# Review of: "Increased carvone production in Escherichia coli by balancing limonene conversion enzyme expression via targeted quantification concatamer proteome analysis"

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A general problem which has plagued researchers involved in microbial pathway engineering is that different levels of each enzyme in an introduced pathway are required for optimal conversion. Balancing the level of each enzyme is thus the goal.

Yoshida et al. focused on the bioconversion of (-)-limonene into (-)-carvone by three enzymes: a cytochrome P450 hydrolase, a cytochrome P450 reductase (CPR) and carveol dehydrogenase (CDH). (-)-carvone is one of the major components of spearmint oil (from Mentha spicata), although the chemical is also found in many other essential oils, most notably caraway (Carum carvi).

In this study, initially the three enzymes were expressed in E. coli. Their observations of by-product formation (dihydrocarveol and dihydrocarvone) led the authors to segregate the expression of the enzymes by using two plasmids in two cells, one containing P450/CPR, and a second with only CDH. By mixing different quantities of the two cells expressing these plasmids into the reaction medium, the maximum carvone was generated with a ratio of 100-to-1 of the two plasmids, showing that high CDH expression was indeed counterproductive. The authors hypothesized that the expression level of the three enzymes differs and leads to an imbalance in the bioconversion of limonene to carvone. They then applied the quantification concatamer method (QconCAT) to quantify the amount of expression for each of the three proteins in the bioconversion pathway, determining that the optimal P450/CDH ratio was about 16. Using a low-copy plasmid for expressing CDH, the authors were able to improve significantly on the carvone titer from a single cell.

Although the QconCAT methodology is promising, one wonders whether the elaborate approach, requiring C-13 labeling, is necessary, and under what circumstance it would be most applicable. Protein expression could be optimized by other means, such as through varying promoter strengths, and then completing a purely statistical-based analysis to determine the optimal expression. Alternatively, because the authors conducted a bioconversion rather than forming product during the course of cell growth on a substrate such as glucose, the authors could merely mix different quantities of cells/enzymes as was initially accomplished in this study. Indeed, because one or both of the primary by-products appear to be endogenous, another approach might be to use purified or partially purified enzymes to avoid the presence

of those problematic endogenous enzymes altogether. Because the limonene-to-carvone process does not require a growth substrate, the enzyme expression process which requires growing cells (which is likely optimal under one set of conditions) could be separated from the bioconversion process. In other words, the bioconversion process could be optimized independently of cells and their plasmids.

An inexplicable but interesting result is that dihydroxycarvone was observed primarily in cells not expressing CDH, although dihydroxycarvone is proposed to be derived from the product of CDH, (-)-carvone. If the native enzyme is present at a consistent activity in the various experiments, then more substrate (carvone) would be anticipated to lead to more product (dihydrocarvone). So, it is not clear why high carvone formation (including the ultimate optimized conditions) did not lead to more dihydrocarvone. An interaction might exist between the presumptive native dehydrogenase and the heterologous enzymes, or the proposed pathways to the by-products are incomplete. Ideally, the specific enzyme(s) responsible for by-product formation could be identified.