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Outline of Research Result

Enhancement of a platform chemical (catechol) production from cellulosic materials

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Research aims

In this study, we focused on improving the production of catechol (CL), which is a platform chemical for the synthesis of pesticides, pharmaceuticals, antioxidants etc. It has been reported that CL can be produced by shunting the carbon flow to protocatechuate from 3-dehydroshkimate (DHS) in the shikimate biosynthetic pathway (Fig. 1).[1] The produced protocatechuate is then decarboxylated to catechol. However, the decarboxylation step has been reported to be a bottleneck in CL production, and to the best of my knowledge, no study till date has been able to resolve this issue. The aim of this study is to find this solution, and develop an efficient strategy for catechol production from renewable cellulosic feedstocks.

Methods

Identification of an enhancer-like protein for protocatechuate decarboxylation and characterization of the enhanced Pdc

The deletion clones of the region encoding KpdBCD, a 4-hydroxybenzoate decarboxylase complex, were prepared, and an *Escherichia coli* XL-1 Blue recombinant harboring a plasmid to express AroY was transformed with each deletion clone. The resulting transformants were used to evaluate *in vivo* PCA decarboxylation activity. The cells expressing both AroY and KpdB were ground using an ice-cold pestle and mortar with alumina. Phosphate buffer (100 mM, pH 7.5) was used to extract enzymes from the cells. After centrifugation, the supernatant was collected as a crude extract, and it was used to evaluate the initial characteristics of the enhanced Pdc.

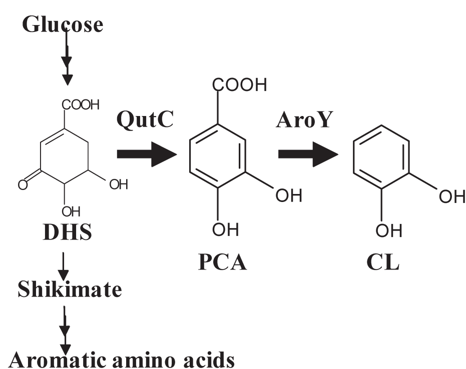

Fig. 1. CL production by carbon flow shunting in the shikimate pathway.

Construction and evaluation of a catechol-producing strain with the enhanced Pdc activity

A *pheA*-deficient mutant, *E. coli* strain JD23488 (obtained from NBRP-*E. coli*), was transformed with pTS066 to express $aroF^{FBR}$ and *qutC*. The recombinant, JD23488/pTS066 was further transformed with either pTS036 (to express *aroY*) or pTS052 (to express *aroY* and *kpdB*). JD23488/pTS066+pTS036 and JD23488/pTS066+pTS052 were cultured in M9 medium containing 1 mM IPTG and 10 μM phenylalanine in addition to appropriate antibiotics, and evaluate the effect of the enhanced Pdc activity on CL production. Glucose was used as the sole carbon source.

Results

Identification of an enhancer-like protein for protocatechuate decarboxylation

Other decarboxylases of aromatic compounds such as vanillate demethylase (VdcBCD) and a 4-hydroxybenzoate decarboxylase (BsdBCD) are hetero-oligomeric enzymes consisting of three subunits. Pdc has been reported to be a homo-oligomeric enzyme, and its activity was low and unstable.[1–3] Methods for enhancing the activity to increase the productivity of CL and other valuable chemicals are required; however, no efficient techniques have been reported till date.

KpdC, a subunit of the 4-hydroxynenzoate decarboxylase from *Klebsiella pneumoniae* NBRC14940 (KpdBCD), showed 24% identity with AroY. We hypothesized that AroY could make a hetero-oligomeric protein complex with KpdB and KpdD, thereby increasing Pdc activity. Deletion clones of the KpdBCD-encoding region were prepared and their effects of their expression on Pdc activity were evaluated. Pdc activity increased when both KpdB and KpdD were expressed with AroY, and it was also enhanced with the coexpression of only KpdB and AroY (Fig. 2).

