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Iterative metagenome mining leads to expanded substrate scope of the LEH for biocatalytic epoxide opening

AUTHORS

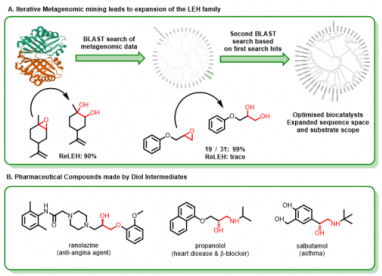
Bethany HOGG / UNIVERSITY OF MANCHESTER, MANCHESTER INSTITUTE OF BIOTECHNOLOGY, MANCHESTER
Emily KEMPA / ASTRAZENECA, CHARTER WAY, MACCLESFIELD, CHESHIRE
Charlotte MORRILL / UNIVERSITY OF MANCHESTER, MANCHESTER INSTITUTE OF BIOTECHNOLOGY, 131 PRINCESS STREET, APARTMENT 510, MANCHESTER
Rhys BARKER / UNIVERSITY OF MANCHESTER, MANCHESTER INSTITUTE OF BIOTECHNOLOGY, 131 PRINCESS STREET, APARTMENT 510, MANCHESTER
James FINNIGAN / PROZOMIX LTD, WEST END INDUSTRIAL ESTATE, HALTWHISTLE
Martin HAYES / ASTRAZENECA, PEPPAREDSLEDEN 1, GOTHENBURG
Christian SCHNEPEL / KTH ROYAL INSTITUTE OF TECHNOLOGY, BRINELLVÄGEN 8, STOCKHOLM
Corresponding author : Nicholas TURNER / nicholas.turner@manchester.ac.uk

PURPOSE OF THE ABSTRACT

Recently, the use of metagenome mining to discover novel enzymes has become of increasing interest in biocatalysis. The process has been integral in accelerating the field through identification of enzymes for specific molecule synthesis, particularly drug target synthesis, and exploring sequence space. Interrogating genomic data from environmental samples and comparing these against known, previously characterised sequences through a bioinformatics platform has previously allowed for screening and characterisation of a 384-panel of imine reductases, thus offering a broad screening tool for the synthesis of chiral amines. More recently, this method is being applied to find novelty in well-studied enzyme classes, in the early stage functionalisation of high value intermediates.

In this work, we describe the discovery of more than 50 novel limonene epoxide hydrolase (LEH) enzymes from interrogating metagenomic data, adding to this small subset of the epoxide hydrolase (EH) family. Furthermore, we discovered that some candidates demonstrated high activity towards a vastly different substrate scope and displayed good tolerance for more sterically hindered epoxides. These ‘hit' enzymes introduce novelty to a well-studied but poorly classified enzyme class. Computational studies rationalised the high activity of two both LEHs in particular, which prompted structural analysis by X-ray crystallography revealing substrate and product binding modes which are currently harnessed for further enzyme engineering. A second search of the metagenomic data using the most active LEHs from the first panel as reference points generated a second panel of homologs, which possessed more active and enantioselective candidates for the aforementioned substrate scope. This shows that iterative metagenome mining can be used to walk along protein sequence space and be a powerful, complementary tool in directed evolution and biocatalysis.

FIGURES



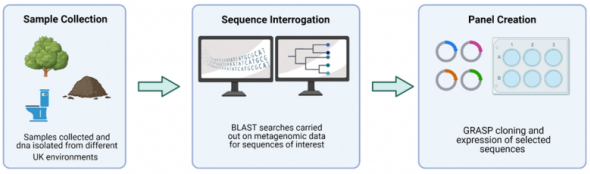


FIGURE 1

Figure 1

Iterative metagenome mining leads to expanded enzymes families and novel biocatalysts.

FIGURE 2

Figure 2

Flowchart showing metagenomic panel creation from the initial round of mining metagenomic data.

KEYWORDS

metagenome mining | biocatalysis | LEH | Epoxide Openeing

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