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WORLD BREWING CONGRESS 2016

LC/MS Metabolomic Profiling of an Amber Ale Fermented with Four Different Yeast Strains

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World Brewing Congress
August 13-17, 2016
Sheraton Downtown Denver
Denver, CO 80202, U.S.A.

Overview

Metabolomic profiling of beer by LC/MS has largely focused on the hops used[1] or compositional changes that occur during storage[2], yet brewers yeast is known to contribute over 500 flavor-active compounds to beer. Here we profiled a 20-barrel batch of an amber ale that was equally divided and fermented by White Labs with Bedford British ale yeast, Dusseldorf alt yeast, Tennessee whiskey yeast and Abbey ale yeast. Untargeted metabolomic profiling was conducted by positive and negative ion ESI LC q-TOF MS. Mass Profiler Professional was used for differential analysis and metabolic pathway analysis. Pathways related to carbohydrate and amino acid metabolism, which are known to contribute to flavor and aroma in beer, were of primary interest.

Objectives

Short-term objectives:
- To identify metabolic pathways that differentiate the yeasts: Bedford British ale, Dusseldorf alt, Tennessee whiskey and Abbey ale
- To obtain Level 1 identifications (retention time and MS/MS spectral matching to authentic standards) for metabolites

Long-term objectives:
- To correlate differences in yeast metabolism to the different flavor profiles
- To assimilate genomic and metabolomic data obtained for the four different yeast strains

Brewing & Fermentation

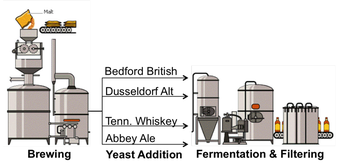


Figure 1. Fermentation with different yeast strains.[3] After brewing, a 20-barrel batch of amber ale was divided and fermented with four different yeast strains.

Untargeted Metabolomic Workflow

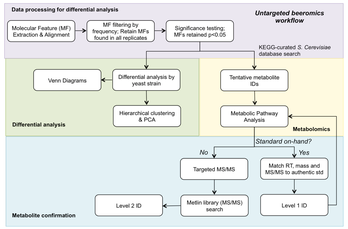


Figure 2. Untargeted metabolomics workflow. Data was collected on an Agilent 6530 q-TOF MS in both positive and negative ESI modes. Agilent's Mass Profiler Professional software was used for differential analysis and for metabolic pathway analysis.

Results

Differential Analysis by Yeast Strain

Principle Component Analysis

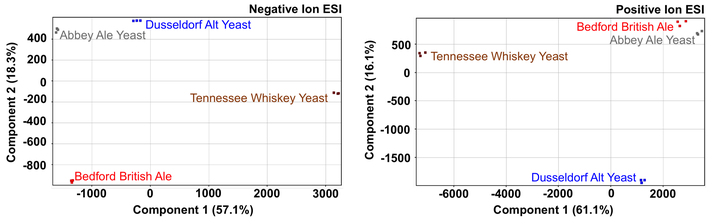


Figure 3. PCA plots for positive (right) and negative (left) ESI data. The Tennessee whiskey yeast was least similar to the other strains in P1, largely due to the increased number of features unique to this yeast (see Figure 4). In P2, the Dusseldorf Alt and Abbey ale yeasts cluster together in the negative ion data and the Bedford British and Abbey ale yeasts cluster together in the positive ion data. Generally, basic compounds are ionized in positive ESI and acidic compounds are ionized in negative ESI.

Venn Diagrams

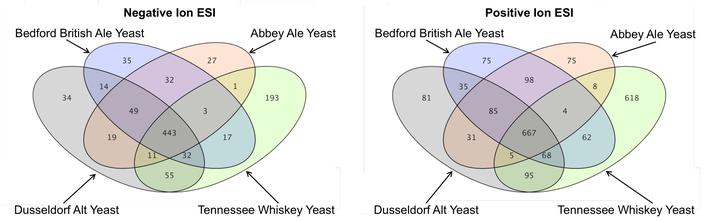


Figure 4. Venn diagrams for positive (right) and negative (left) ESI data. After filtering and significance testing there were 2007 and 965 MFs in the positive and negative ion data, respectively. 33% (pos) and 46% (neg) MFs were found in all yeast strains and replicates (n=12). The Tennessee Whiskey yeast has the most unique MFs.

Hierarchical Clustering

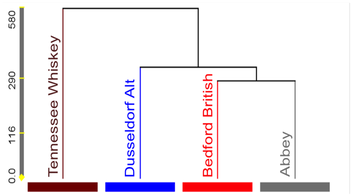


Figure 5. Hierarchical clustering for positive ion ESI data. The Abbey ale and Bedford British yeasts exhibited the greatest compositional similarity, while the Tennessee Whiskey (as seen in Figs. 3-4) is the most different. The same clustering was observed in the negative ion data (not shown). The Tennessee Whiskey yeast was isolated from a distilling setting while the other yeasts were isolated from a brewing setting. This may explain why the Tennessee Whiskey yeast is so different.

Metabolic Profiling

Table 1. Metabolic pathways with two or more metabolites matched (based on accurate mass) to KEGG-curated *S. cerevisiae* library.

