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Discovery of novel split transketolases for application in biocatalysis

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PURPOSE OF THE ABSTRACT

Biocatalysis represents today a major resource in the industrial sector for several reasons, including the possibility of implementing more sustainable chemical processes, the high stereoselectivity of enzymes, and their potential to be modified through protein engineering for new biocatalytic reactivities. Transketolases (TKs) are thiamine pyrophosphate dependent enzymes with huge potential in the field of industrial biocatalysis, due to their ability to catalyse the transfer of carbon units in a stereoselective manner. The most well-known and characterised transketolases are encoded as proteins of around 650 amino acids. However, in many organisms from the archaeal and bacterial domains transketolases consists, instead, of two proteins of around 300 amino acids, aligning one with the N-terminal half of full-length TKs, and the other with their C-terminal half. For this reason, these enzymes are also named as ‘split-gene' transketolases (or split TKs).

Up to now only one example of split TK has been biochemically and structurally characterised [1]. The reconstituted biosynthetic pathway for the production of a phosphonate with herbicidal properties has been found to involve a split TK homologue [2]. Understanding characteristics and peculiarities of split TKs could be beneficial to explore their applicability in biocatalysis and possibly discover novel reactivities, as well as to gain insights about their structure-function relationship, in comparison to full-length transketolases.

In this project genome and metagenome mining techniques were applied to broaden the panel of split transketolases available for investigation and use in biocatalysis. Thus far, 70 putative split TKs were retrieved both from (meta)genomic online repositories and from 4 different in-house metagenomes. These were assembled into a phylogenetic tree to investigate their spread across archaeal and bacterial domains and their relatedness to full-length transketolases. From a first selected group, 11 putative split TKs were cloned and expressed in Escherichia coli in soluble form. The preliminary characterisation of some of these biocatalysts, including the reaction with glycolaldehyde and lithium hydroxypyruvate for the production of erythrulose and their temperature optimum, allowed the identification of interesting hits to be further explored in the future and exploited for application in biocatalysis.

FIGURES

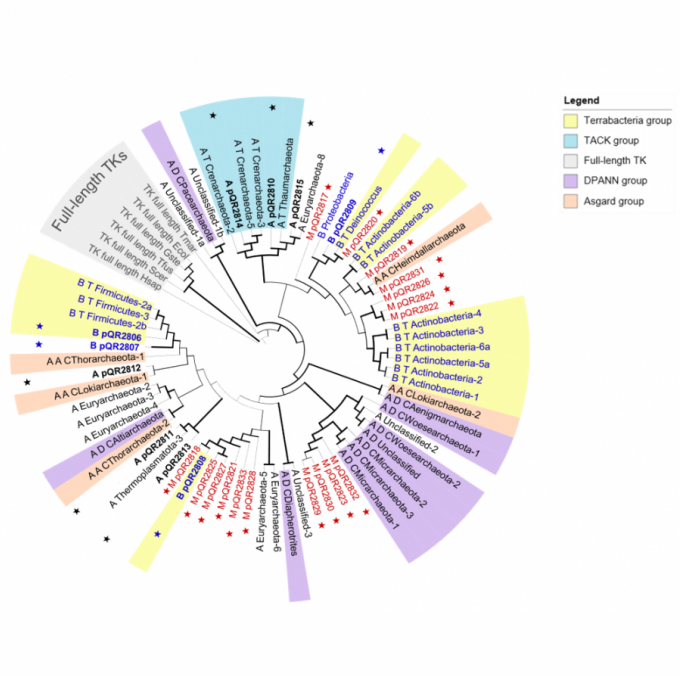

FIGURE 1

Phylogenetic tree including 76 sequences of different transketolases, mainly split transketolases.

FIGURE 2

KEYWORDS

biocatalysis | transketolase | metagenomics

BIBLIOGRAPHY

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