

Supplementary Figures

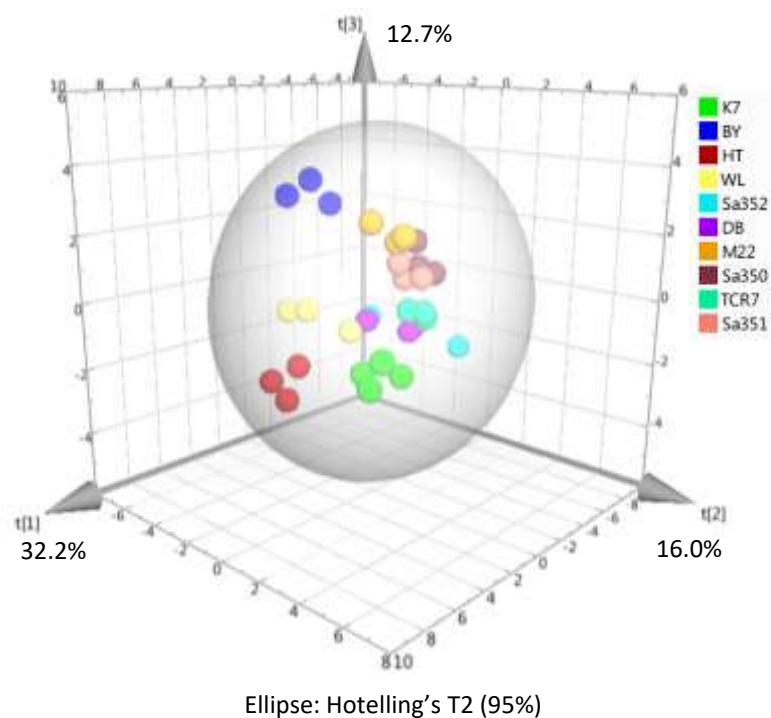


Figure S1. 3D PCA Score Plot of the *Saccharomyces* Yeast Endogenous Metabolites. The PCA plot has three PCs representing 32.2%, 16%, and 12.7% of the sample variances. Yeast strains are labeled by color.

Table S1. Endogenous Metabolite Loading Matrix.

Endogenous Metabolite	M1.p[1]	M1.p[2]
serine1	0.298183	0.151295
threonine1	0.270912	0.24956
nicotinic	0.084131	0.304491
glycine	0.164934	0.323333
succinic	0.046036	0.125748
uracil	0.150956	-0.29857
fumaric	0.296116	0.167411
serine2	0.307159	0.029229
threonine2	0.295011	0.035795
methionine1	0.209894	-0.26806
aspartic1	0.243726	-0.16329
malic	0.258265	0.198813
aspartic2	0.265834	-0.28568
pyroglutamic	0.249486	-0.30179
phenylalanine1	0.187078	-0.24547
glutamic acid2	0.171301	0.008042
citric	0.270136	-0.03397
adenine1	0.134758	-0.00263
glucose1	-0.1288	-0.25644
glucose2	-0.13053	-0.21503
m-inositol	0.076818	-0.02
sucrose	-0.05538	0.249083
trehalose	-0.0911	0.189166

S. cerevisiae M22

S. arboricola 350

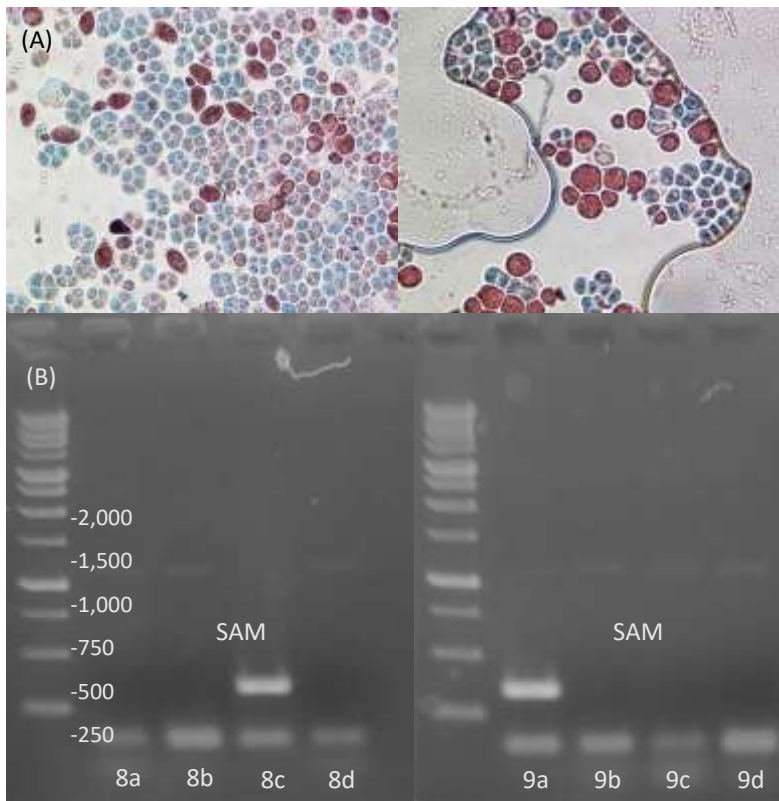


Figure S2. Spore Staining and PCR DNA Confirmation. (A) Light microscope images of spores stained by malachite green with vegetative cells counter stained with safrain. **(B)** PCR confirmation of hybrid status by species specific primers. SAM 8c and SAM 9a were confirmed by having bands for both *S. cerevisiae*, 150bp, and *S. arboricola*, 340bp.

Table S2. Connecting Letters Report of Significance for Sake Fermentations. Samples that have matching letters are not significant, samples that do not have the same letter are significant (alpha= 0.05).

