Approaches to Increasing Terpenoid Production in *Zymomonas mobilis* by Improving Activity of IspG and IspH of the MEP Pathway

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Project Goals: Our goal is to improve the production of terpenoids in *Zymomonas mobilis* by the MEP pathway through the engineering of IspG and IspH. These enzymes are the bottleneck in the pathway due to the nature of their active site that contains an iron-sulfur cluster. To improve IspG and IspH activity we propose to engineer the proteins itself, as well the ability of *Z. mobilis* to produce and deliver the iron-sulfur clusters.

Terpenoids can be used as a substitute for petroleum in the production of plastics and biofuels. The bacterium *Zymomonas mobilis* produces terpenoid precursors, dimethylallyl diphosphate (DMADP) and isopentenyl diphosphate (IDP) from glucose via the methyl erythritol phosphate (MEP) pathway. Because the MEP pathway is proposed to be limited by the activity of the enzymes IspG and IspH, we are testing ways to increase their activity in *Z. mobilis*. Both IspG and IspH contain a [4Fe-4S] cluster in their respective active sites, which makes them prone to O_2 damage, and recent data indicate that pathway intermediates accumulate in response to O_2. We have initiated several lines of investigation to study the role of IspG/H activity in MEP pathway function. To test the O_2 sensitivity of the *Z. mobilis* IspG and IspH variants, we took advantage of *E. coli* tester strains that conditionally require IspG or IspH activity for growth. We found that under either aerobic or anaerobic conditions, *Z. mobilis* IspG fully complemented growth of the relevant *E. coli* tester strain. In contrast, *Z. mobilis* IspH only fully restored growth of the relevant strain under anaerobic conditions. These results suggest that *Z. mobilis* IspH is more O_2 sensitive than its *E. coli* counterpart. To test this hypothesis, we will compare the effect of O_2 on the stability of the [4Fe-4S] cluster in isolated *E. coli* and *Z. mobilis* IspH, use this tester strains to identify O_2 resistant IspH variants, and test the ability of these variant proteins to increase the ability of *Z. mobilis* to produce terpenoids by the MEP pathway. In a second approach, we are testing if improving [4Fe-4S] cluster delivery or synthesis would increase the occupancy, and accordingly, the activity of IspH. When each of the 3 predicted iron-sulfur cluster carriers from *Z. mobilis* were co-expressed with *Z. mobilis* IspH in the *E. coli* tester strain, none of them improved growth under aerobic conditions. We are also testing if expression of the Fe-S cluster biosynthetic machinery affects *Z. mobilis* IspH activity, since this bacterium contains a homolog of the *E. coli* [2Fe-2S]-containing transcription factor, IscR. Purification of the *Z. mobilis* IscR homolog has shown that it carries an iron-sulfur cluster, which is destroyed

by O_2. To test the role of this IscR homolog in expression of the Fe-S cluster-biosynthetic machinery, we are constructing a strain of *Z. mobilis,* which lacks this gene. Combined, we predict that these studies will allow us to test if *Z. mobilis* strains with increased Fe-S cluster occupancy of IspH can be used to improve terpenoid production.

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