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Valorization of agricultural waste streams by adapting established enzyme cascades using high-throughput screening systems.

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PURPOSE OF THE ABSTRACT

The utilization of agricultural waste streams as potential feedstock for enzyme cascades in biocatalytic processes has gained significant attention in recent years. These residual streams are typically low-cost or even waste products of agricultural processes and represent an abundant and renewable source of carbon as well as other nutrients that can be utilized in enzyme cascades to yield a variety of valuable products. These cascades involve the sequential action of multiple enzymes to convert various substrates into desired products and offer several advantages over traditional methods of chemical catalysis. In previous studies, our group has established enzyme cascades for the production of various commodity and platform chemicals such as ethanol, isobutanol, butanediol or α-ketoglutarate from glucose or other monosaccharides [1-3]. The aim of the study presented here is to add specific enzyme-catalysed reaction steps to these core cascades in order to incorporate e.g. lactose obtained from agricultural waste streams. To achieve this, enzymes like galactosidase for the hydrolysis of lactose to D-glucose (D-Glu) and D-Galactose (D-Gal) need to be engineered for high catalytic efficiency in the reaction environment of the substrate containing waste streams (salt and substrate concentrations). Microfluidic droplet screening has emerged as a powerful tool for high-throughput screening of enzyme libraries, allowing rapid identification of enzymes with improved catalytic properties for the target application, such as cascade activity, selectivity and stability. In previous studies, our group has successfully applied absorbance and fluorescence-activated droplet sorting systems to improve the catalytic properties of different enzymes [4]. By modifying and optimizing assay setups (Figure 1) and the technical environment of these systems, the enzymes of the extended cascades can be adapted to the above-mentioned reaction conditions using directed evolution techniques.

FIGURES

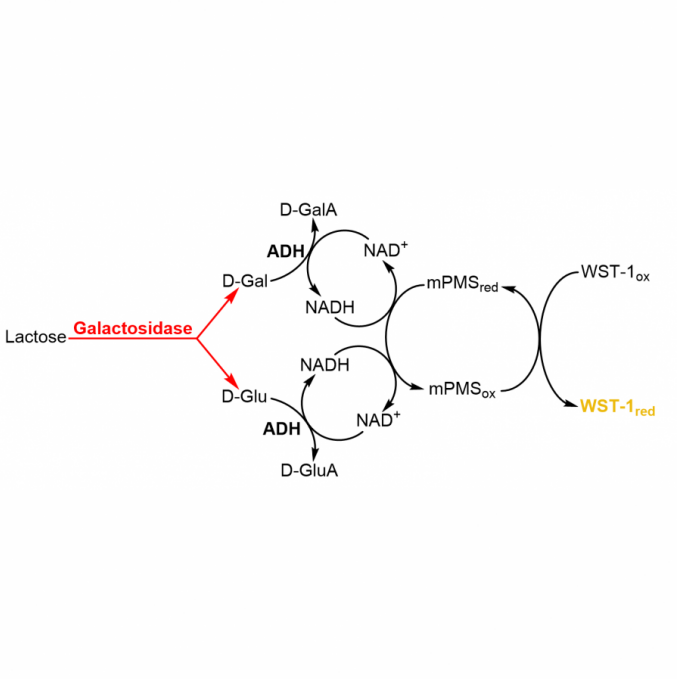

FIGURE 1

Assay scheme for droplet-based galactosidase screening.

Figure 1: Assay scheme for droplet-based galactosidase screening. D-Glu and D-Gal are oxidized to the respective sugar acid by alcohol dehydrogenase, NADH is reducing WST-1 to the absorbant formazan form via the electron mediator mPMS.

FIGURE 2

KEYWORDS

Galactosidase | Microfluidics | EnzymeEngineering | EnzymeCascade

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