Improving Iron-Sulfur Cluster Stability in *Zymononas mobilis* to Increase Terpenoid Production

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Project Goals: Our goal is to increase the production of terpenoid precursors in the bacterium *Zymomonas mobilis.* These molecules are synthesized from glucose via the MEP pathway. This pathway has known rate-limiting steps catalyzed by the iron-sulfur dependent enzymes IspG and IspH. Our work focuses on increasing the activity of these steps to engineer *Z. mobilis* strains for terpenoid production.

Terpenoids can substitute for petroleum in the production of compounds of economic value, including drugs, flavoring agents, and biofuels. The bacterium *Zymomonas mobilis* uses glucose to produce the terpenoid precursors dimethylallyl diphosphate (DMADP) and isopentenyl diphosphate (IDP) via the methyl erythritol phosphate (MEP) pathway. Thus, *Z. mobilis* has the potential to become an important engineering platform for terpenoids. However, recent data showed that O_2 exposure reduces terpenoid precursor production, accompanied by an accumulation of intermediates prior to the iron-sulfur (FeS) cluster-dependent enzymes IspG and IspH. These enzymes have been previously identified as the pathway's limiting steps. Since Fe-S clusters are known targets of oxidative damage, we are investigating if Fe-S cluster lability explains the effect of O_2 on flux through the enzymes IspG and IspH in *Z. mobilis* and accordingly, develop approaches to improve these enzymes' O_2 stability. We have taken two strategies to achieve this goal. First, we have taken advantage of a well characterized *E. coli* platform to examine *Z. mobilis* IspG and IspH activity under aerobic conditions compared to the native *E. coli* enzymes. Our results suggest that *Z. mobilis* IspH is more O_2 sensitive than either *E. coli* IspH or *Z. mobilis* or *E. coli* IspG. Further we found that the O_2 sensitivity of IspH function can be rescued by coexpression of IspG, indicating IspG and IspH may have co-evolved for optimal O_2 stability. In a second complementary approach, we are testing if flux through the MEP pathway can be improved by co-overexpressing IspG and IspH orthologs in *Z. mobilis.* To successfully accomplish this goal, we will need to provide strains with sufficient Fe-S cluster biogenesis machinery to assemble active proteins. Surprisingly, overexpression of the *Z. mobilis suf* genes, encoding the Suf Fe-S biogenesis machinery induces an O_2 sensitive phenotype in *Z. mobilis*. To have more refined control over *suf* operon expression, we are investigating its native regulation. We have found that *suf* expression is controlled by a transcription factor, RsuR, that is a homolog to [2Fe-2S]-IscR from *E. coli,* which regulates Fe-S cluster biosynthesis. Unlike E. coli IscR, RsuR binds a [4Fe-4S] cluster. Using DNaseI footprinting, we have identified the DNA sequence that RsuR binds to in the *suf* operon promoter region. DNA binding is cluster dependent. O_2 eliminates DNA binding and induces cluster degradation, which implies a sensing mechanism for the transcription factor. In summary, our diverse strategy will generate new knowledge relative to the Fe-S cluster dependent enzymes in the MEP pathway and insight into engineering strategies for boosting synthesis of Fe-S cluster enzymes in *Z. mobilis.* This new knowledge will allow us to generate a more robust strain of *Z. mobilis* with improved terpenoid production.

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