

Biosynthesis of Hop Bitter acids (Lupulone and Humulone) by Engineered *E. coli*

Jingling Huang^a, Nan Zhang^a, Guoqing Zhang^a, Xueni Ren^a, Huibin Zou^{a,b,*}

^aCollege of Chemical Engineering, Qingdao University of Science and Technology, Qingdao 266042, China

^bCAS Key Laboratory of Bio-based Materials, QIBEBT, Chinese Academy of Sciences, Qingdao 266101, China

*Corresponding author: zouhb@qibebt.ac.cn, huibinzou@hotmail.com

Objective

The lupulone and humulone are multiprenylated chemicals derived from hops and are known for their contribution to the bitterness of beer. These compounds also present pharmacological activities, such as antibacterial, anti-oxidant, sedative drugs, as well as anti-tumor activities reported. The bitter acid biosynthesis pathway has been studied but full biosynthesis of humulone has not been reported yet.

Methods

We engineered the genes of carboxyl coenzyme A ligase (CCL2), pentenone synthase (VPS) and prenyltransferase (PT2), isopentenyl pyrophosphate isomerase (IDI), prenyltransferase (PT1) in *E. coli*. In addition, we also screened feasible humulone-forming monooxygenase (MO), which is crucial in the humulone biosynthesis pathway. After fermentation tests and HPLC-MS, NMR assay, the results showed that variable engineered strain could successfully produce lupulone and humulone from glucose.

Results

After fermentation tests and HPLC-MS, NMR assay, the results showed that variable engineered strain could successfully produce lupulone and humulone from glucose.

