Research Progress Report – Washington State Grape and Wine Research

Project Title: Microbiology and Chemistry of Washington Wines
10A-3057-0522
13B-3057-4846

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Summary:

Studies related to the ability of *Brettanomyces* to metabolize phenolics present in grape cultivars. Overall, these experiments demonstrated that (a) *Brettanomyces* are not able to metabolize tartaric acid esters of phenolic acids present in red wines, (b) culturability of *Brettanomyces* may or may not indicate formation of 4-ethylphenol/4-ethylguaiacol (*e.g.*, culturable cells can be recovered but volatile phenols are not produced), and (c) growth and metabolism by the four strains studied was more dependent on the strain rather than the wine. Significant interactions between ethanol (12 to 16% v/v) and storage temperature (12° to 21°C) were observed for two strains of *Brettanomyces*. In general, strains grew well in wines containing 12% to 14% ethanol at ≥15°C. Culturabilities of strains decreased in all wines kept at 12°C or in wines containing 16% ethanol. At 15%, growth depended on strain with I1a more resistant to this concentration of ethanol (growth at 18° and 21°C) than F3. Chitosan was observed to be ineffective against killing two strains of *Acetobacter* isolated from commercial Washington red wines. Finally, research with non-*Saccharomyces* yeasts isolated from Washington grapes was initiated. In general, the non-*Saccharomyces* yeasts evaluated (*C. californica*, *C. oleophila*, *Mt. pulcherrima*, *My. caribbica*, *My. guillermondii*, or *W. anomalus*) reached populations in excess of 10^7 cfu/mL

but did not completely metabolize glucose or fructose, unlike that of fermentations conducted by *Saccharomyces*. Based on an initial principal component analysis, differences in aroma were noted between *C. californica*, *My. caribbica*, and *S. cerevisiae*.

Objectives (2013):

1. *Brettanomyces* and *Acetobacter* spoilage of wines.

   (a) Complete study of phenolics metabolized by *Brettanomyces*, focusing on impact of lactic acid bacteria (*Pediococcus*) on metabolism of precursors to volatile phenols (*e.g.*, 4-ethylphenol, 4-ethylguaiacol, and 4-ethylcatechol).

   (b) Ascertain the effectiveness of chitosan against *Brettanomyces* and *Acetobacter* under vinification conditions.

   (c) Investigate the relationships between alcohol concentration and temperature on *Brettanomyces*.

   (d) Examine whether new technology (so-called "electronic tongue") can be used for detection of *Brettanomyces* spoilage.

2. Role of non-*Saccharomyces* yeasts on wine quality.

   (a) Screen individual isolates for fermentative ability/sensory impact including mannoprotein formation.

Summary of Major Research Accomplishments and Results:

<u>Objective 1</u>

Objective 1a: Red wines from Washington and Oregon were inoculated with a range of strains of *B. bruxellensis* and concentrations of hydroxycinnamic acids and their tartaric acid esters were monitored by HPLC. Besides consuming *p*-coumaric and ferulic acids, strains I1a, B1b, and E1 isolated from Washington wines metabolized 40 to 50% of caffeic acid, a finding in contrast to strains obtained from California wines. Higher molar recoveries of 4-ethylphenol and 4-ethylguaiacol synthesized from *p*-coumaric and ferulic acids, respectively, were observed in Washington Cabernet Sauvignon and Syrah but not Merlot. This finding suggested that *Brettanomyces* either (a) utilized vinylphenols formed during processing of some wines or (b) metabolized other unidentified phenolic precursors. None of the strains of *Brettanomyces* studied metabolized caftaric or *p*–coutaric acids present in wines from Washington or Oregon.

This portion of the research with phenolic precursors was completed and published in 2013 (Schopp et al., 2013). Additional research investigating the ability of other microorganisms (*i.e.*, lactic acid bacteria) to metabolically utilize phenolics is being conducted in collaboration with Oregon State University.

Objective 1b: Acetobacter aceti strains N6B1 and B7E were originally isolated from commercial winery in Washignton State. The strains were maintained in glycerol (-80°C) or using modified

apple Rogosa agar (4°C). Four different formulations of chitosan were obtained from Lallemand: Kiofine-B and three other formulations (#1, #2, and #3).

Five colonies were aseptically transferred to 50 mL sterile Erlenmeyer flasks fitted with foam stoppers containing 25 mL 5% YEPE media (20 g/L yeast extract, 40 g/L peptone, 5% v/v ethanol, pH 3.8). Cultures were incubated in an orbital shaker at 125 rpm and 30°C until approximately 10^7 cfu/mL. Cultures were diluted with 0.2 M phosphate buffer and added to experiments to achieve approximately 10^4 cfu/mL.

