AIP – Activity 9 Final report: Classic breeding of low volatile acidity and low hydrogen sulfide producing yeast strains

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Descriptive Analysis and Quality Evaluation of three Experimental Wines

We have successfully bred a low VA (acetic acid) and low H2S wine yeast strain using classic mutations. These two yeast strains were backcrossed and a low VA/H2S yeast was obtained. Details from these experiments have been fully reported. We then produced experimental wines at Calona Wines in the Okanagan with the help of Howard Soon, winemaker. The wines were vinified using the same Chardonnay must and three distinct yeast strains. Two of the three yeast strains utilised were parental strains bred for their low VA and low H2S phenotypes, respectively. The third yeast, and offspring of the two parental strains, was produced through backcross breeding; the aim was to obtain a wine yeast with low VA as well as low H2S characteristics. An expert panel of 8 judges evaluated the wines in duplicate.

The blind tastings took place at the B.C. Wine Information Society Sensory Centre located at Okanagan College's Penticton campus. Wines were rated for 14 sensory attributes on an unstructured 10-cm linear scale. The panelists also scored the wines for perceived quality (scores out of 20 points) using a scoring sheet adapted from the UC-Davis 20-point scoring sheet.

The chosen design of experiment for this study was a balanced block design with two replications; the judges tasted all wines in duplicate. The wines were served concurrently and assessed sequentially. ISO wine glasses were labeled with 3-digit codes and presented to panelists in a randomised order; Petri dishes were used to cover the wines prior to the evaluation. Wines were poured at a temperature of 18°C during the first evaluation, and 45 minutes prior to sensory analysis. The wine temperature was recorded to be a few degrees warmer (20°C) at the time of the assessment on the second replication. Each sample consisted in approximately 30 ml of wine. The descriptive profiling and quality assessment were conducted separately in order to avoid the introduction of biases in the sensory judgement. Samples were first evaluated by olfaction then assessed (by mouth) and expectorated.

Three-way Analysis of Variance (ANOVA) computations (Minitab 16) showed that the wines differed in their acidity ($p=0.004$, full data set) and floral character ($p=0.053$ computed with the scores of 5 judges). All other attributes were not significant ($p\leq0.05$). A graphical representation of the wines' sensory profiles is shown in Figure 1 (oak-related descriptors are not represented since the wines were perceived to be unoaked). Least Significant Difference (LSD) calculations demonstrated that the wine made with the offspring yeast (H4-10A) was the most acidic of the flight and differed significantly from the wine made with the low H2S-producing yeast. In addition, the floral character of the wine produced with the Bv197-bb3, low acetic acid-producing yeast, was significantly more intense than the wine made with the pr-bwa1 (low H2S) yeast.

The quality assessment revealed that the 3 experimental wines achieved good overall quality scores ranging from 15.53/20 to 16.15/20. A small number of defects were identified in all three wines. The wine made with the Hybrid yeast was slightly effervescent. Two members of the panel reported that both the wine made with the H4-10A (Hybrid) yeast and wine produced with Bv197-bb3 (low acetic acid-producing yeast) exhibited slight sulfur defects. There were also four reports that the wine made with pr-bwa1 (low H2S-producing yeast) was characterised by volatile acidity.

