Transfer-to-Excellence (TTE) Program

Analysis of the Mevalonate and DXP Pathway Intermediates

Cindy Lei[1], Edward Baidoo[2]
[1]University of California Berkeley; [2]Joint BioEnergy Institute

Abstract:

The mevalonate and non-mevalonate pathway to isoprenoid production are essential in many types of plant, bacteria, and living organisms. Many applications have been aimed at isoprenoid production, given their wide range of functions from primary and secondary metabolism. Such areas have included carotenoids, vitamins, plant defense, and communication [1]. In addition, commercial and industrial uses involving isoprenoids have accounted for the expansion of corporate enterprise, from the production of perfumes to natural rubber or flavorings. However, when introducing a heterologous isoprenoid pathway into a microbial host, major challenges still occur due , such as the accumulation of metabolic intermediates. To identify these potential bottlenecks, Hydrophilic Interaction Liquid Chromatography was utilized to measure these metabolic intermediates in both pathways. Since this method was recently developed, it requires validation in order to be applied to biological extracts.

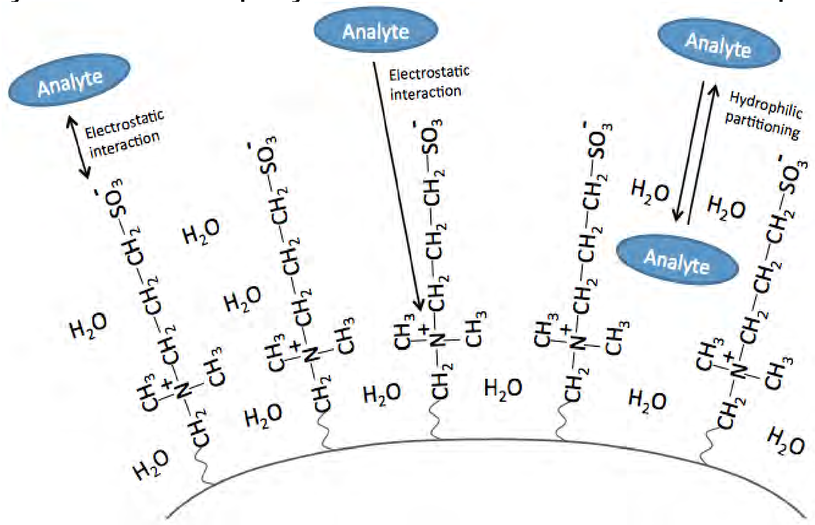
Introduction/Background

The fundamental precursors to isoprenoids are isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) via the mevalonate or non-mevalonate (DXP) pathway. In the mevalonate pathway, acetyl-CoA is transformed to IPP, followed by an IPP isomerize that balances IPP and DMAPP [1]. In the non-mevalonate pathway, pyruvate and glyceraldehyde-3-phosphate is used to synthesize IPP and DMAPP. IPP and DMAPP can be used to form monoterpenes (via the C10 pryophosphate geranyl diphosphate, i.e. GPP), sesquiterpenes (via the C15 farnesyl diphosphate, i.e. FPP), and diterpenes (via the C20 geranylgeranyl diphosphate, i.e. GGPP).

However, when introducing a heterologous isoprenoid pathway into a microbial host, major challenges still occur, such as the accumulation of metabolites. These bottlenecks will affect the flux of the biosynthetic pathways as downstream intermediates begin to accumulate. The cell's ability to survive can be affected as an imbalance arises in producing the desired product over its natural survival needs. As a result, cellular growth inhibition or cellular toxicity occurs.

Methods

To identify any bottlenecks that affect the flux through the mevalonate and DXP pathway, a Hydrophilic Interaction Liquid Chromatography (HILIC) method was developed. This chromatographic method was coupled to time-of-flight mass spectrometry via electrospray ionization to measure compound separation.



HILIC retention is based on the hydrogen bonding between the water layer on the surface of the stationary phase and the analyte and through the zwitter-ionic side chains via electrostatic interaction [2].