For experiments in media, 5% YEPE was used while experiments in wine were performed in a modified commercial Merlot (13.2% v/v ethanol). Wine was diluted with deionized water to achieve 10% v/v ethanol, pH was adjusted to 3.8 with 10 M NaOH, and 30% (v/v) H_2O_2 was used to eliminate SO_2. Glucose (2 g/L) and yeast extract (0.1 g/L) were added prior to sterile filtration (0.22 µm, Millipore PES Express^{TM}Plus).

For all experiments, 25 mL of wine or 5% YEPE was aseptically transferred to sterile 50 mL Erlenmeyer flasks fitted with sterilized foam stoppers. Chitosan formulations were suspended in sterile deionized water (10 g/L) and added to flasks to achieve concentrations of 0, 4, or 10 g/hL. *A. aceti* were inoculated at approximately 10^4 cfu/mL. Flasks were shaken in an orbital shaker at 125 rpm and incubated at 30°C with sampling every 48 hr to monitor culturability. All treatments were performed in triplicate. Culturable populations were determined using an Autoplate 4000 spiral plater (Spiral Biotech) and MR agar. Plates were incubated at 30°C until countable colonies were obtained.

The two strains of *Acetobacter* originally isolated from Washington wines were not affected by any of the chitosan preparations, either in a synthetic medium (Figures 1-2) or in Merlot wine (Figure 3). After inoculation at approximately 10^4 cfu/mL, populations increased to $>10^7$ cfu/mL in all treatments. Thus, while various chitosan preparations were successful in reducing populations of *Brettanomyces* (refer to previous reports), these were not at all effective against *Acetobacter* under various conditions.

Objective 1c: Brettanomyces bruxellensis strains I1a and F3 were acquired from the Washington State University culture collection and grown on Wallenstein Laboratory differential medium agar plates (Difco, Detroit, MI). Single colonies were transferred to 10 mL of YM broth (pH 3.85, Difco). Inocula were prepared by transferring 100 µL of these cultures to 50 mL of YM broth containing 5% v/v ethanol). Cells were harvested in late exponential growth phase by centrifuging samples at 2000 x *g* for 20 min and washed twice in 0.2 Na_2HPO_4 (pH 7.0) buffer.

A 2011 commercially prepared Merlot wine (pH 3.45, ethanol 13.2%) with total SO_2 removed using an equal molar amount of 30% (v/v) H_2O_2. A 5 x 4 x 2 factorial experiment with ethanol (12, 13, 14, 15, or 16% v/v), incubation temperature (12˚, 15˚, 18˚, or 21°C), and strain (I1a and F3) was designed with replication in triplicate. Solutions containing different ratios of absolute ethanol/deionized water were added to the wines to achieve various ethanol concentrations. Wines were supplemented with 0.5 g/L glucose, 0.5 g/L fructose, and 0.1 g/L yeast extract and adjusted to pH 3.75 using 10 M NaOH prior to filtration through 0.22 µm filters (Millipore, Billerica, MA) into autoclaved 100 mL milk dilution bottles. After incubation of replicates at

specific temperatures for 24 hours, all wines were inoculated with 10^4 CFU/mL of *B. bruxellensis* strain I1a or F3.

Wines were sampled twice per week for the first four weeks and once per week thereafter. Culturability was determined by plating on Wallenstein Laboratory Differential medium (WLD, Difco) incubated at 27°C using the spread plate technique and an Autoplate 4000 spiral plater (Spiral Biotech, Bethesda, MD). After 100 days, volatile acidities were measured using segmented flow analysis (Astoria-Pacific, Clackamas, OR) while 4-ethylphenol and 4-ethylguaiacol were measured using gas chromatography mass spectroscopy. Specifically, 4-ethylphenol and 4-ethylguiaicol were analyzed using a headspace-solid phase microextraction method with an 85 µm polyacrylate fiber (Supelco, Bellefonte, PA). The fiber was thermally desorbed at 280°C for 3 min by the injection port of a GC-MS/MS (Varian model 4000, Walnut Creek, CA). Separation was achieved using a DB-5MS capillary column (0.18 mm ID x 20 m) with 0.18 µm film thickness obtained from J&W/Agilent Technologies (Wilmington, DE). The carrier gas, helium, was held at a constant flow of 0.8 mL/min. The temperature program consisted of: 40°C held for 2.0 min, increased 20°C/min to 160°C and held for 0.0 min, and then increased 50°C/min to 300°C and held for 0.2 min. The volatile phenols were identified by retention times as well as fragmentation patterns compared to chemical standards.

A two-way analysis of variance (ANOVA) and Tukey's LSD was carried out for mean separation at probability $p \leq 0.05$ using XLSTAT software (Addinsoft, New York, NY).