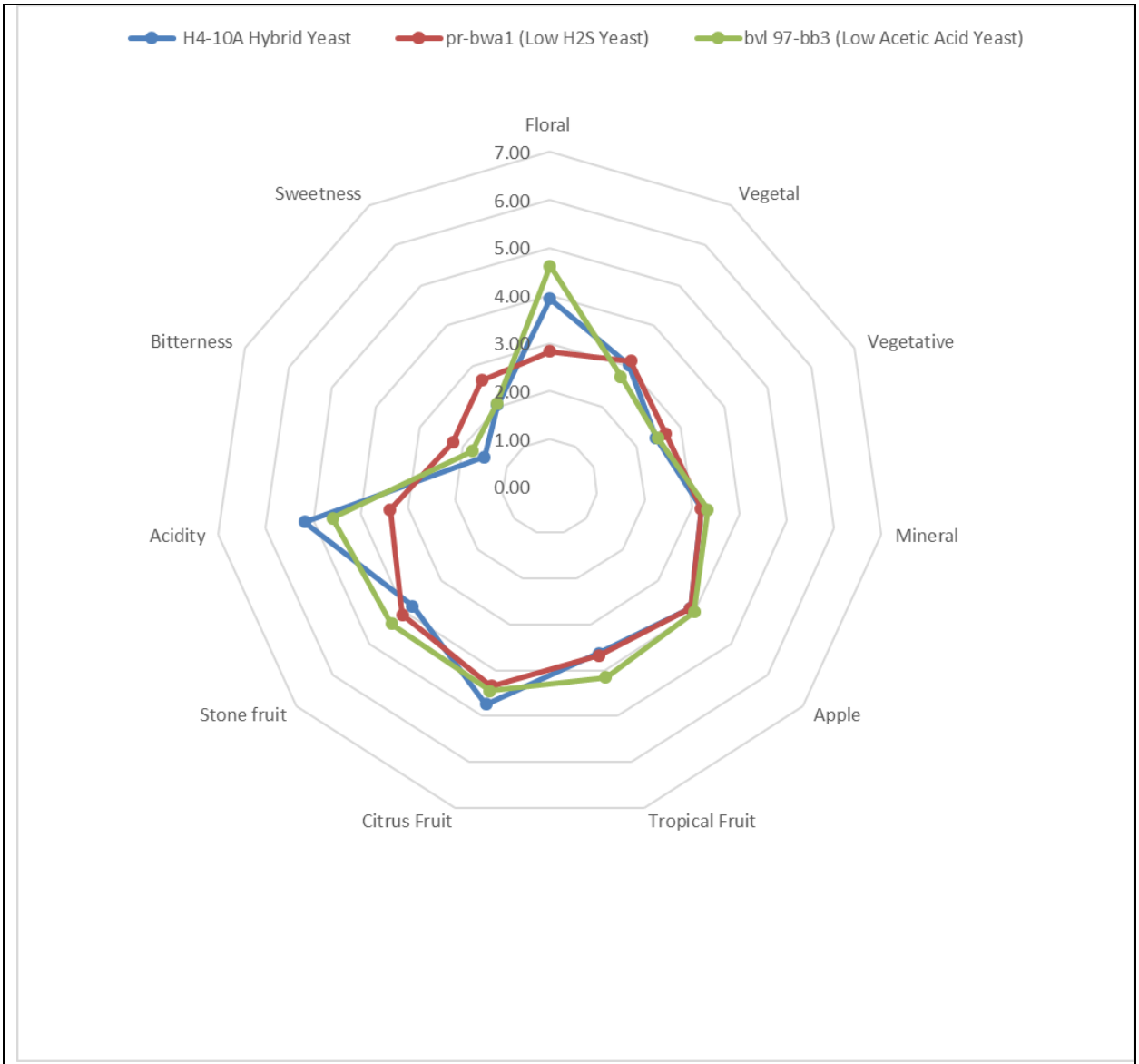


Figure 1: Spiderweb diagram of mean sensory scores (N=16), (N=10 for "floral" descriptor) for 11 sensory attributes (max score= 10) for 3 experimental wines. "Acidity" and "Floral" are significant attributes (p-values of 0.004 and 0.053 respectively).

We are now considering patenting of the lowVA/H2S yeast and licensing of this yeast to a yeast manufacturer. This yeast will be very valuable to winemakers in Canada producing ice wine and normal table wines and I am planning that this yeast be available to winemakers in Canada in 12 months. We will present these data at the BCWGC conference to be held in the Okanagan. We are in the process of writing a publication that will be submitted to the American Journal of Enology and Viticulture.

3. What is your target audience for sharing information about the results of your project? Describe your strategy and success in reaching this target audience.

Initially, I will present the information to winemakers in Canada through lectures at wine conferences to be held. I will also write a report in Canadian wine magazines read by many winemakers. Finally I intend to present talk at the Wine Conference in California and publish a scientific paper in the American Journal of Enology and Viticulture.

Background Information

Hydrogen sulfide and acetic acid are common spoilage compounds produced by yeast during fermentation in conditions of stress and nutrient deprivation. Yeast assimilable nitrogen (YAN) present in grape juice, is an important nutrient required for yeast assimilation of amino acids. Grape juice containing low levels of YAN can be supplemented with ammonium salts such as Diammonium phosphate but can lead to yeast production of the carcinogen ethyl carbamate (Coulon et al, 2006). Nitrogen deficient grape must can lead to yeast production of hydrogen sulfide (H_2S), known for the "rotten egg" aroma in finished wine (Linderholm et al, 2010). Production of acetic acid, the major component of volatile acidity (VA), produces a "vinegar" aroma and flavor in wine, and has also been linked to nitrogen deficient grape must (Cordente et al, 2013). Previously, wine yeasts with the ability to withstand these conditions were collected from nature or genetically manipulated. A different approach may involve selection of strains that carry these beneficial traits, and integrating each trait

into a single novel strain through a classical breeding strategy. To perform a large screen and select for a low VA producing strain, small micro scale wine fermentation trials can be performed (Liccioli et al, 2011). Furthermore, the differential yeast growth media BiGGY agar can be utilized to select for a strain of yeast lacking sulfate reductase activity (Linderholm et al, 2010). In this study we utilized both methods to screen the yeast progeny previously produced from crossing a low acetic acid producer and a low H2S producer. Through a series of laboratory and winery based fermentation trials, our results conclude that the hybrid strain H4-10A consistently produces minimal levels of H2S and acetic acid in Chardonnay wine without any nutrient supplementation, an elegant solution for winemakers to avoid problematic fermentations.

Concise analysis

In this analysis we screened the offspring previously derived from mating the low H2S producer: pr-bwa1, and the low VA producer: brl97-bb3. To test for the low H2S phenotype, the hybrid spores were spotted onto BiGGY agar to identify those unable to reduce sulfate to sulfide. Micro-titre plate fermentation trials in Chardonnay grape juice were performed to assess a large number of hybrid spores for the production of acetic acid at the end of fermentation. A single haploid spore that carried both the low H2S and low VA phenotype was backcrossed with the low VA parental strain brl97-bb3.

Lastly, the hybrid and parental strains were assessed for production of VA and H2S in 30 litre fermentation trials of Chardonnay at Sandhill winery in Kelowna, BC. Acetic acid was measured throughout the course of fermentation. Finished wine produced from each strain was assessed for H2S and other volatile sulfide based compounds. Triangular taste tests were performed on the finished wine to determine if a difference in flavor/aroma was present.

Main conclusions:

Following growth on BiGGY agar the hybrid strain H4-10A consistently lacked sulfate reductase activity (white colony color) as observed in the low H2S parental strain pr-bwa1. Micro-fermentation trials of H4-10A resulted in less acetic acid (0.24 g/L) present at the end of fermentation then the low H2S strain pr-bwa1 (0.89 g/L) and a similar to the low VA parental strain brl97-bb3 (0.15 g/L).
In assessing acetic acid production at the end of 30L Chardonnay pilot fermentation trials, we found that the hybrid strain produced 0.16 g/L of acetic acid, less than that of the parental strain pr-bwa1 (0.37 g/L), and similar to brl97-bb3 (0.12 g/L). Testing for sulfide compounds resulted in no detectable amount of hydrogen sulfide present in wine produced by either the parental strains or the hybrid strain (<0.5 ppb). Although no difference in hydrogen sulfide was present between the 3 strains, the low VA parental strain brl97-bb3 was not defective in the sulfate reductase pathway as seen by brown colony color development on BiGGY agar, indicating the possibility to form H2S in

wine is indeed present in this strain.

