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Exploration of metagenomic sequence space for novel specificities from the PEP-dependent N-acetylneuraminic acid synthase protein family

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PURPOSE OF THE ABSTRACT

Carboligases are one of the most desirable enzyme classes in organic synthesis since they form carbon-carbon bonds. PEP lyases (or synthases) are of particular interest as their mechanism of action involves the cleavage of phosphoenolpyruvate with subsequent release of phosphate, thus making their reactions irreversible given the strong driving force for product formation. There are three categories or PEP lyases, (i) N-acetylneuraminic acid synthases (sialic acid synthases), (ii) 2-keto-3-deoxyoetosonic acid 8-phosphate synthases (KDO synthases) and (iii) 3-deoxy-D-arabino-heptulosonic acid 7-phosphate synthases (DAHP synthases)[1].

This project is focused on the ubiquitous, yet largely uncharacterised, sialic acid synthases, where to date research has been limited to genetic, metabolic, biochemical and/or structural studies[2–5]. Thus the fundamental and commercial potential of additional novel specificities harboured by this family are as yet unknown. Towards investigating that end, a screening panel of 112 novel / maximum diversity members of this family was designed through metagenomic mining. All targets were selected using N-acetylneuraminic acid synthase (NeuB) from Neisseria meningitidis (PDB code 1XUZ) as reference sequence, and evenly cover at the phylogenetic level the whole protein family. All target sequences were mined from Prozomix Limited's proprietary metagenomic libraries using "ProzomiGo" software. Preliminary biochemical screening experiments with 58 soluble recombinant products have indicated novel specificities indeed reside within this family and are currently being confirmed.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

metagenomics | biocatalysis | enzyme discovery | novel activity

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