References

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Support Information
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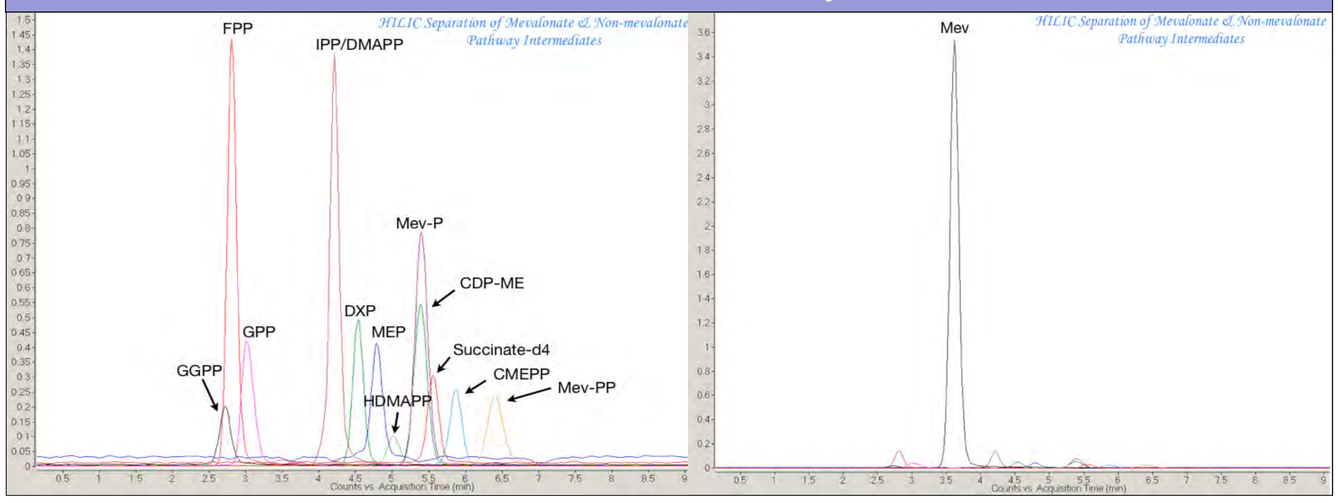
Contact Information
Email: CindyyLei@Gmail.com

Results/Data Analysis



Method Validation

Compound	Retention Time (RT, min)	*Reproducibility (%RSD, based on RT, n=5)	Efficiency (N)	Resolution (R)	LOD (µM)	LOQ (µM)	R^2
Mev	3.615	2.27	2204.1	6.13	0.02	0.06	0.991
Mev-P	5.403	1.88	2486.8	0.40	0.14	0.48	0.996
Mev-PP	3.000	3.04	3891.8	7.62	0.65	2.18	0.997
DXP	4.538	0.76	2412.6	2.95	0.21	0.70	0.997
MEP	4.788	1.02	1346.7	2.43	0.91	3.03	0.998
CDP-ME	5.865	1.78	1293.4	0.76	0.20	0.67	0.999
CMEPP	4.999	0.54	2378.8	1.27	0.38	1.26	0.997
HDMAPP	4.211	1.34	2033.2	3.43	0.84	2.79	0.997
IPP/DMAPP	6.403	0.53	1209.8	1.89	0.19	0.63	0.999
GPP	2.807	3.07	1200.9	8.46	0.06	0.21	0.997
FPP	2.730	3.09	900.0	8.19	0.30	1.01	0.998
GGPP	5.403	3.17	2890.3	0.42	0.23	0.76	0.989

LOD - Limit of Detection

LOQ - Limit of Quantification

*Reproducibility - five replicates were used

**R^2 - two calibration curves were used

Discussion/Conclusion

- The retention time was used to identify the compounds, along with it's mass to charge ratio.
- The relative standard deviation was calculated to assess the reproducibility of the method based on how consistent the retention time was over a measurement of five days.
- The chromatographic efficiency, *N*, was used to measure the ability of the HILIC method to separate the aforementioned compounds. That is, the narrower the peaks are, the higher the efficiency of separation [3].
- The resolution was used to measure how well the HILIC method separated the metabolic intermediates. For quantitative analysis, a resolution value greater than 1.5 is highly desirable [3].
- The LOD & LOQ was used to measure the lowest amount of compound that could be detected and quantified, respectively.
- The R^2 was used to measure the linearity of the HILIC method. The high R^2 coefficient values observed indicate that the method is suitable for the quantification of metabolites.
- Based on our data, the validation of our analytical method proved to be acceptable.

Future Applications

We aim to apply our validated HILIC method to biological *E. coli* extracts.

Acknowledgements

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