Success Story - A success story presents a significant result or an important milestone achieved. It is intended to showcases achievements in applied research. Focus on research results, successful technology transfer, potential for pre-commercialization, and/or potential impact. A Success Story is not a progress report for each activity (suggested length 2 – 3 paragraphs).

In this study we screened the progeny of 2 previously mutated and commercially significant wine strains that produced ether low acetic acid or low H_2S in Chardonnay wine. After culturing the hybrid spores on BiGGY agar and in small volumes of Chardonnay wine juice (126 mg/L N), we isolated the strain H4-10A that lacked sulfate reductase activity as seen in the low H_2S parental strain, and produced low acetic acid as seen in the low VA parental strain.

We assessed the ability for the hybrid strain H4-10A to produce acetic acid in 30L fermentation trials in Chardonnay wine juice at Sandhill winery. We found that the acetic acid present at the end of fermentation was 0.16g/L, a similar level as seen in the low VA parental strain brl97-bb3 (0.12 g/L). Wine produced with H4-10A further contained undetectable amounts of hydrogen sulfide (<0.5 ppb). Triangular taste tests performed at Sandhill winery determined that H4-10A produced wine that tasted significantly different then that produced by strain pr-bwa1 (p- value=0.07), and different from brl97-bb3 (p-value = 0.2). A full sensory evaluation of wines will be done by Sirocco consulting in the Okanagan.

2. Objectives/Outcomes (technical language is acceptable for this section)

Provide a brief summary that includes introduction, objectives, approach/methodology, deliverables/outputs, results and discussion, and any Ph.D or Master students recruited to work on the project.

Introduction

The most popular strains of yeasts to use in wine production are those that result in unique and satisfying flavors and aromas in finished wine. Yeast strains are further chosen based on their tolerance to the conditions of oenological stress encountered during fermentation. One such stress is the availability of nutrients in the grape juice for yeast to utilize. Yeast development requires nitrogen and sulfur for the assimilation of sulfur containing amino acids. In nitrogen deficient grape juice, yeast will continue to reduce sulfate to sulfide via the sulfate reductase pathway, which is rather converted into the spoilage compound H_2S than incorporated into amino acids. (Linderholm et al, 2010). Yeast production of the spoilage compound acetic acid can also reach high levels in the presence of nitrogen deficiency, along with other factors such as sugar content and pH of the grape juice (Cordente et al, 2013). Previously, wine yeasts with the ability to withstand these conditions were

collected from nature or genetically manipulated; the former requiring a level of effort, the latter becoming an unpopular commodity. A more efficient approach can involve breeding strains of yeast together that carry these desirable traits, to produce an enologically important hybrid strain. Screening a multitude of hybrid spores can be performed using micro-scale fermentation trials previously found to achieve significant results in volumes of wine as small as 1 millilitre of culture medium (Liccioli et al, 2011).

In this study we screened the yeast progeny of a low acetic acid and a low H_2S producer, to find a single haploid spore that produced low levels of both compounds after fermentation in Chardonnay grape juice. We utilized micro-scale fermentation trials to assess yeast production of acetic acid. Yeast sulfate reductase activity was assessed by growth and color development on BiGGY agar. Lastly, winery based fermentation trials were conducted with the hybrid and parental strains to assess production of acetic acid and H2S in 30L volumes of chardonnay wine. Our results conclude that the hybrid strain H4-10A contains both the low acetic acid and low H2S phenotype of the parental strains pr-bwa1 and brl97-bb3, respectively. We found that H4-10A produced only 0.16g/L of acetic acid in 30L volumes of chardonnay wine, similar to the parental strain brl97-bb3 (0.12 g/L), and less then pr-bwa1 (0.37 g/L). The hybrid strain H4-10A not only produced minimal acetic acid, but also and undetectable levels of hydrogen sulfide (<0.5 ppb) in finished wine.

Approach/methodology

BiGGY analysis and micro-fermentation trials of F1 hybrid spores

Each diploid hybrid colony was cultured on 2% potassium acetate media for 5-10 days to induce sporulation. At the appearance of tetrads, the cultures were treated with zymolase and spotted onto YPD agar plates for tetrad dissection. After incubation at 30 degrees Celsius for 3 days, each spore was spotted onto BiGGY agar to identify those pure white in color. Each white spore was then inoculated into 3ml of liquid YPD and incubated for 18 hours with agitation. Cultures were then diluted to an initial OD of 0.2 into fresh 3ml of liquid YPD, and grown to a final OD of 1. The cultures were pelleted, washed twice, and inoculated at a cell concentration of 10^6 cells/mL in a final volume of 2 mL of chardonnay grape juice in micro-titre plates in triplicate. Each plate was covered with sterile breathable sealing membranes, and left in an anaerobic jar at 20 degrees Celsius. After approximately 8-12 days, the wine cultures were sterile filtered and analyzed for residual sugar, and acetic acid by HPLC.

Backcrossing H5-39b with brl97-bb3

The hybrid spore H5-39b was back-crossed to retrieve a strain more genetically similar to the parental strain brl97-bb3. To track mating, brl97-bb3 was transformed with plasmid pYC conferring resistance to Hygromycin, and H5-39b was transformed with the plasmid phVX2 conferring resistance to

Geneticin. Sporulation of both strains was ensued on 2% Potassium acetate agar for 7 days at 19 °C. Both cultures were treated with Zymolase at the appearance of tetrads, and incubated together for 24 hours in liquid YPD for mating. The mated culture was streaked on antibiotic plates containing 300 µg/mL of Hygromycin and 300 µg/ml of Geneticin to select for hybrid offspring. Once growth was achieved, sporulation was induced in the hybrid culture by growing the culture on 2% Potassium acetate media for 7 days. At signs of tetrad formation the cultures were treated with Zymolase for use in tetrad dissection and analysis of haploid spores. Screening the spores for the low H2S and low VA phenotypes was performed as described earlier.

