services including the following.

Project planning

Isomerase advises partners who have a biocatalytic process or target enzyme class in mind but want advice on what potential technical solutions are possible.

Identification of candidate enzymes

We can advise synthesis and process chemistry teams on biotechnological solutions to their chemistry problems. It may be replacing a chemistry step with a biocatalytic step, or it might be suggesting a route change if that is a better solution. Isomerase can advise on starting points for candidate enzyme screening.

Freedom to Operate (FTO) analysis

Isomerase can assess the patent and non-patent literature around a particular target process or enzyme and propose processes or enzyme sequences for testing that can avoid patent claims or have the potential to be patentable or proprietary.

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 leading commercial software ensuring optimal decision making and compliance needs. We excel in advising on CAPEX/OPEX needs or sourcing CDMOs that are tailored to our partners' specific processes.

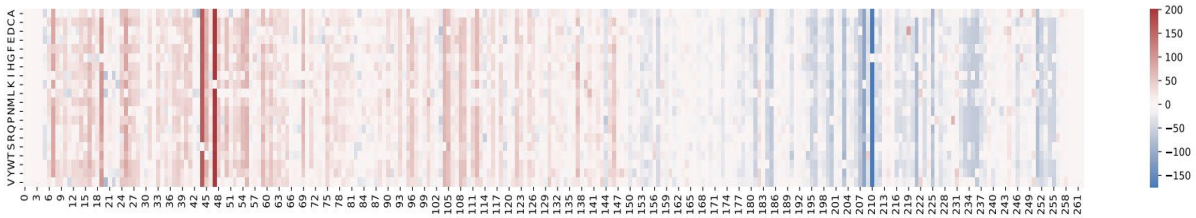
Process Risk Assessment

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?

EvoSelect®

EvoSelect® is Isomerase’s proprietary software that uses evolutionary information contained within both public and proprietary enzyme sequence databases to improve directed evolution studies, such as those directed at improving the properties or changing the substrate or product of an enzyme, reducing the cost and time taken to complete a study. It can also be used to support generation of IP-free or novel protein sequences with similar activity to a patent-protected sequence, but outside existing claims.



A sample output from EvoSelect®. The x-axis shows positions along a protein, y-axis amino acid variants at a given position, and the colour shows the beneficial (red) or detrimental (blue) impact.

Isomerase’s use of EvoSelect® in a partner project does not require the Partner to take a license to EvoSelect® but they still own intellectual property in protein sequences generated from its use on their behalf.

HiMASS

HiMASS is Isomerase’s High-throughput Microtiter Analytical Screening System. This proprietary screening platform enabling cultures at microscale to achieve high cell densities using a controlled fed batch fermentation. Using this platform, it is possible to screen hundreds of conditions producing grams per litre of product in liquid chemistry that mimics correlates closely to stirred tank reactor environments. Our clever system can control the rate of microbial growth and find the ideal conditions for high titre and productivity.

Pichia strains

Isomerase has developed fast and robust engineering tools for *Pichia* and has generated a growing collection of proprietary *Pichia* strains, including *Mut*$^{+}$ and *Mut*^{S} (useful for tuning expression in methanol-induced systems), auxotrophic mutants (enabling increased antibiotic-free plasmid retention)

and protease knockouts (reducing breakdown of desired protein products) and methods for methanol-free production.

Isomerase's use of our proprietary *Pichia* strains in a Partner project to produce protein for a Partner, does not require the Partner to take a license if the deliverable is the protein produced, but would require a product-specific license, considering the amount the Partner has already spent with Isomerase, if the deliverable was Isomerase's *Pichia* strain engineered to produce their protein. Licenses to Isomerase's *Pichia* strains from companies not otherwise working with Isomerase are available on commercial terms.

What are representative examples of past projects?

CASE STUDY

LABORATORY-BASED VALIDATION OF LITERATURE OR *IN SILICO* HYPOTHESIS

Request

Partner requested the establishment of a biocatalytic process, based on literature evidence that suggests potential enzyme activity within various filamentous fungi.

Our approach

Isomerase obtained strains from its own and public collections, establishing protocols for analytical work, fermentation, and biotransformation. This confirmed the targeted biocatalytic activities in the strains. Due to the strains producing a variety of products from multiple enzyme activities, Isomerase sequenced and analysed genomes from selected strains to pinpoint genes responsible for the desired biotransformation. A list of potential fungal genes was synthesised and expressed in *Pichia pastoris*, with each strain evaluated for the specific enzyme activity. One strain demonstrated the correct biotransformation, becoming the lead candidate for further development and scale-up.

Value added

Isomerase was able to use a combination of protein expression and assay development, bioinformatics, genomics and understanding of microbial metabolism to identify genes encoding for enzymes with valuable activities. Isomerase successfully generated expression strains and processes for further development of biocatalytic processes.

CASE STUDY

LOWER COST OF GOODS THROUGH PROCESS IMPROVEMENT

Request

To improve the upstream and downstream processing for production of a biocatalytic enzyme in *E. coli* BL21 (DE3) for use in generating a peptidic product.

Our approach

Isomerase enhanced enzyme production using Design of Experiments (DoE) for process optimisation and added stabilizers, extending the shelf life of the enzyme. Due to cost of goods (CoG) reasons, the partner preferred minimal processing, resulting in minimal DSP which led to degradation of peptidic product due to a peptidase in BL21 crude lysate. Through systematic testing, Isomerase identified and knocked out the problematic peptidase gene, stabilising the product without compromising growth rates after optimising the process through further USP DoE. The improved method, ensuring product stability with reduced processing costs, was efficiently transferred to the partner for scale-up at a selected CDMO.

Value added

The initial task was to enhance a production process using a partner's strain. Isomerase not only achieved this but also identified and addressed another process issue together with the partner, employing a newly engineered strain that subsequently became an additional asset for the partner.

CASE STUDY

IDENTIFICATION, EVALUATION, AND SCALE UP OF ENZYMES WITH DESIRED ACTIVITY

Request

Partner required enzyme sourced to perform specific biocatalytic activity, initially for Proof of Concept, and then followed by scaleup for an accessible Cost of Goods process.

Our approach

Isomerase used bioinformatic approaches to analyse potential candidate enzymes capable of performing the desired activity, shortlisting 20 of the most likely options. These candidate sequences were adapted for expression in various chassis organisms and their activities screened by chemical analysis. After confirming the correct activity in one of the candidate enzymes, the process was further optimised for scale up in *Pichia*, from which a working freeze-dried lysate could be obtained to satisfy the partner's onward requirements.

Value added

Ability to identify enzyme capable of the desired biocatalysis, express successfully in host systems, proving activity via chemical analysis, with subsequent scale up for practical product for partner.

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