Pathway	Total number of metabolites/pathway	# database hits Positive ESI	# database hits Negative ESI	# database hits Both Pos/Neg	% Coverage
Isoleucine, leucine, and valine biosynthesis	8	2	0	0	25%
Arginine degradation	9	1	1	0	22%
Isoleucine and valine biosynthesis	8	2	0	0	25%
Methionine degradation	16	3	1	0	25%
Phenylalanine, tyrosine, tryptophan biosynthesis	17	1	0	3	24%
Phenylalanine and tyrosine biosynthesis	10	0	0	2	20%
Phenylalanine degradation	7	1	0	1	29%
Polyamine biosynthesis	6	2	0	0	33%
Tryptophan biosynthesis	8	1	0	1	25%
Tryptophan degradation dia kynurenine	8	0	1	1	25%
Tryptophan degradation	5	0	0	2	40%
Sulfur amino acid biosynthesis	15	2	1	0	33%
Histidine biosynthesis	9	1	1	0	22%
Threonine and methionine biosynthesis	11	1	1	0	18%
De novo NAD biosynthesis	11	1	1	1	27%
NAD salvage pathway	17	1	1	1	18%
Principle pathways of carbon metabolism	37	0	5	0	14%
TCA cycle	11	0	4	0	36%

Amino Acid Metabolism

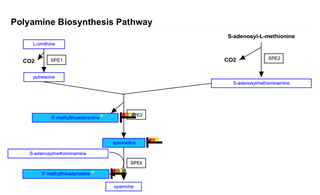


Figure 6. Initial efforts focused on polyamine synthesis due to its involvement in the production of 5-methylthioadenosine (5-MTA), which may serve as a marker of beer aging during storage.[2] Putrescine can be formed by L-ornithine by ornithine decarboxylase. Spermidine (matched to library but not confirmed with a standard) is formed by the addition of a propylamine moiety to putrescine, catalyzed by spermadine synthase. The source of the propylamine group is decarboxylated S-adenosyl-L-methionine. The other product of the aminopropyltransferase reaction is 5-MTA, which is recycled back to L-methionine.[4] *ID confirmed by RT-matching to authentic standard.

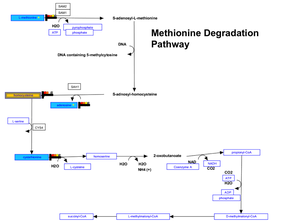
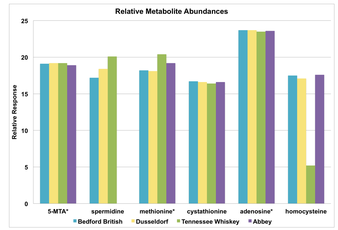


Figure 7. Three metabolites from the methionine degradation pathway[5] were matched to the KEGG-curated library: methionine, adenosine and cystathionine. Methionine and adenosine (*) were RT-matched to standards.

Figure 8. Relative abundance (log scale) of metabolites (matched to KEGG-curated library) from polyamine biosynthesis (Figure 6) and methionine degradation (Figure 7) pathways. The metabolites (with the exception of spermidine) were found in all yeast strains at fairly consistent concentrations. The greatest difference was observed for homocysteine; its concentration was 70% less in the ale made with Tennessee whiskey yeast. Metabolites methionine, cystathionine and homocysteine are also involved in the sulfur amino acid biosynthesis (Table 1). Methionine and homosysteine are also metabolites in threonine and methionine biosynthesis (Table 1).



Yeast Genomics

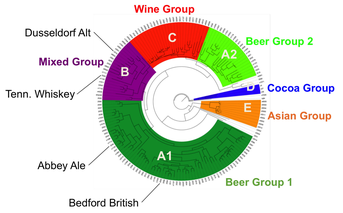


Figure 9. Phylogenetic tree for White Labs yeast strains. The Abbey ale and Bedford British yeasts both fall in Beer Group 1, although in different branches. These two strains cluster together in Figure 5. Dusseldorf alt and the Tennessee whiskey yeasts both fall into the mixed group. While they are in different branches in the hierarchical cluster (Figure 5), they are neighbors.

Conclusions

- The composition of the amber ale made from the Tennessee whiskey yeast differs significantly from the beers made from the other yeasts. This is likely the result of its isolation in a distilling setting vs. a brewing setting.
- The pathways investigated thus far (polyamine synthesis and methionine degradation) do not differentiate the yeast strains. Perhaps the other pathways in Table 1 will afford more differentiation.
- Similar clustering was observed for metabolomic (Figure 5) and genetic data (Figure 9) that warrants further investigation (see Future Work).

Future Work

- Use untargeted metabolomics to investigate how volatile (GC/MS) and nonvolatile (LC/MS) metabolites change during fermentation for beers brewed with genetically different yeast strains.

References

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2. Heuberger, A. L., Broeckling, C. D., Lewis, M. R., Salazar, L., Bouckaert, P., & Prenni, J. E. Food Chem. 2012, 135, 1284–1289.
3. Figure adapted from http://www.calhouns.com/microbrewery.html (accessed on March 7, 2016).
4. http://pathway.yeastgenome.org (accessed on March 7, 2016).
5. http://www.wikipathways.org/index.php/Pathway:WP46 (accessed March 3, 2016)

Acknowledgments

Agilent Technologies
NSF CHE-0922935 (MRI)
NSF CHE-1046630 (MRI)