Sandhill 30L Chardonnay wine production

Liquid yeast cultures for H4-10A, brl97-bb3, and pr-bwa1 were produced by inoculating a single isolated colony of each strain into 50 ml liquid YPD and incubated aerobically at 30 degrees Celsius. After 18 hours, an aliquot of each 50 ml culture was added to 500ml of fresh liquid YPD at an initial OD of 0.2 and grown to a final OD of 1 at 30 degrees Celsius. An aliquot of the 500mL culture was then inoculated at an initial OD of 0.2 into 1L of yeast growth media containing 50% sterile filtered Chardonnay grape juice, 50% sterile filtered water, 1% yeast extract, and 1% Bacto-peptone media. Cultures were grown aerobically at 30 degrees Celsius and harvested at an OD of 20, equivalent to achieve a final concentration of10^7 cells/mL in 30 L of grape juice. Cultures were centrifuged at 4000rpm, washed twice with sterile water, and left for storage at 4 degrees Celsius in sterile 50 ml plastic centrifuge tubes covered with sterile water. The liquid cultures were transported in an ice box for 24 hours at a temperature of 4 degrees Celsius to Kelowna, BC. Liquid cultures were stored in a refrigerator at Sandhill winery for 5 days at a temperature of 2.7 degrees Celsius. Sandhill Chardonnay grapes (Clone 76 from Block 11-South) were harvested on September 1st, 2016, and brought to Sandhill winery for whole cluster press. The juice was cold settled at 4 degrees Celsius in tank 9A-08 for 24 hours, and racked to tank 10-01. 1 Litre of juice was taken from tank 10-01, added to 3 separate 2 Litre glass flasks, and left to come to room temperature. The liquid yeast cultures were added to each of the 2L glass flasks, and fitted with an airlock. 30 litres of juice was taken from the top of tank 10-01, added to each of the 50 L fermentation tanks, and sealed with their floating lids for transport to an isolated room for inoculation of the yeast starter cultures. After 2 hours, each starter culture was added to 30 litres of Chardonnay grape juice at a temperature of 15 degrees Celsius. Cooling Blankets, previously purchased from Cool Brew, were connected to a tap water source and wrapped around each 50L tank to maintain a fermentation temperature of 13-15 degrees Celsius. Samples of fermenting juice (10mL) were taken from each tank from days 1-5, and every 3 days thereafter. Samples were analyzed for residual sugar, acetic acid, and YAN using a Gallery Auto-analyzer. Brix was measured using an Anton Paar-DMA- 35 Density meter. Measurements were logged in Sandhill winery log books after analysis. Fermentation was considered complete when residual sugar reached less than 2 g/L. At dryness, the wine was racked into 23 L sterile glass carboys, bentonite added at a rate of 0.2 g/L, and left to settle for 2 days at room temperature. After settling, each glass carboy was left to cold settle at 4 degrees Celsius for 6 days. The wine was then racked into

new glass carboys, and assessed for pH, free and total sulfites. Sodium meta-bisulfate was added to produce a final concentration of 50ppm prior to bottling. Wine from each carboy was filtered using a Buon Vino mini superjet fitted with number 2 pads. The filtered wine was stored in the 23L glass carboys at 4 degrees Celsius for 5 days with addition of dry ice to minimize headspace. Each wine carboy was racked into 750ml glass bottles and corked for storage. Bottles were left for 2 weeks prior to triangular taste testing at Sandhill Winery. Each wine was sent in duplicate to ETS laboratories in California, USA, for GC-MS analysis of sulfides.

Triangular taste tests

To determine if a difference in flavor was present between wine produced via the Hybrid strain H4-10A and the parental strains; a series of preliminary wine tastings were conducted. The 10 tasting panelists were selected from Sandhill winery, and contained a combination of winemakers, laboratory technicians, and entry level sommeliers. Each panelist was given 4 sets of tastings. Set "A" contained 2 glasses of wine produced by brl97-bb3, and 1 glass of wine form H4-10A. Set "B" contained 2 glasses of H4-10A wine, and 1 glass of pr-bwa1 wine. Tasting set "C" contained 2 glasses of wine produced by Sandhill winery chardonnay yeast, the other glass produced by H4-10A. The last tasting set contained 2 glasses produced by pr-bwa1 and 1 from brl97-bb3. Each set was randomly ordered; the order recorded by the experimenter. Each panelist was not aware of the order of the wines, and was instructed to taste each set of wines to determine which wine was different among the 3.

Results/Discussion

To isolate a single hybrid strain that contained both parental phenotypes, we tested approximately 100 individual hybrid spore's on BiGGY agar and in micro-fermentation trials of chardonnay wine. Of all the spores, we found that the spore H5-39B produced the same white colony color as the low H2S parental strain pr-bwa1, while the low VA parental strain produced a brown color (FIG.1). H5-39B also produced the least acetic acid (0.25 g/L) compared to 0.23 g/L produced by the parental strain brl97-bb3, while the parental strain pr-bwa1 produced 0.79 g/L acetic acid (FIG.1). From these results we concluded that H5-39B contained the low H2S and low VA phenotypes as seen in the parental strains. In order to retrieve a hybrid strain more genetically similar to the low VA parental strain, H5-39b was crossed back with the parental strain brl97-bb3. Mating the two strains together successfully produced hybrid culture as seen by growth on YPD agar containing Hygromycin and Geneticin (FIG. 2). From testing the individual haploid spores of the mated culture on BiGGY agar, and performing a multitude of micro-fermentation trials we identified the backcrossed spore H4-10A. We found that H4-10A produced the pure white color on BiGGY agar, and the least acetic acid (0.24 g/L) as compared to the parental strain brl97-bb3 (0.15 g/L), and 0.89 g/L produced by pr-bwa1 (FIG.3).
To assess the performance of H4-10A in producing wine in larger volumes significant for commercial wine production, 30 L Chardonnay fermentation trials were conducted at Sandhill winery harvest

2016. We found that the strain brl97-bb3 was faster in metabolizing the sugars present in the grape juice (FIG.4). The hybrid strain proceeded at a slower rate than brl97-bb3, but faster than pr-bwa1 (FIG.4). All 3 strains were able to reach dryness (<2g/L) after 29 days of fermentation. Ethanol concentration at the end of fermentation for brl97-bb3, pr-bwa1, and H4-10A was 13.19%, 12.98% and 12.98 %, respectively.

