NEW GLUCOSE ISOMERASE – FIT FOR BIOREFINERY CHALLENGE

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Biofuel is not the bio-product with the highest value-addition, especially with the currently low oil price in mind. Modern concepts of biorefineries therefore tend to include production of more valuable products than ethanol, such as bioplastics. Hydroxymethyl furfural (HMF) is considered to be a central platform chemical for biomaterials production. HMF can be produced from hexose sugars, however the conversion is much more efficient ketoses ketoses such fructose than with aldehydes (glucose, mannose and so on). Glucose is the only sufficiently abandoned sugar monomer to potentially become a row material for commodity chemicals manufacturing, wood being the most likely source of glucose. Glucose can be then enzymatically converted to its ketose isomer – fructose using xylose (glucose) isomerase. The glucose isomerases are widely commercially available: they are one of the largest in volume in the industrial enzyme market for their production of widely-used High Fructose Syrups (HFS) for food applications. However, the currently available commercial enzymes are highly sensitive to the substrate sugar purity, which is well acceptable in food industry application. Typically, even sugar produced from starch requires activated carbon filtration, ion exchange chromatography and degasification before it can proceed to isomerization reaction. Sugars produced in 2nd generation biorefinery (especially from wood) have much more impurities than starch derived sugar, including lignin, extractives, etc, and required level of purity is not justified for the technical sugar. Taking this challenge, we set to develop and industrial glucose isomerase that can work directly in lignocellulosic biomass hydrolysate.

To address the choice of enzyme prototypes covering most structurally diverse groups, we obtained a custom made 3dm database (ordered from Bio-Prodict BV, Netherlands). The database contained around 25 000 protein sequences from public databases aligned and uniformly numbered based on structural alignment or strong homology. Database, where annotation of proteins was not taken in account while building it, eventually contained xylose isomerases L-rhamnose isomerases, hydropiruvate isomerase, innosose isomerases, D-tagatose epimerases, L-ribulose-phosphate epimerases, mannonate dehydratases and endonucleases.
This tool gave a general view on structural diversity of known characterized and just annotated xylose isomerase, and helped to find a representative pool of prototypes to test for our special requirements. Among over 20 tested candidates, we found a new extremely robust enzyme, which outperformed every reference enzyme in glucose isomerization in crude lignocellulosic hydrolysate.

The broad scope of proteins represented in 3dm database allowed unprecedented opportunity to analyze the proteins sharing the same fold in terms of what makes them functionally distinct. Focusing our attention on xylose isomerases, we were able to identify several positions, which make a protein with such fold a xylose isomerase. Some of those positions have never been mentioned in the literature as mutational hot spots or as residues essential for the function. We constructed focused libraries with variation in these positions and were able to find enzyme variants with strongly altered substrate preferences between glucose and fructose and enabled doubling the efficiency of glucose isomerization by the new enzyme. The enzyme production in E.coli was scaled up to industrial scale.

Thus using bioinformatics approach combined with protein engineering, we developed an industrial enzyme that enables sugar valorization and platform chemicals production in biorefinery streamline.
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