Initial characterization of the enhanced protocatechuate decarboxylase

The effects of pH and temperature on Pdc activity under the condition that AroY was expressed either with or without KpdB are shown in Fig. 3 (a and b). Regardless of the

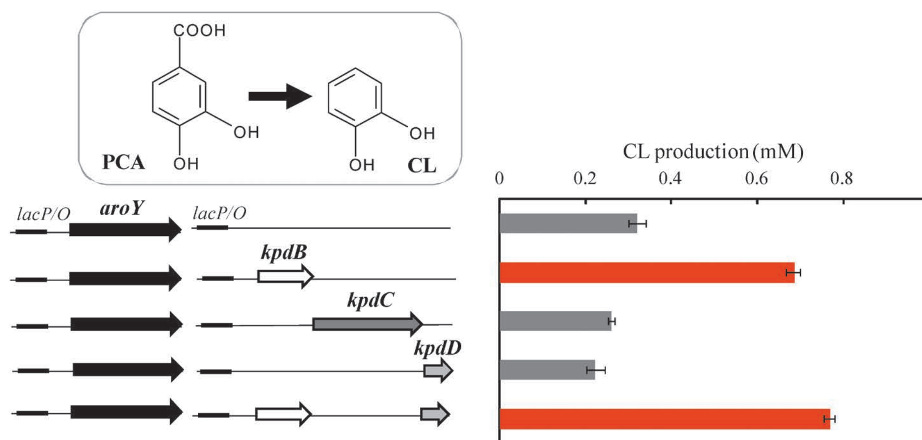

Fig. 2. Effects of the expression of tne subunits of KpdBCD on PCA decarboxylation.

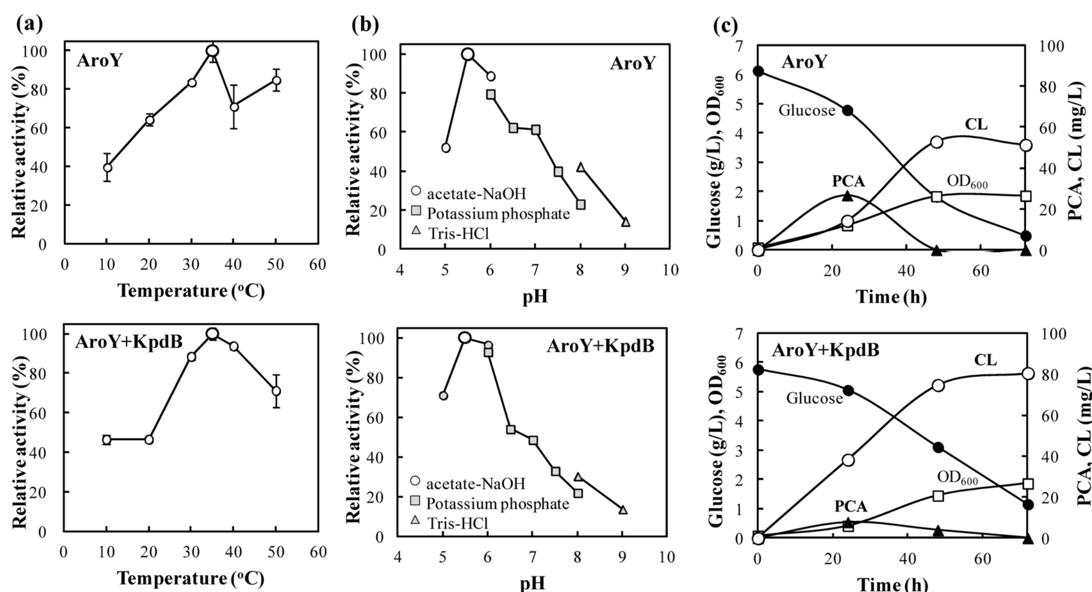

Fig. 3. Effects of KpdB expression on Pdc activity (a, pH; b, Temperature) and CL production (c).

expression of KpdB, the optimal pH and temperature for Pdc activity were 5.5 and 35°C, respectively, although Pdc activity increased approximately 20-fold than that without the KpdB expression. AroY and KpdB were separately eluted at different ion concentrations via DEAE-anion chromatography. These results suggested that AroY and KpdB would not form a hetero-oligomeric protein complex like VdcBCD and BsdBCD, and the activity may be enhanced by a different and novel mechanism.

Effect of the enhanced Pdc activity on catechol production

The expression of the enhanced Pdc (AroY+KpdB) solved the temporary accumulation of PCA, as observed when the ordinary Pdc (AroY) was used to decarboxylate PCA (Fig. 3c). The enhanced Pdc-expressing strain produced catechol with 2-fold higher yield than the ordinary strain.

Conclusion

In this study, a novel factor, which enables efficient production of CL by enhancing Pdc activity, was identified. CL is converted to muconic acid via *ortho* cleavage reaction, and muconic acid also acts as a platform chemical for adipic acid and terephalic acid, which are in great demand in the polymer industries.[4] Our results will also contribute to efficient muconate production.

References

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