ENGINEERING *CORYNEBACTERIUM GLUTAMICUM* TO PRODUCE THE BIOGASOLINE ISOPENTENOL FROM PLANT BIOMASS HYDROLYSATES.

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Many microbes used for the rapid discovery and development of metabolic pathways have sensitivities to final products and process reagents. Isopentenol, a biogasoline candidate, has an established heterologous gene pathway but is toxic to several microbial hosts. Reagents used in the pretreatment of plant biomass, such as ionic liquids, also inhibit growth of many host strains. We explored the use of *Corynebacterium glutamicum* as an alternative host to address these constraints. We found *C. glutamicum* ATCC 13032 to be tolerant to both the final product, isopentenol, as well to three classes of ionic liquids. A heterologous mevalonate-based isopentenol pathway was engineered in *C. glutamicum*. Targeted proteomics for the heterologous pathway proteins indicated that the 3-hydroxy-3-methylglutaryl-coenzyme A reductase protein, HmgR, is a potential rate-limiting enzyme in this synthetic pathway. Isopentenol titers were improved via three routes: media optimization; substitution of an NADH-dependent HmgR homolog from *Silicibacter pomeroyi*; and development of a *C. glutamicum* Δ*poxB* Δ*ldhA* host chassis. We describe the successful expression of a heterologous pathway in the gram-positive industrial microorganism, C. glutamicum, for the production of the biogasoline candidate, isopentenol. We identified and optimized critical genetic and media parameters required to produce 1.25 g/L isopentenol in defined minimal media with D-glucose as the carbon source and similar titers (1 g/L) using sorghum biomass hydrolysates as a carbon source.