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Cell-Free Production of α-Ketoglutarate from D-Galacturonic Acid via a Multi-Enzymatic Cascade

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PURPOSE OF THE ABSTRACT

Recently, D-glucuronate was converted to α-ketoglutarate (aKG) via a multi-enzymatic cascade using five enzymes, namely UDH, D-glucarate dehydratase (GlucD), KdgD, αKgsaDH and NOX. In this context, aKG represents an important chemical building block.[1] In this study, we wanted to use D-galacturonic acid as substrate for the enzymatic production of aKG (Scheme 1). D-galacturonic acid is the main component of pectin, which is present in most primary cell walls.[2] The utilization of biogenic raw materials like uronic acids for the production of base chemicals is of crucial importance to develop a bio-based economy.

The conversion of D-galacturonate to aKG required the identification of a D-galactarate dehydratase (GalcD) to convert the intermediate galactarate to 5-keto-4-deoxyglucarate. Therefore, galactarate dehydratases from Actinobacillus succinogenes 130Z (As), Escherichia coli (Ec), Salmonella typhimurium (St) and Sodalis ligni (Sl) were selected via a data bank serach. The dehydratases were produced in E. coli with N-terminal His6-tag. All enzymes displayed activity toward D-galactarate. However, the EcGalcD and the AsGalcD were only active in the presence of 10-50 mM DTT and thus not suitable for the cascade. StGalcD and SlGalcD were active without the addition of a reducing agent and were applied in the cascade. With the StGalCD 20 mM (80%) aKG were obtained from 25 mM D-galacturonate.

In the future, the efficiency of the cascade will be improved by combining process engineering and enzyme engineering approaches. Furthermore, the addition of a GlucD to the cascade may allow the simultaneous conversion of galacturonate and glucuronate to aKG in one cascade.

FIGURES

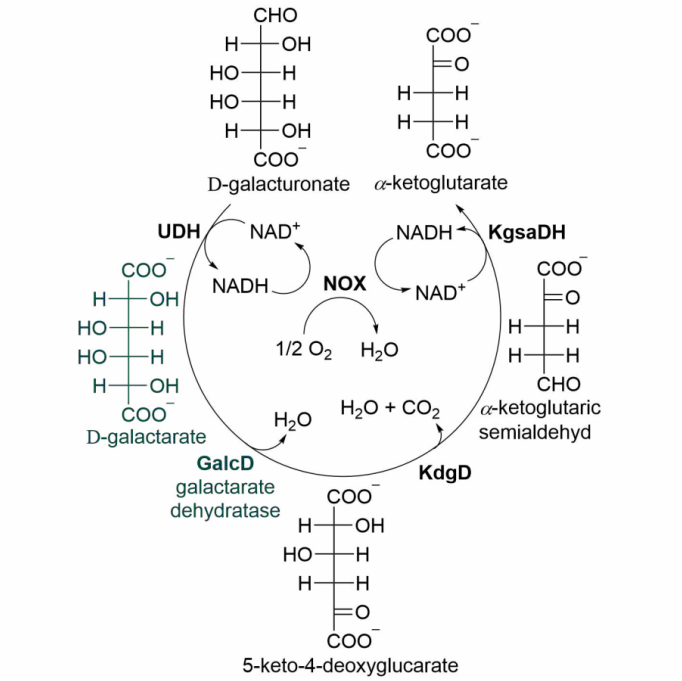

FIGURE 1

Scheme 1.

Multi-enzymatic conversion of D-galacturonate to aKG. UDH: uronate dehydrogenase; GalcD: galactarate dehydratase; KdgD: 5-keto-4-deoxyglucarate dehydratase; KgsaDH: a-ketoglutaric semialdehyde dehydrogenase; NOX: NADH oxidase.

FIGURE 2

KEYWORDS

Enzymatic Cascade | Biomass | Dehydratases | Biocatlysis with Oxygen

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