Wine produced by H4-10A and the parental strains pr-bwa1 and brl97-bb3 was assessed for its production of acetic acid throughout the entire period of fermentation. We found that at day 2 of fermentation, H4-10A produced a peak level of 0.36 g/L acetic acid that was later metabolized to a final amount of 0.16g/L (FIG.5). In comparison, brl97-bb3 produced a consistent low level of acetic acid, finishing fermentation at 0.12 g/L (FIG.5). The parental strain pr-bwa1 produced the most acetic acid with a peak level of 0.52 g/L, and a final amount of 0.37 g/L (FIG.5).

To conclude that H4-10A was indeed a low H2S producer, we assessed the finished wine for Hydrogen sulfide and other sulfide based compounds. We found that both the hybrid strain H4-10A and the parental strains produced no detectable amount (<0.5 ppb) of Hydrogen sulfide (FIG.6). No detectable amounts of methyl mercapten, ethyl mercapten, dimethyl disulfide, diethyl sulfide or diethyl disulfide were also found in any of the 3 wines (FIG.6). Wine produced with the hybrid strain resulted in more dimethyl sulfide (5.9 ppb), than the parental strains brl-97bb3 (4.3 ppb) and pr-bwa1 (5.65 ppb) (FIG.6).

To determine whether H4-10A produced a wine that tasted different from the two parental strains, triangular taste tests were performed. From a series of tastings, 6 out of 10 panelists correctly identified the wine produced by H4-10A as different from that produced by pr-bwa1 ($p=0.07$). In comparing H4-10A to wine produced by brl97-bb3, only 5 out of 10 panelists correctly identified the two as different ($p=0.2$). In comparing the flavor between the wine produced by the parental strains, 9 out of 10 panelists correctly identified the difference ($p <0.05$). The wine produced from H4-10A was also correctly identified by 10 out of 10 panelists as different from chardonnay produced from Sandhill winery.

In this study we found that H4-10A produced low levels of acetic acid, similar to that of the low VA parental strain, in both micro-scale fermentation trials and large scale winery based fermentation trials. We also found that H4-10A lacks sulfate reductase activity and produced undetectable amounts of hydrogen sulfide in finished wine, as seen in the parental strain pr-bwa1. As predicted the parental strain pr-bwa1 produced undetectable amounts of hydrogen sulfide, and produced the most acetic acid of the 3 strains. Although the parental strain brl97-bb3 produced undetectable amounts of hydrogen sulfide as well, this strain still contained sulfate reductase activity and therefore may produce H2S in other conditions encountered in wine production. Furthermore, H4-10A produced the most dimethyl sulfide of the 3 strains; significantly less than the sensory threshold (30 ppb) and at a level which has been found to rather impart “roundness” and “fruitiness” in wine (ETS laboratories, 2016). Our results conclude that H4-10A can provide winemakers with a tool to avoid producing wine spoiled by yeast production of acetic acid or hydrogen sulfide in conditions of nutrient deprivation.

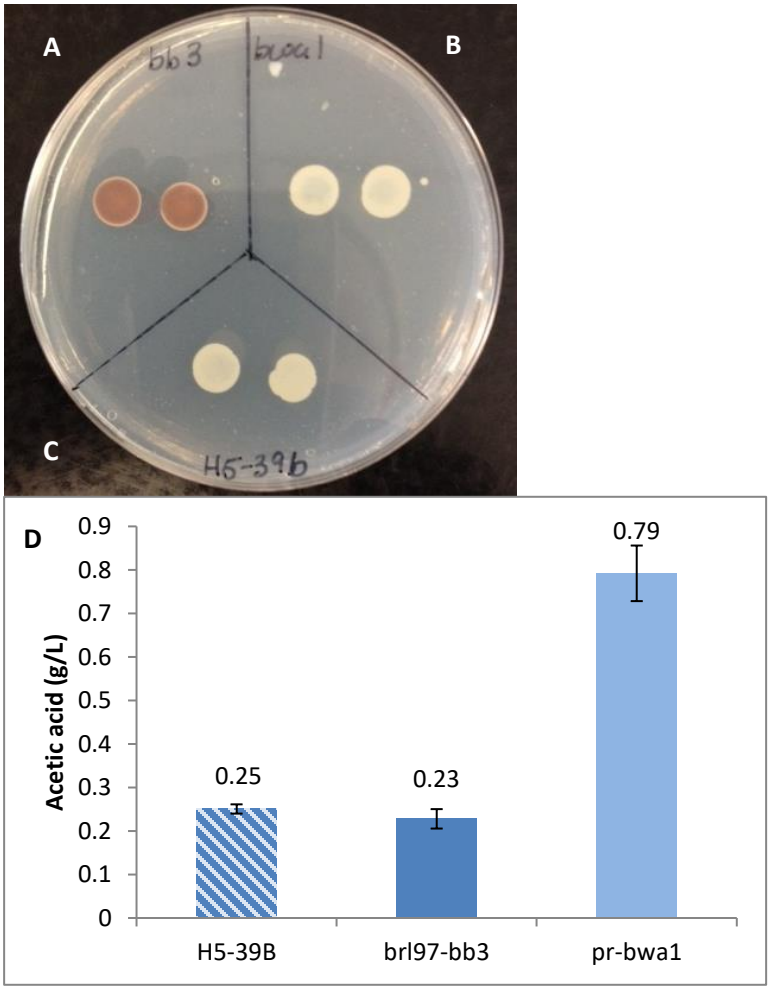


Figure 1. Colony color development of parental strains brl97-bb3 (A), pr-bwa1 (B) and Hybrid spore H5-39B (C) on BiGGY agar. Production of acetic acid at the end of fermentation by H5-39B, brl97-bb3 and pr-bwa1 in 2 mL of Chardonnay wine juice in triplicate (D).

