Thermostable Isomerase Processes for Biotechnology

Juan M. Gonzalez¹, Nils-Kåre Birkeland², Antonio García-Moyano³, Peter Schönheit³

¹ Institute of Natural Resources and Agrobiology, Spanish Council for Research, IRNAS-CSIC, Sevilla, Spain
² Department of Biology, University of Bergen, Norway
³ Christian Albrechts-University of Kiel, Germany

Introduction
Isomers are molecules with identical atomic composition but with different structural and/or functional characteristics and isomerases are the enzymes catalyzing the conversion between different types of isomers (Figure 1). The TIPs project (Thermophilic Isomerase Processes for Biotechnology) focuses on the provision of novel thermostable isomerases from thermophilic microorganisms and metagenomes and their biotechnological applications (Table 1).

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).

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>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar isomerase</td>
<td>5.1.3.-</td>
<td>Production of new sugars as building blocks</td>
</tr>
<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>
</tr>
</tbody>
</table>

Cherry-picked enzyme candidates will be cloned and recombinantly produced. Expression bias will be minimized with codon-optimized synthetic genes, a multiple vector system for high-throughput expression analysis as well as several streamlined expression hosts (Figure 4). Purified enzymes will then be functionally and structurally characterized (Figure 5) and further optimized towards their biotechnological application.

Acknowledgements
TIPs (ERA-IB-16-049) is funded through the ERA-IB2, 7th joint call, Industrial Biotechnology for Europe: an integrated approach. Partners receive funding from their own countries: Germany (Bundesministerium für Bildung und Forschung), Norway (Forskningsrådet) and Spain (PCIN-2016-129; Ministry of Economy, Industry and Competitiveness).