Expression vectors

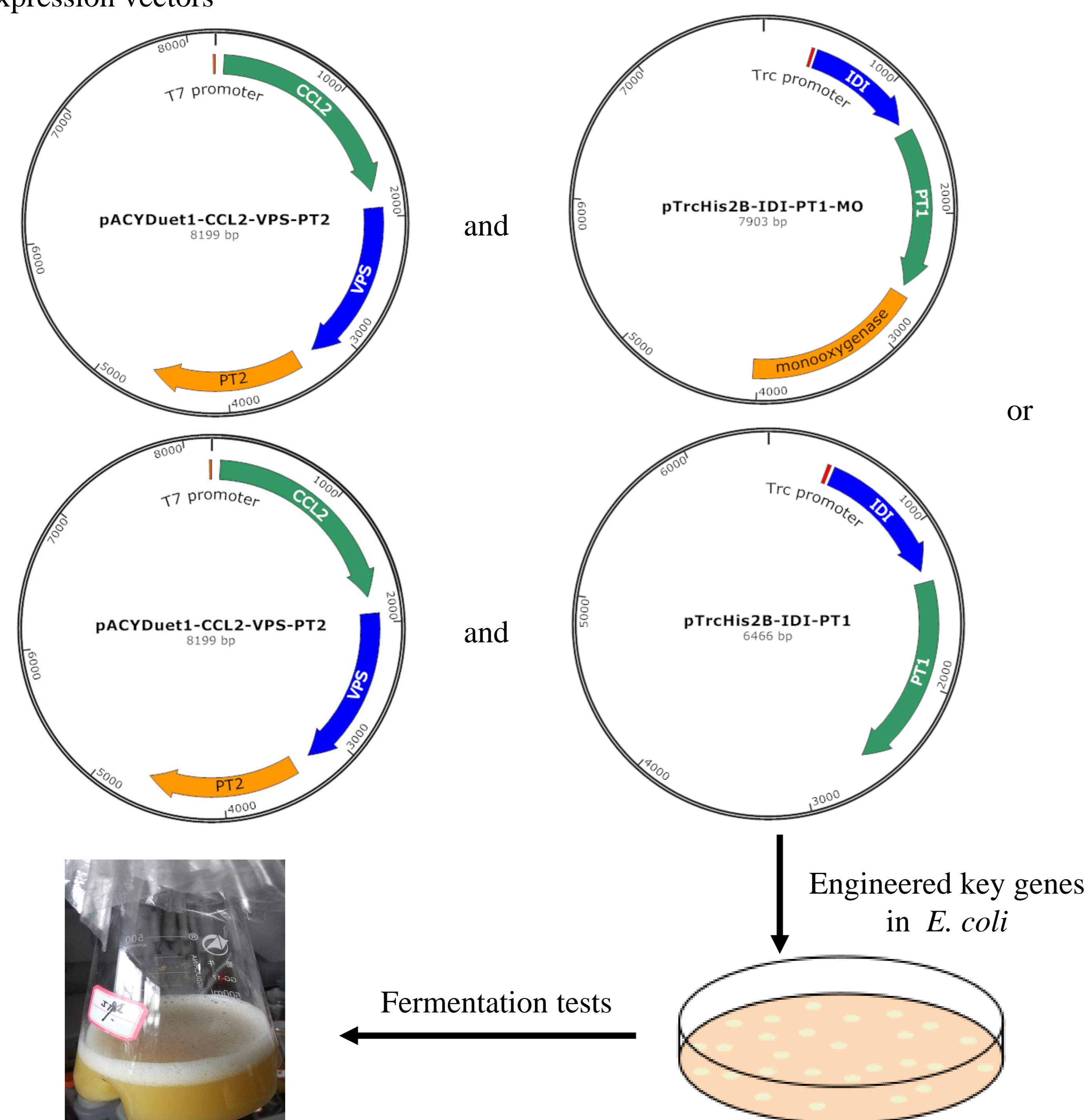


Fig. 1. Key gens of the pathway of lupulone and humulone were engineered in *E. coli*.

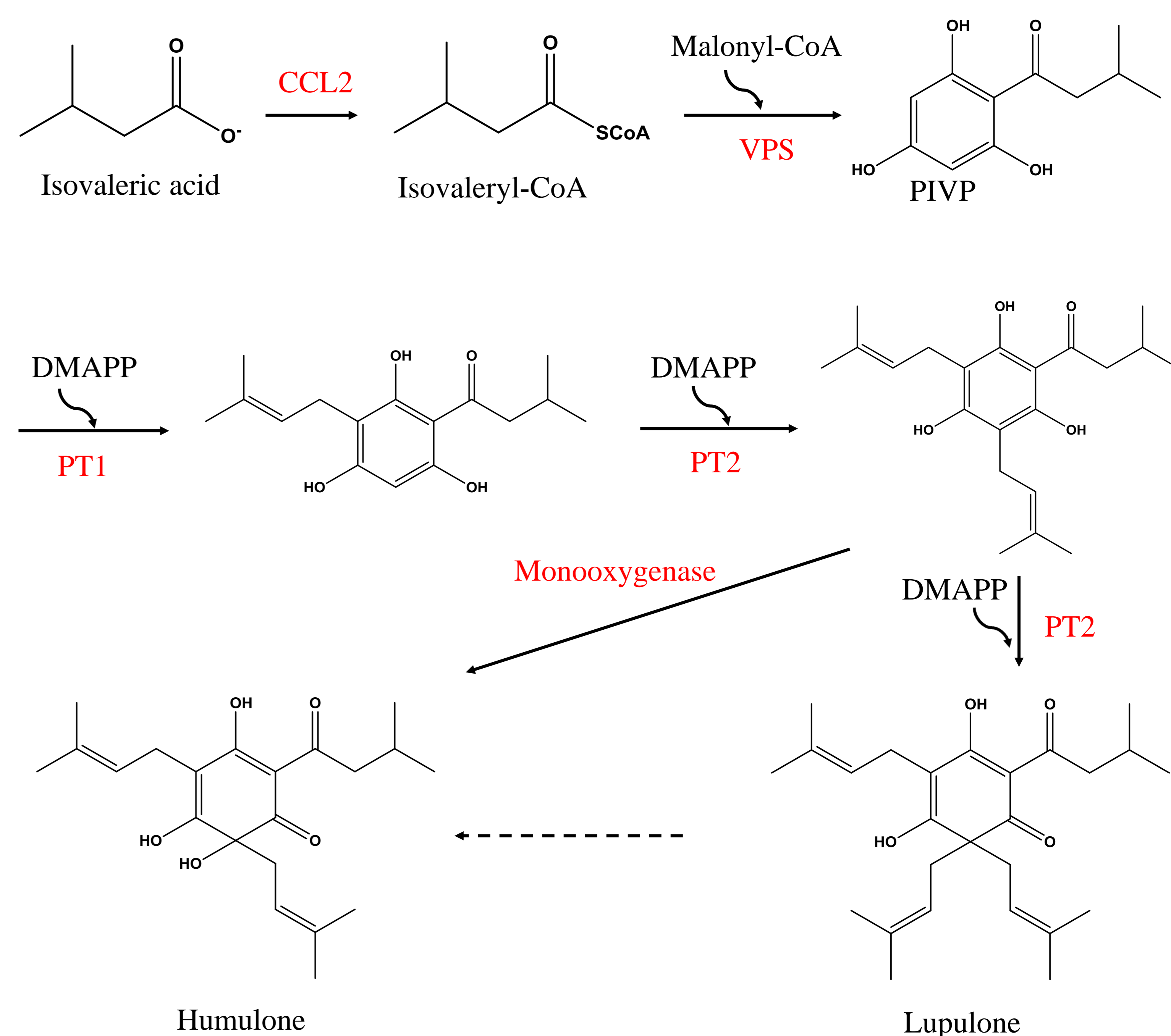


Fig. 2. Metabolic pathway of exogenous enzyme.