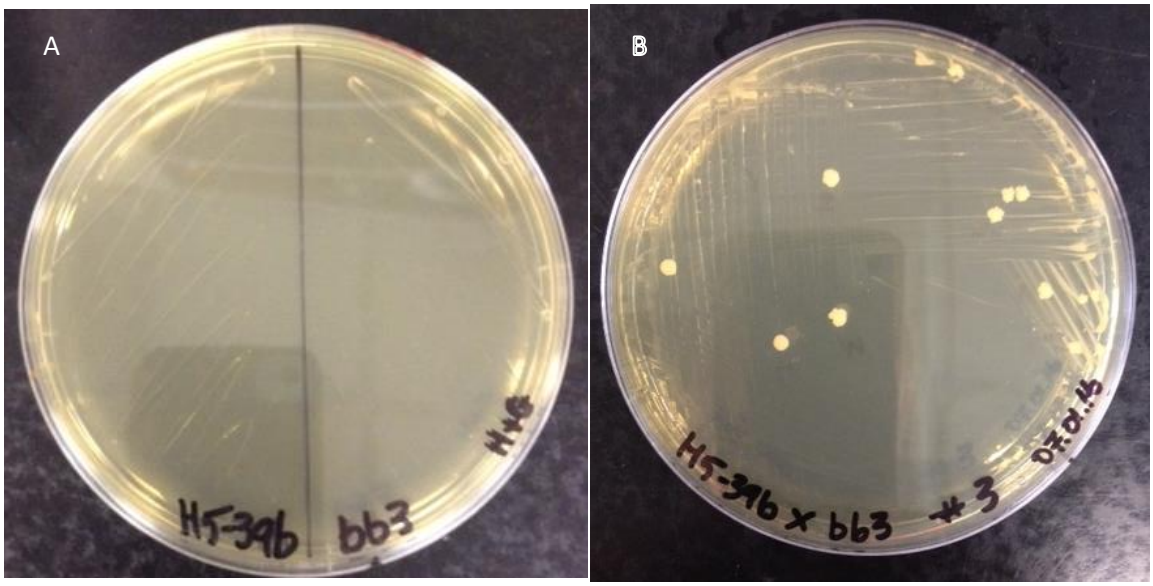
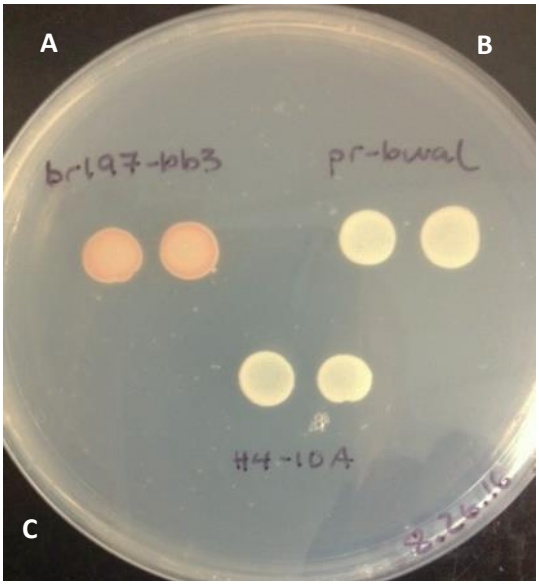


Figure 2. Backcross of H5-39B with parental strain brl97-bb3. H5-39B transformed with plasmid phVX2 (Geneticin resistance), and brl97-bb3 transformed with plasmid PYC (Hygromycin resistance) does not grow on YPD containing 300ug/mL Geneticin and Hygromycin (A). Mated culture produces growth on media containing 300ug/mL Geneticin and Hygromycin (B).



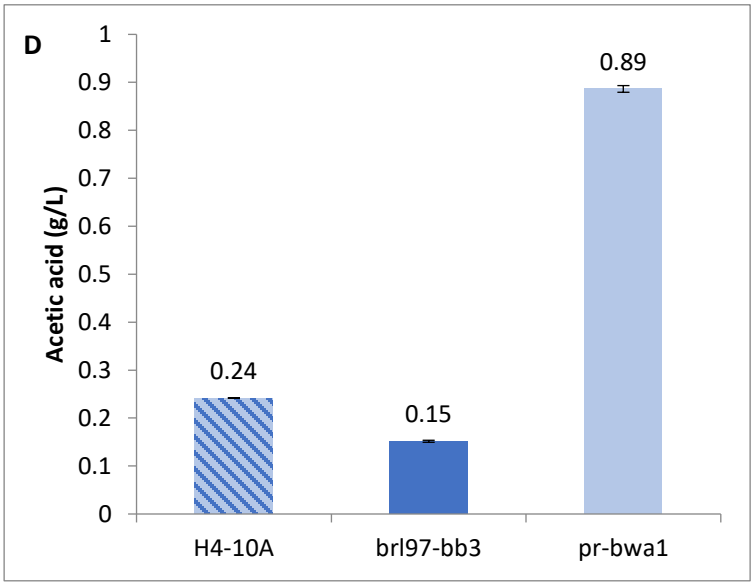


Figure 3. Colony color development of parental strains brl97-bb3 (A), pr-bwa1 (B) and Backcrossed hybrid spore H4-10A (C) on BiGGY agar. Production of acetic acid at the end of fermentation by H4-10A, brl97-bb3 and pr-bwa1 in 2 mL of Chardonnay grape juice in triplicate (D).

