Thermostable Isomerase Processes for Biotechnology

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The TIPs project (Thermophilic Isomerase Processes for Biotechnology; ERA-IB-16-049) is an ERA-IB-2 (Industrial Biotechnology for Europe: an integrated approach) project that focuses on the identification and characterization of novel thermostable isomerases from thermophilic genomes and metagenomes and their biotechnological applications (Table 1).

Introduction

Isomerases are molecules with identical atomic composition but with different structural and/or functional characteristics. Isomerases are the enzymes catalyzing the conversion between different isomeric forms of a variety of molecules (Figure 1). Thermostable isomerases have the potential to withstand harsh industrial process conditions, including heating and exposure to organic solvents. Their use at elevated temperatures can enhance substrate accessibility and solubility.

Description and goals

Three types of isomerases have been targeted:

- Sugar isomerases
- Disulfide isomerases
- Chalcone isomerases

Several thermophilic sugar isomerases, e.g. L-Lyxose isomerase, have been identified and characterised. A selection of thermophilic disulfide isomerases has been over-expressed in *Escherichia coli* and are being characterised. Chalcone isomerases from three distinct groups (Archaea, Proteobacteria and Firmicutes) are being studied. The X-ray structure of a thermophilic Firmicutes chalcone isomerase has been determined. These newly identified thermostable isomerases provide new enzymes to add to the industrial biocatalytic ‘tool box’.

Approaches

TIPs includes comparative bioinformatic analyses of sequence data to identify different classes of thermostable isomerases of industrial interest. Both sequence similarity networks (SSN) and genome neighborhood networks (GNN) allow protein sequences to be gathered into clusters with a single function (Figure 2). Further phylogenetic analysis of these isofunctional clusters will help to select the most promising candidates (Figure 3).

Selected enzyme candidates resulting from data mining are being codon-optimized and recombinantly produced in a multiple vector system for high-throughput expression using multiple expression hosts (See poster No. P65). Purified enzymes are functionally and structurally characterized (Figure 4) and further optimized towards their biotechnological application.

Table 1. Three types of isomerases will be targeted under TIPs. Thermostable isomerases are desired because they possess high resistance and durability.

<table>
<thead>
<tr>
<th>Target enzyme</th>
<th>EC number</th>
<th>Application</th>
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<tbody>
<tr>
<td>Sugar isomerase</td>
<td>5.1.3.-, 5.3.1.-</td>
<td>Production of new sugars as building blocks</td>
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<tr>
<td>Disulfide isomerase</td>
<td>5.3.4.-</td>
<td>Improve protein folding and stability</td>
</tr>
<tr>
<td>Chalcone isomerase</td>
<td>5.5.1.6</td>
<td>Production of secondary metabolites</td>
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</tbody>
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Figure 1. Schematic representation of different isomers. Isomerases catalyse the transformation between different isomers.

Figure 2. Sequence similarity network (SSN) analysis of protein disulfide isomerases (InterPro IPR033954). Clustering was obtained with an alignment score of 20.

Figure 3. Phylogenetic relationships of bacterial and archaeal putative chalcone isomerases retrieved from genome databases indicating the three major lineages.

Figure 4. Examples of molecular structures for the three types of isomerases targeted in TIPs. From left to right: - Arabinoose isomerase from *Escherichia* (2HXG), - Protein disulfide isomerase from *Salmonella* (3L8S), and - Chalcone isomerase from *Eubacterium* (4C9S).