Microbial production of isoprenoid using the archaeal mevalonate pathway

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Research objective

Isoprenoid is the collective name of natural compounds that are biosynthesized from isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) and contain C5 isoprene units in their structures. Tens of thousands of isoprenoid compounds have been reported so far, making them the biggest family of natural compounds. Hormones and nutrients such as steroid, lipid soluble vitamins (vitamin A, D, E, and K), and ubiquinone (coenzyme Q), which are essential for human health, are included in the group. Moreover, isoprenoid contains important natural drugs and lead compounds for synthetic drugs, as represented by an anti-malarial drug, artemisinin (whose discoverer Dr. Tu Youyou received the Nobel prize in Physiology or Medicine in 2015), and a cancer chemotherapy drug, paclitaxel (taxol). In addition, many isoprenoid compounds are utilized as perfumes or industrial materials.

There are two pathways discovered so far for the biosynthesis of IPP and DMAPP. One is the mevalonate (MVA) pathway, which has been reported in eukaryotes, archaea, and some bacteria. The pathway starts from acetyl-CoA, proceeds via MVA, produces IPP first, and then isomerizes it to form DMAPP. Another is the methylerythritol phosphate (MEP) pathway, which has been reported in major bacteria and in the chloroplast in plants. It starts from pyruvate and glyceraldehyde 3-phosphate, proceeds via MEP, and yields IPP or DMAPP in the final step. We have worked on the MVA pathway in archaea, and recently discovered a modified MVA pathway that proceeds via a novel compound, *trans*-anhydromevalonate phosphate (tAHMP) [1]. Since this pathway is conserved in most archaea, it was named "the archaeal MVA pathway". The prominent feature of the pathway is that it consumes only two ATP molecules for the production of one IPP, whereas the usual MVA pathway requires three ATP molecules. This is because a dehydration/decarboxylation step, which is catalyzed only by an ATP-dependent decarboxylase in the usual MVA pathway, is catalyzed by two novel enzymes, i.e., phosphomevalonate dehydratase (PMDh) and tAHMP decarboxylase (AMPD), in an ATP-independent manner. From this property, the archaeal MVA pathway is expected to be applicable to isoprenoid bioproduction as an energy saving metabolic pathway.

The objectives of this study are to show the usefulness of the archaeal MVA pathway by constructing an isoprenoid production system involving the pathway in the host *Escherichia coli* cells [2] and to search for enzymes of the pathway, which are more suitable for

isoprenoid production, by taking advantage of the system.

Methods

The genes of MVA kinase (MVK), PMDh, AMPD, isopentenyl phosphate kinase (IPK), IPP isomerase (IDI), which catalyze the biosynthetic reactions from MVA to IPP and DMAPP, and that of prenylated FMN synthase that catalyzes the production of a coenzyme for AMPD, were inserted into pBAD18, a plasmid for *E. coli*, to construct pBAD-mMP7 and pBAD-aMP7. MVK and IPK genes from a mesophilic methanogen *Methanosarcina mazei* and the IDI gene from *E. coli* were used. The other genes for the construction of pBAD-mMP7 were from *M. mazei*, and from *A. pernix* for pBAD-aMP7. Each of the plasmids was introduced into *E. coli* harboring a plasmid, pACYC-IBE, containing carotenoid biosynthetic genes (Figure 1A). The transformants were cultivated in 5 mL of LB medium containing 5.0 mg/mL of mevalonolactone (MVL). In addition to general aerobic shaking cultivation, a semi-anaerobic cultivation was performed in a medium overlayered with paraffin. After harvesting, lycopene, a colored carotenoid, was extracted from the cells with acetone and then quantified based on absorption.