Day	<i>S. c</i>	<i>S. a</i>	Hybrid
1	A	A	A
2	A	A	A
3	A	A	A
4	A	A	A
5	A	A	A
6	A	A	A
7	A	A,B	B
8	A	A,B	B
9	A	A,B	B
10	A	A,B	B
11	A	A,B	B
12	A	A,B	B
13	A	A,B	B
14	A	A,B	B

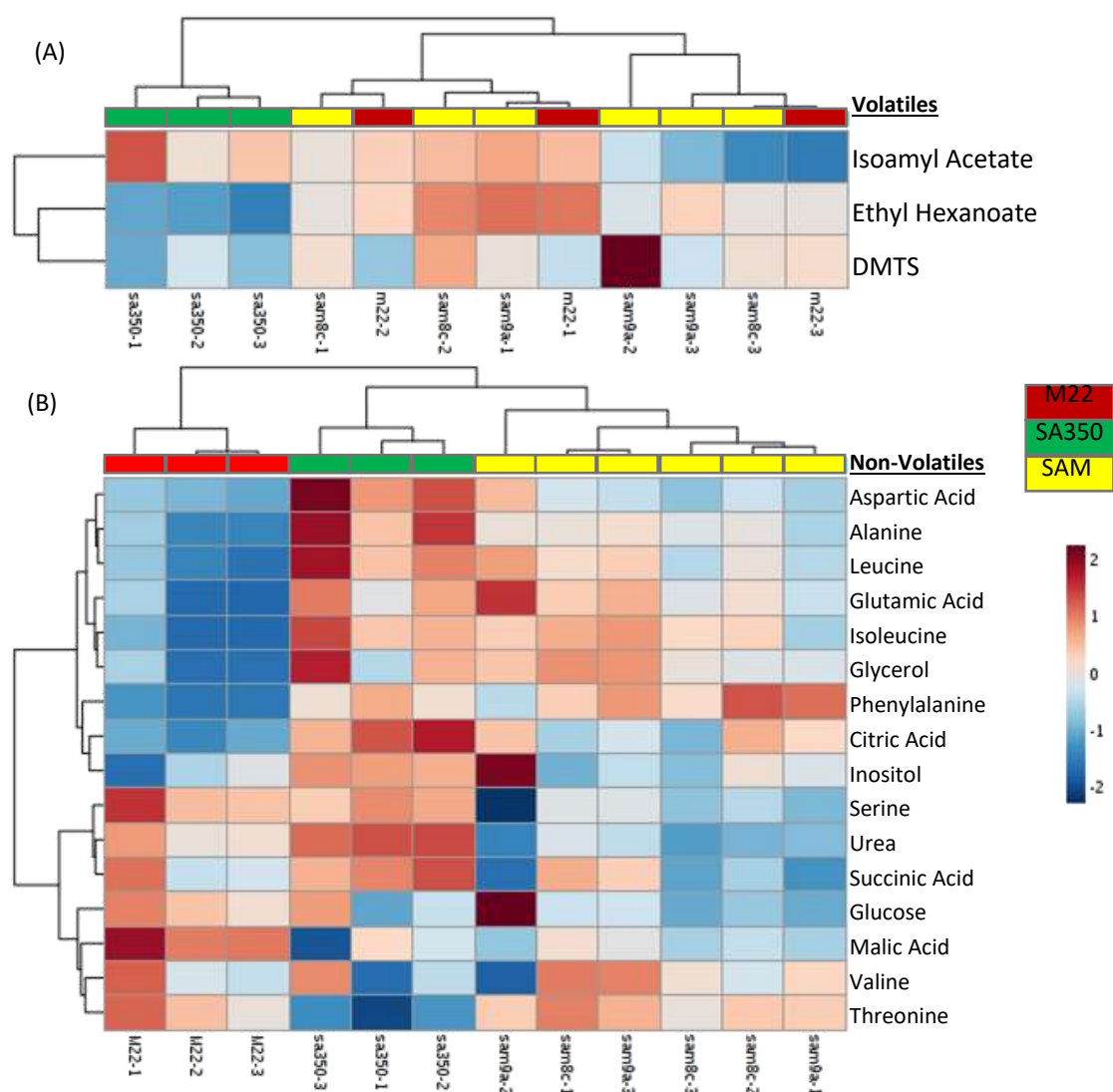


Figure S3. Sake Fermentation Metabolic Profile of *S. arboricola* Interspecific Hybrids and Parental Strains Sample by Sample. (A) Hierarchical clustering heatmap of sake fermentation volatile profiles between strains and species. (B) Hierarchical clustering heatmap of sake fermentation non-volatile profiles between strains and species. Relatively high compound concentrations were shown in varying degrees of red. Relatively low compound concentrations were shown in varying degrees of blue. Shown as individual sample concentrations of three biological replicates each.

Table S3. Sake Metabolite Loading Matrix

Sake Metabolite	M1.p[1]	M1.p[2]
valine	0.039308	-0.03906
alanine	-0.32672	0.026332
leucine	-0.32873	0.000774
isoleucine	-0.29454	-0.11937
urea	-0.0258	0.28709
glycerol	-0.26263	-0.12227
succinic acid	-0.15761	0.335353
threonine	0.253427	-0.0692
aspartic acid	-0.31879	0.115173
malic acid	0.246756	0.290595
glutamic acid	-0.28849	-0.11192
serine-3TMS	0.00122	0.4507
phenylalanine	-0.16184	-0.3145
citric acid	-0.28593	0.003946
glucose	0.046795	0.311003
inositol	-0.27947	0.047063
isoamylacetate	-0.12037	0.165932
ethyl caproate	0.268673	-0.20688
DMTS	0.121216	-0.31587
ethanol	-0.05329	-0.29592