ENGINEERING OF AN ENVIRONMENTAL ISOLATE OF BACILLUS MEGATERIUM FOR BIOCHEMICAL PRODUCTION UNDER SUPERCRITICAL CO_2

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Continuous processing is a mainstay for chemical production but is far less common for biochemical processes. The increase in productivity and corresponding decrease in costs make continuous processing an intriguing option for bulk chemicals where price is a major consideration. Among the various challenges of continuous bioprocessing are the risks of contamination and the toxicity of the target products. Supercritical carbon dioxide ($scCO_2$) may provide a means to address both of these issues. $scCO_2$ is an attractive substitute for conventional organic solvents due to its unique transport and thermodynamic properties, its renewability and labile nature, and its high solubility for compounds such as alcohols, ketones and aldehydes. $scCO_2$ is also known for its broad microbial lethality. The isolation and engineering of a microbe that is capable of growth and production in the presence of $scCO_2$ thus represents an opportunity to create a production environment that is both resist to contamination and capable of sequestering toxic products through phase separation. Using a targeted bioprospecting approach by sampling fluid from a natural, deep-subsurface $scCO_2$ well, a strain of *Bacillus megaterium* was isolated that is able to germinate and grow in the presence of $scCO_2$. Transformation is possible using a protoplast-based method, which permitted the identification of promoters capable of inducible heterologous protein expression in both aerobic and anaerobic conditions. A xylose-inducible promoter was evaluated under $scCO_2$ and found to have similar expression under both conditions. We engineered the *B. megaterium* strain to produce isobutanol from 2-ketoisovalerate by introducing a two-enzyme pathway (2-ketoisovalerate decarboxylase (KivD) and alcohol dehydrogenase (Adh)). Due to the strong partition of the aldehyde to the $scCO_2$ phase, we tested five homologous Adh enzymes and found that YqhD from *E. coli* resulted in greater than 85% conversion when grown aerobically. Isobutanol production was also observed when our recombinant strain was cultured under $scCO_2$. Finally, we have developed a process model for an integrated bioprocess and have found conditions that are comparable if not better than existing *in situ* extraction techniques such as gas stripping.

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