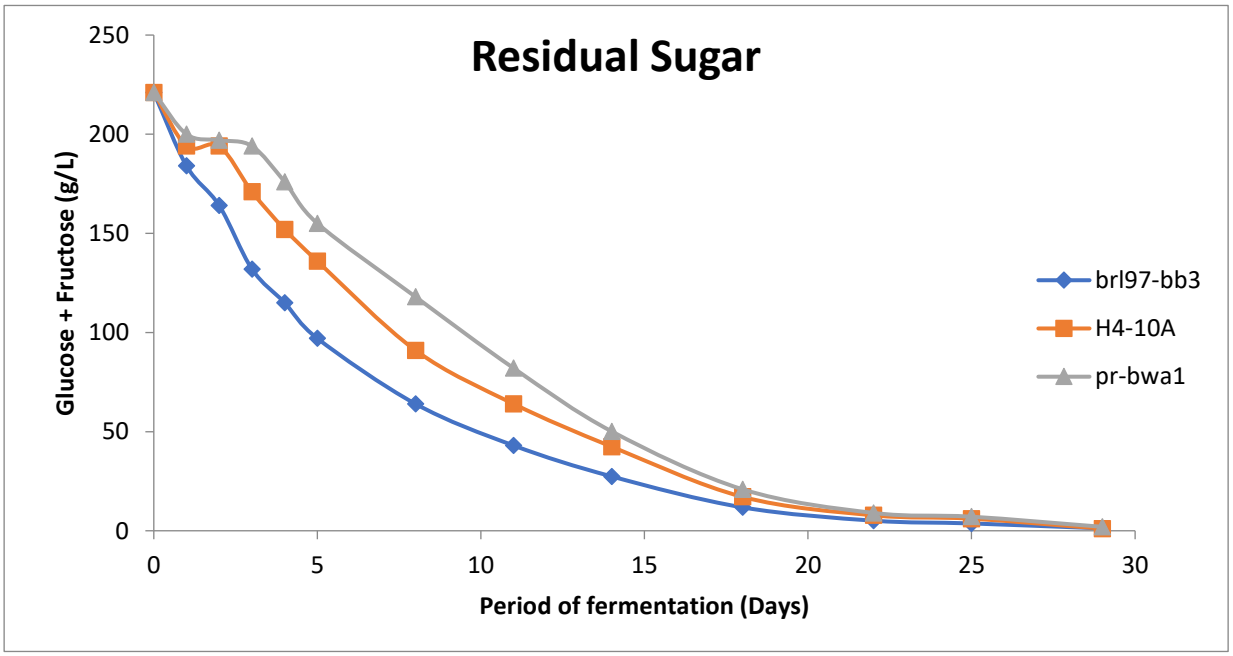


Figure 4. Fermentation rate of parental strains brl97-bb3, pr-bwa1, and the hybrid strain H4-10A in 30L of Sandhill Chardonnay grape juice. Residual sugar is displayed as glucose and fructose (g/L). Fermentation temperature was held at 12 degrees Celsius, and considered complete when residual sugar <2 g/L.

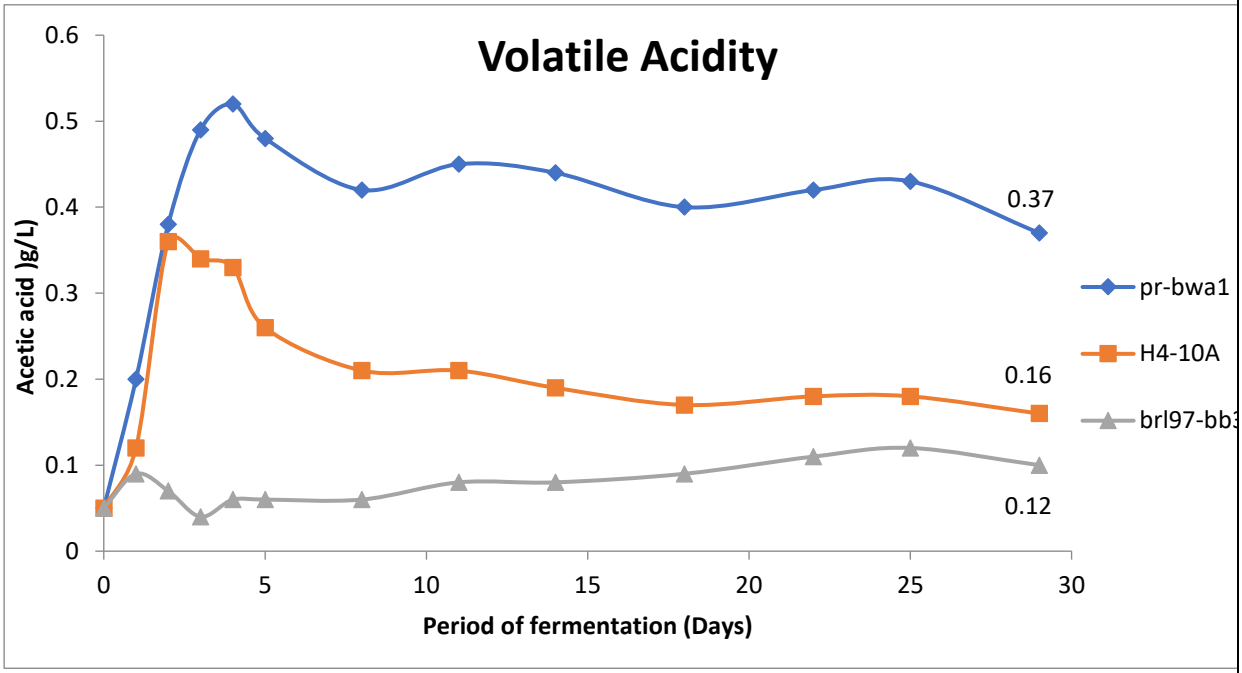


Figure 5. Acetic acid production by parental strains brl97-bb3, pr-bwa1 and hybrid strain H4-10A, during the course of fermentation in 30L of Sandhill Chardonnay grape juice. Fermentation was considered complete at a residual sugar concentration < 2g/L. Temperature was maintained at 12 degrees Celsius.