The strains of *Brettanomyces* studied (I1a and F3) grew well in red wines that contained 12% (Figure 4), 13% (Figure 5) and 14% (Figure 6) ethanol if temperatures were ≥15°C (59F). However, as the ethanol concentration increased, both strains exhibited a longer lag phase with a lower temperature. For example, strain I1a reached 10^6 cfu/mL within 20 days of inoculation into the 12% ethanol wine maintained at 15°C. This same strain required approximately 70-75 days to reach the same population when incubated at the same temperature but in 14% ethanol wine. In wines containing 12% to 14% ethanol, culturabilities of both strains of *Brettanomyces* slowly decreased and recovery was slowed (12 and 13%) or nonexistent (14%).

Strain responses differed between I1a and F3 in the 15% ethanol wines (Figure 7). Here, F3 appeared to be more inhibited at this ethanol concentration than I1a. As evidence, F3 never reached populations in excess of 10^4 cfu/mL even when wines were maintained at 21°C (70°F) or 18°C (64°F), unlike that of I1a which required 60 days to reach 10^6 cfu/mL. Both strains died off and never recovered when incubated at 12°C (54°F). Neither strain of *Brettanomyces* survived inoculation into 16% ethanol wines regardless of temperature of incubation (Figure 8).

Concentrations of volatile acidity (VA), 4-ethylphenol (4-EP), and 4-ethylguaiacol (4-EG) determined on day 100 mirrored growth patterns observed in Figures 4 to 7. Overall, low concentrations of VA or volatile phenols were found in wines stored at 12°C or in 16% ethanol wines for I1a (Table 1) or F3 (Table 2). In fact, even though populations of F3 were between 10^2 and 10^4 CFU/mL for wines with 15% ethanol (Figure 7), very limited amounts of 4-EP or 4-EG were produced. At this concentration of ethanol, strain I1a synthesized large amounts of 4-EP and 4-EG but only when temperatures were ≥18°C.

Objective 1d: Please refer to the progress report by C. Ross regarding the use of the electronic tongue for application towards detection of *Brettanomyces* and other wine quality parameters.

Objective 2

Initial fermentations were conducted with a 2011 Chardonnay grape must (pH 3.34, 22.3 °Brix) obtained from commercial sources. After total SO_2 was removed using hydrogen peroxide, the must was sterile filtered through a 0.2 µm c Nylon membrane cartridge membrane housed in an autoclaved filter housing (Pall, Port Washington, NY). Diammonium phosphate (50 mg/L) and 20 µ Sigmacell cellulose (1% w/v, Sigma-Aldrich, St. Louis, MO) were aseptically added to the musts prior to transfer into sterile glass dilution bottles (100 mL). *C. californica*, *Mt. pulcherrima, My. caribbica, My. guillermondii, W. anomalus,* or *S. cerevisiae* were then inoculated at initial populations of 10^4 to 10^5 CFU/mL. Fermentations were conducted in triplicate at 18°C.

In another fermentation, a 2012 Chardonnay grape must (pH 3.64, 23°Brix) was obtained a commercial source. The must was transferred into sterile glass carboys (18 L). *C. californica, My. carribica,* or *S. cerevisiae* were inoculated (10^4-10^5 CFU/mL) in triplicate and incubated at 18°C. On day 40, environmental temperatures were increased to 22° and 24°C before inoculating *S. cerevisiae* (10^7 CFU/mL). Diammonium phosphate (50 mg/L), thiamin (0.5 mg/L), pantothenic acid (250 µg/L), and folic acid (0.2 mg/L) were also added at this time. Upon completion of fermentation, wines were filter sterilized as described previously and 578 mg/L of potassium metabisulfite was added. These wines were then stored at 4°C in sterile vessels until further analyses.

Culturabilities were monitored by spiral plating onto Wallenstein Laboratory agar (WL, Difco, Detroit, MI) and Lysine agar (Lysine Medium, Oxoid, Hampshire, England) using an Autoplate 4000 (Spiral Biotech, Bethesda, MD). Volatile acidity was measure by Cash still (Ough and Amerine 1988) while glucose and fructose were determined enzymatically (Yellowline Kit, r-Biopharm, Darmstadt, Germany). For sensory analysis, a trained panel was conducted to quantify individual aromatic attributes. WSU students and staff (n=7, 5 female and 2 male) were recruited and trained for a total of eight one-hour sessions. The aromas to be evaluated were chosen by an informal aromatic evaluation (n=5) of the wines to be tested. Panelists evaluated the intensity of each aromatic attribute for the three wines, in duplicate, using Compusense software (Ontario, Canada).

