Engineering of the Enzymes IspG and IspH from *Zymononas mobilis* to Increase Terpenoid Production

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Project Goals: Our goal is to engineer *Zymomonas mobilis* strains with increased terpenoid production. Terpenoids precursors in *Z. mobilis* are synthesized by the MEP pathway. In this pathway the last two steps, catalyzed by the enzymes IspG and IspH, present a bottleneck. We hypothesize that this is due in part to the stability of the iron-sulfur cluster located in each enzyme's active site. To improve IspG and IspH activity we propose to engineer these enzymes, as well as, the ability of *Z. mobilis* to produce and deliver iron-sulfur cluster cofactors to these enzymes.

Terpenoids can substitute for petroleum in the production of compounds of economic interest ranging from vitamins and perfumes to biofuels. Bacteria, such as *Zymomonas mobilis*, produce the terpenoid precursors dimethylallyl diphosphate (DMADP) and isopentenyl diphosphate (IDP) from glucose via the methyl erythritol phosphate (MEP) pathway. It has been proposed that the activity of the MEP pathway is limited at the last two steps, which are catalyzed by the enzymes IspG and IspH. Both enzymes carry a [4Fe-4S] cluster in their active sites, which, for some orthologs, makes them prone to O_2 damage. Additionally, recent data indicate that intermediates preceding IspG and IspH accumulate in response to O_2. Thus, our objective is to determine if Fe-S cluster lability explains the accumulation of IspG and IspH precursors in the presence of O_2 in *Z. mobilis* and if engineering approaches can improve the robustness of these enzymes. To achieve this goal, we are studying several aspects of both enzymes. First, to test the effect of O_2 on *Z. mobilis* IspG and IspH activity, we took advantage of *E. coli* strains that conditionally require IspG or IspH activity for growth. Using this *E. coli* strain, we have been able to replace the *E. coli* IspG and IspH enzymes with those from *Z. mobilis*. Under anaerobic conditions, *Z. mobilis* IspH and IspG complement the *E. coli* growth requirements for these enzymes but strains carrying the *Z. mobilis* IspH show poor growth under aerobic conditions. This result suggests that *Z. mobilis* IspH is sensitive to O_2. Current experiments are focused on isolating O_2-resistant variants of IspH and purifying the [4Fe-4S] form of IspH to test whether the Fe-S cluster is O_2-labile. We also found that co-expression of *Z. mobilis* IspG and IspH improves the growth of the *E. coli* strain lacking IspH, suggesting that *Z. mobilis* IspG and IspH might form a complex that protects their Fe-S clusters from O_2-mediated damage. Another approach to increase IspG and IspH protein activity is through protein overexpression. However, since the proteins require Fe-S cofactors, it may also be necessary to increase the production of the required cofactor to drive Fe-S cluster occupancy. Thus, we are also studying the function

and expression of the *Z. mobilis* Fe-S cluster biosynthetic machinery. In *Z. mobilis* the Fe-S cluster machinery is encoded by the *suf* operon. We have showed that expression of the genes encoding the *Z. mobilis* Suf pathway can replace the function of the *E. coli* pathway. We have also identified a homolog of the [2Fe-2S]-containing transcription factor, IscR, that regulates Fe-S cluster biosynthesis in *E. coli.* The *Z. mobilis* IscR homolog carries a [4Fe-4S] cluster that is highly sensitive to O_2. This protein binds to the promoter region of the *suf* operon *in vitro*. Currently, we are developing an *in vivo* reporter system that allows us to probe the role of the IscR-homolog in *Z. mobilis.* In summary, we predict that our multipronged strategy will generate new knowledge concerning the role of IspH Fe-S cluster stability in MEP pathway function and allow us to generate a strain of *Z. mobilis* with a more robust MEP pathway and improved terpenoid production.

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