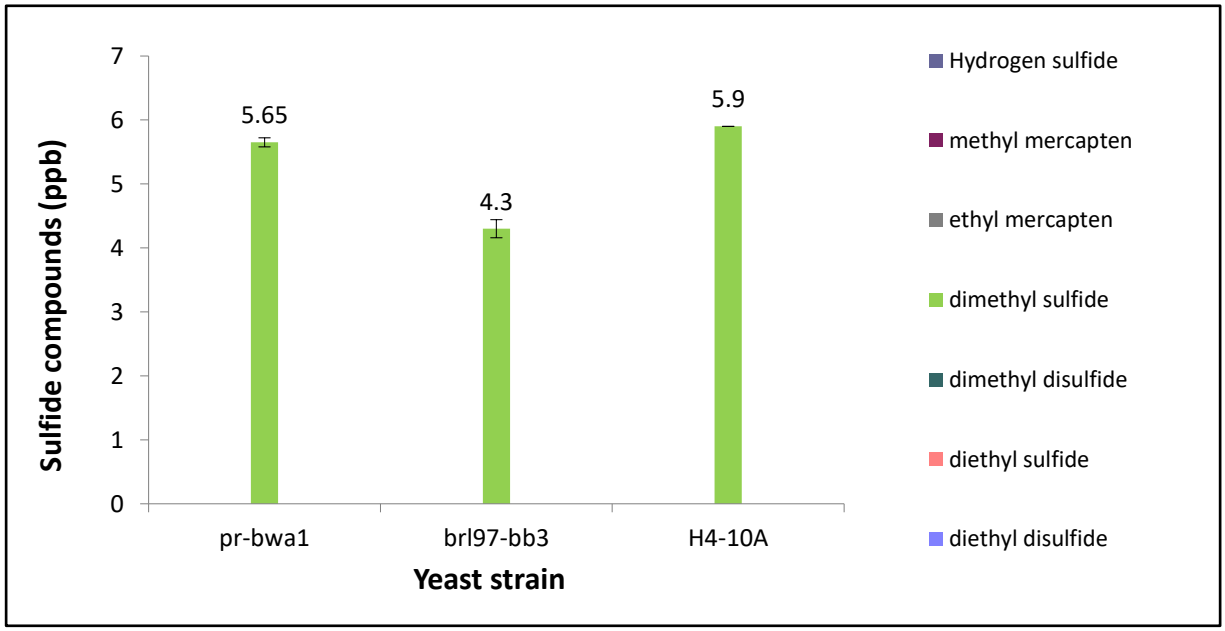


Figure 6. Sulfide compounds present in finished wine produced from parental strains pr-bwa1, brl97-bb3, and the hybrid strain H4-10A in 30L of Sandhill Chardonnay grape juice. Sulfides were measures by ETS laboratories using GC-MS in duplicate. Detection limit for all sulfide compounds except

dimethyl disulfide (1 ppb) was 0.5 ppb.

Literature cited:

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5. Linderholm AL, Dietzel K, Hirst M, Bisson LF (2010) Identification of MET10–932 and characterization as an allele reducing hydrogen sulfide formation in wine strains of*Saccharomyces cerevisiae*. Appl Environ Microbiol 76:7699–7707

3. Issues

- Describe any challenges or concerns faced during the project. How were they overcome or how do you plan to overcome?
- Describe any potential changes to the work plan and the budget. How were or how will they be managed?

The main issue faced during this study was to find the hybrid spore that contained both the parental phenotypes. As both parental strains were mutated via chemical/UV mutagenesis, they contained mutations that decreased spore viability. As such, sporulation and tetrad dissection of the diploid hybrid strain resulted in 2 of 4 viable spores available for testing. This reduced the probability of finding the spore that contained both the parental phenotypes. To achieve our goal, a multitude of tetrad dissections were performed, and a large screen was conducted using small volume micro-fermentation trials. This method allowed approximately 50 spores to be tested at a single time, while utilizing the least amount of grape juice for fermentation. These small scale trials also allowed our

fermentations to be conducted within 8-12 days rather than the common fermentation period of 30 days.

4. Lessons Learned:

Describe the key lessons learned gained as a result of executing the project (e.g., a more efficient approach to performing a specific task for activity / project).

A key lesson learned in this project was the connection between yeast production of H_2S and activity of the sulfate reductase pathway. We saw that in the strain brl97-bb3, consistent brown colony color development occurred on BiGGY agar, indicating that the strain indeed has the ability to reduce sulfate to sulfide and thus has the ability to produce H_2S. Although this pathway was present, it did not lead to production of H_2S after fermentation in 30L of chardonnay wine.

5. Future Related Opportunities:

Describe the next steps for the innovation items produced by the activity/project. Is additional research required? Is there potential for commercialization or adoption?

Once the research is completed, we will consider patenting and commercialization of the hybrid strain and its use in industrial wine production. Sensory analyses of wines will be pursued to assess the flavor and aroma in wine produced by the hybrid strain H4-10A. GC/MS analyses will be conducted to compare volatile compounds produced by all three strains. Studying the genetic pathways of H4-10 may provide more insight into the mutation resulting in the low production of H_2S and acetic seen in the finished wine.