Results

When cultivated aerobically, *E. coli* strains harboring pBAD-mMP7 or pBAD-aMP7 decreased lycopene production compared with the negative control strain harboring pBAD18. Therefore, no positive effect of the introduction of the archaeal MVA pathway was observed. In contrast, when cultivated semi-anaerobically, the strain harboring pBAD-mMP7 significantly increased lycopene production, while that harboring pBAD-aMP7 only showed a slight increase (Figure 1B). The removal of MVL from the culture medium caused a decrease in lycopene production at a level comparable with that of the negative control. Since *E. coli* originally exhibits the MEP pathway but not the MVA pathway, the increased lycopene production in the presence of MVL was considered to be derived from IPP/DMAPP biosynthesized from the introduced archaeal MVA pathway. The reason for the difference between the culture conditions could be because the ATP-saving archaeal MVA pathway took advantage of the semi-anaerobic conditions that result in low ATP production, or because the oxygen-sensitive enzymes in the pathway could not show enough activities under the aerobic conditions.

When deficient plasmids, which were constructed by removing the PMDh or AMPD gene from pBAD-mMP7, were used, lycopene production did not increase. The lycopene production was recovered when the removed gene was inserted back into each of the deficient plasmids. Therefore, the deficient plasmids can be used to detect the activity of PMDh and AMPD. Using this system, we have identified PMDh and AMPD from multiple organisms

and are now trying to search for enzymes that have superior properties such as oxygen-resistance. In addition, we are constructing an evaluation system for isoprenoid production using volatile terpene as an index, instead of carotenoid which is hydrophobic and thus reaches maximum production rapidly.

Conclusion

The archaeal MVA pathway is applicable for the bioproduction of isoprenoid when conditions allow. It has a high potential especially under low oxygen.

References

1) Hayakawa, H. *et al.* (2018) Modified mevalonate pathway of the archaeon *Aeropyrum pernix* proceeds via *trans*-anhydromevalonate 5-phosphate. *Proc. Natl. Acad. Sci. U. S. A.* **115:** 10034-10039.

2) Yoshida, R., Yoshimura, T., and Hemmi, H. (2020) Reconstruction of the "archaeal" mevalonate pathway from the methanogenic archaeon *Methanosarcina mazei* in *Escherichia coli* cells. *Appl. Environ. Microbiol.* **86:** e02889-19.

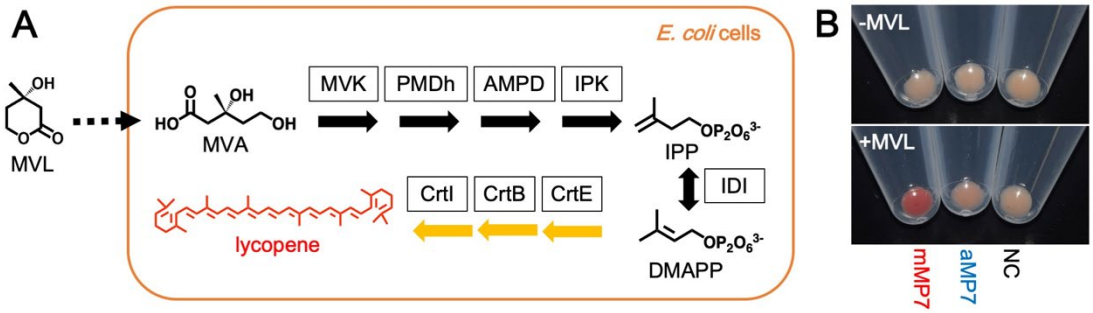

Figure 1 Lycopene production system involving the archaeal MVA pathway

(A) Scheme of the system. CrtI, CrtB, and CrtE are carotenogenic enzymes encoded in pACYC-IBE. (B) *E. coli* cells producing lycopene. mMP7, the strain harboring pBAD-mMP7; aMP7, the strain harboring pBAD-aMP7; NC, the strain harboring pBAD18. Copyright @ American Society for Microbiology, *Appl. Environ. Microbiol.* **86:** e02889-19 doi: 10.1128/AEM.02889-19, 2020.