Metabolic engineering of *Escherichia coli* for production of D-psicose and D-allose from glucose and fructose

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Abstract

D-Psicose and D-allose, a rare keto and aldo-hexose, are not abundant in nature and are difficult to prepare by chemical methods. For the production of D-psicose and D-allose, we used allose operon of *Escherichia coli*, and D-tagatose 3-epimerase of *Agrobacterium tumefeciens* and L-rhamnose isomerase of *E. coli*. In order to use the allose operon, *E. coli* was transformed with pTalsEBK containing *alsE*, *rpiB*, and *alsK*. When *E. coli* harboring pTalsEBK was cultivated in 2YT medium with 30g/L of glucose or fructose, 1g/L of D-psicose was obtained approximately. It seems that *rpiB* can not convert psicose-6-phosphate to allose-6-phosphate. Therefore, psicose-6-phosphate 3-epimerase and allose-6-phosphate isomerase encoded by *alsE* and *rpiB* were purified and the in vitro reaction catalyzed by these enzymes were investigated. Psicose-6-phosphate 3-epimerase can epimerize fructose-6-phosphate to psicose-6-phosphate and has no substrate specificity on unphosphrylated fructose. Allose-6-phosphate isomerase can isomerize D-allose to D-psicose but, not D-psicose to D-allose. The other scheme for production of the rare sugars was performed with D-tagatose 3-epimerase and L-rhamnose isomerase by which fructose was epimerized to D-psicose and then isomerized to D-allose. When recombinant *E. coli* harboring pTTE-rhaA with *Atu4750* and *rhaA* was cultivated in 2YT medium containing 30g/L of fructose, both rare sugars of D-psicose and D-allose were observed. D-psicose was obtained upto 5g/L. These *in vivo* conversion reaction were confirmed *in vitro* by using purified enzymes of

D-tagatose 3-epimerase and L-rhamnose isomerase.

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