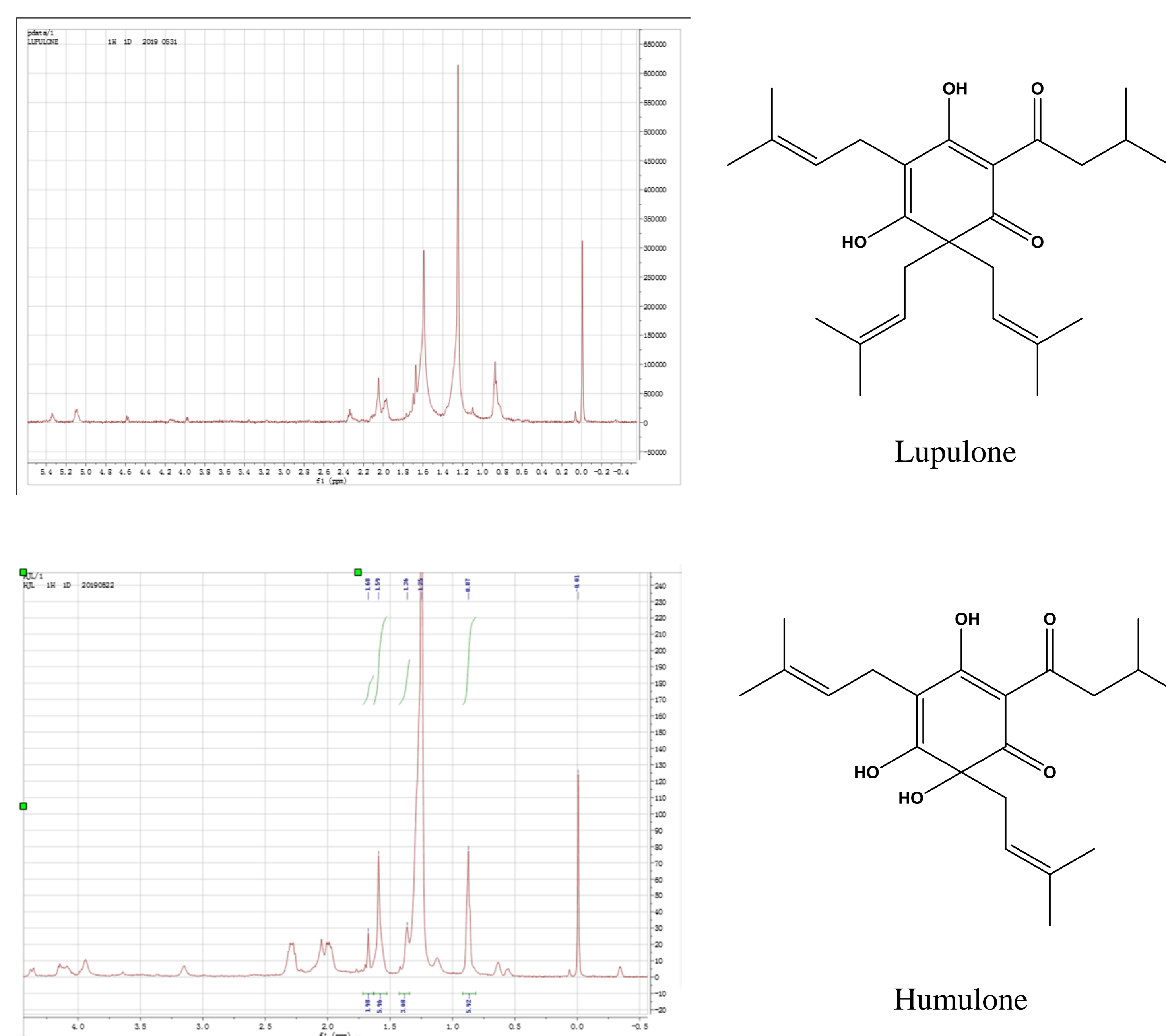


Fig. 3. ¹H NMR assay towards the purified products confirmed that lupulone and humulone were successfully bio-produced from glucose.

Conclusions

This study successfully synthesized lupulone and humulone by engineered *E. coli*, ongoing projects are carrying out to further optimized the strains and improve their productivities.