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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation

Enzymatic Production of Rare 6-Deoxyhexoses from L-Rhamnose and L-Fucose and Characterization of Rare Sugar Producing Enzymes
（L-ラムノースおよびL-フコースからの希少６－デオキシヘキソースの酵素的生産とそれらを生産する酵素の性質に関する研究）

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Enzymatic reaction is a potentially environmentally friendly procedure for production of rare sugars. Interestingly, rare sugar producing enzymes generally have broad substrate specificity which one enzyme is capable to catalyze various substrates containing similar configuration. This study was conducted to elucidate that rare sugar producing enzymes, especially aldose isomerases and ketose 3-epimerase (KE)-family enzymes, which their native substrates are common monosaccharides are able to catalyze the transformation of 6-deoxyhexoses. These enzymes were utilized in production of seven rare 6-deoxyhexoses from commercially available substrates, L-rhamnose and L-fucose. Furthermore, a newly discovered D-allulose 3-epimerase from *Shinella* sp. NN-6 (SDAE), which participated in the production of rare 6-deoxyhexoses, was purified and characterized for a prospective application in a wide-ranging production of rare sugars.

Three rare 6-deoxyhexoses, including 6-deoxy-L-glucose, 6-deoxy-L-altrose, and 6-deoxy-L-allose, were produced from L-rhamnose *via* the intermediate, 6-deoxy-L-allulose. The reactions were catalyzed by means of L-rhamnose isomerase from *Pseudomonas stutzeri* LL172 (PsLRhI), D-tagatose 3-epimerse from *Pseudomonas cichorii* ST-24 (PcDTE), D-arabinose isomerase from *Bacillus pallidus* 14a (BpDAI), L-arabinose isomerse from *Enterobacter aerogenes* IK7 (EaLAI), and L-ribose isomerase from *Acinetobacter calcoaceticus* LR7C (AcLRI). These microbial biocatalysts were used in two difference forms, toluene-treated form (TT-enzyme) and immobilized form (IE-enzyme). L-Rhamnose was converted into L-rhamnulose and 6-deoxy-L-allulose in one-pot reaction of TT-PsLRhI and TT-PcDTE with an equilibrium ratio of 50:35:15. The intermediate 6-deoxy-L-allulose was separated and further epimerized into L-rhamnulose by IE-PcDTE with an equilibrium ratio of 33:67. Pure L-rhamnulose was subsequently transformed into 6-deoxy-L-glucose by TT-BpDAI reactivity which exhibited an equilibrium ratio of 27:73. In contrast, purified 6-deoxy-L-allulose was individually isomerized into 6-deoxy-L-altrose and 6-deoxy-L-allose by means of TT-EaLAI and TT-AcLRI, respectively. Equilibrium ratios between 6-deoxy-L-allulose and 6-deoxy-L-altrose, and between 6-deoxy-L-allulose and 6-deoxy-L-allose were found at 60:40 and 40:60. On the basis of L-rhamnose utilized throughout the process, pure 6-deoxy-L-glucose, 6-deoxy-L-altrose, and 6-deoxy-L-allose were obtained at production yields of 0.9%, 2.3%, and 4.0%, respectively.

Following the similar methodology, another four rare 6-deoxyhexoses,

6-deoxy-L-talose, 6-deoxy-L-sorbose, 6-deoxy-L-gulose, and 6-deoxy-L-idose, were produced from L-fucose by IE-BpDAI, IE-PsLRhI, TT-SDAE, TT-AcLRI, and a commercial D-glucose isomerase (DGI) reactivities. 6-Deoxy-L-talose was directly biosynthesized from L-fucose in one-pot reaction of IE-BpDAI and IE-PsLRhI. This coupling reaction exhibited an equilibrium ratio of L-fucose, L-fuculose, and 6-deoxy-L-talose of 80:9:11. In contrast, L-fucose was sequentially converted into L-fuculose and 6-deoxy-L-sorbose using IE-BpDAI and TT-SDAE. Equilibrium ratios between L-fucose and L-fuculose, and between L-fuculose and 6-deoxy-L-sorbose were 88:12 and 40:60. Furthermore, pure 6-deoxy-L-sorbose was individually isomerized into 6-deoxy-L-gulose and 6-deoxy-L-idose by TT-AcLRI and DGI, respectively. An equilibrium ratio of 6-deoxy-L-sorbose and 6-deoxy-L-gulose was 40:60, whereas it of 6-deoxy-L-sorbose and 6-deoxy-L-idose was 73:27. Eventually, this procedure provided pure 6-deoxy-L-talose, 6-deoxy-L-sorbose, 6-deoxy-L-gulose, and 6-deoxy-L-idose with production yields of 7.1%, 14%, 2.0%, and 2.4%, respectively, based on L-fucosewhich used as a starting material.

Finally, a novel SDAE which took part in the production of rare 6-deoxyhexoses in this study was purified and investigated on its enzymatic properties. Purification by heat treatment at 60°C, hydrophobic column chromatography, and ion-exchange column chromatography provided a final specific activity of 338 U/mg, representing 9.8% yield, and 338 purification fold. The molecular mass of native enzyme is 148 kDa which consists of homotetramer of 37 kDa. SDAE is a thermoacidophilic enzyme which exhibits its highest activity at 80°C and pH 5.5. Moreover, it strongly requires Co^{2+} to activate its activity and enhance its thermostability. Half-life at 60°C of Co^{2+}-treated SDAE was 3-fold higher than metal-free SDAE. Although D-allulose was the most appropriate substrate of SDAE; however, its substrate specificity and catalytic efficiency (k_{cat}/K_m) for 6-deoxyketohexoses, especially L-fuculose and 6-deoxy-L-sorbose, were found in high level. The results indicate that SDAE is suitable to use in the industrial production of rare sugars due to its superior properties in thermostability and broad substrate specificity on ketohexoses and 6-deoxyketohexoses.

These findings clearly demonstrate that PsLRhI, BpDAI, EaLAI, AcLRI, DGI, PcDTE, and SDAE used in this study not only catalyze common monosaccharides, but also effectively catalyze various 6-deoxyhexoses. Seven rare 6-deoxyhexoses were successfully produced from the inexpensive substrates, L-rhamnose and L-fucose, by coupling and sequential enzymatic reactions in acceptable production yields. As the results, these microbial enzymes are probably applied in a wide-ranging production of rare sugars and rare deoxy sugars.