Saccharomyces cerevisiae grew well in the Chardonnay grape must, reaching a population of $>10^7$ cfu/mL and consuming glucose and fructose to concentrations <3 g/L (Figure 9). Similarly, the species of non-*Saccharomyces* yeasts evaluated (*C. californica*, *C. oleophila*, *Mt. pulcherrima*, *My. caribbica*, *My. guillermondii*, or *W. anomalus*) also reached this population (Figure 10). Unlike *Saccharomyces*, none of the non-*Saccharomyces* strains were able to complete fermentation, with residual sugar concentrations ranging between 110 g/L (*Mt. pulcherrima*) up to 155 g/L (*C. californica*) as illustrated in Table 3. *C. oleophilia*, *My. guillermondii*,, and *W. anomalus* produced amounts of volatile acidity higher than that of *Saccharomyces* (0.38 g/L compared to 0.58 to 0.83 g/L) but other species produced similar amounts. Based on an initial principal component analysis, differences in aroma were noted between *C. californica*, *My. caribbica*, and *S. cerevisiae* (Figure 11).

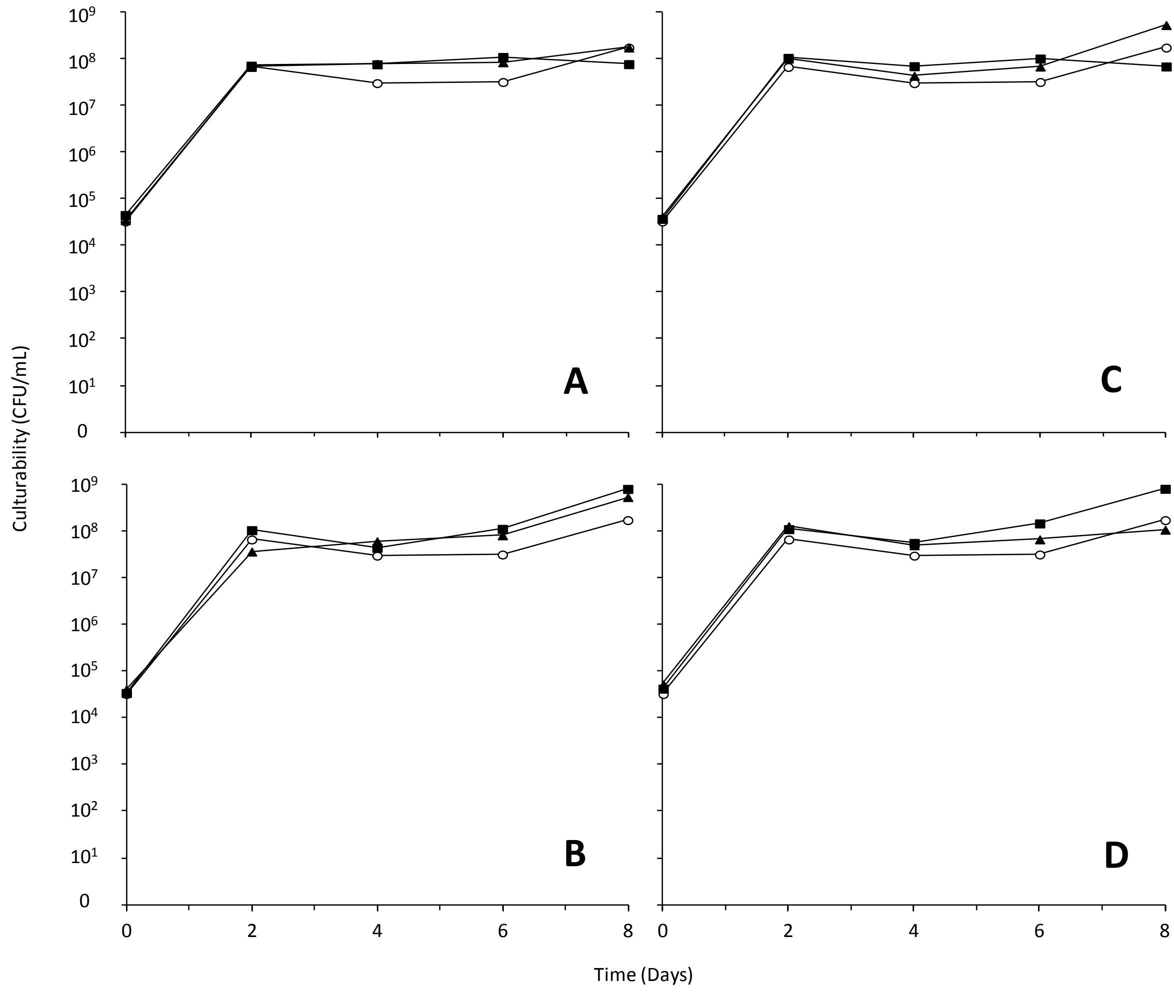

Figure 1. Culturabilities of *A. aceti* N6B1 in 5% YEPE medium with 0 (○), 4 (▲), or 10 g/hL (■) of chitosan preparations (A) Kiofine-B, (B) #1, (C) #2, or (D) #3.

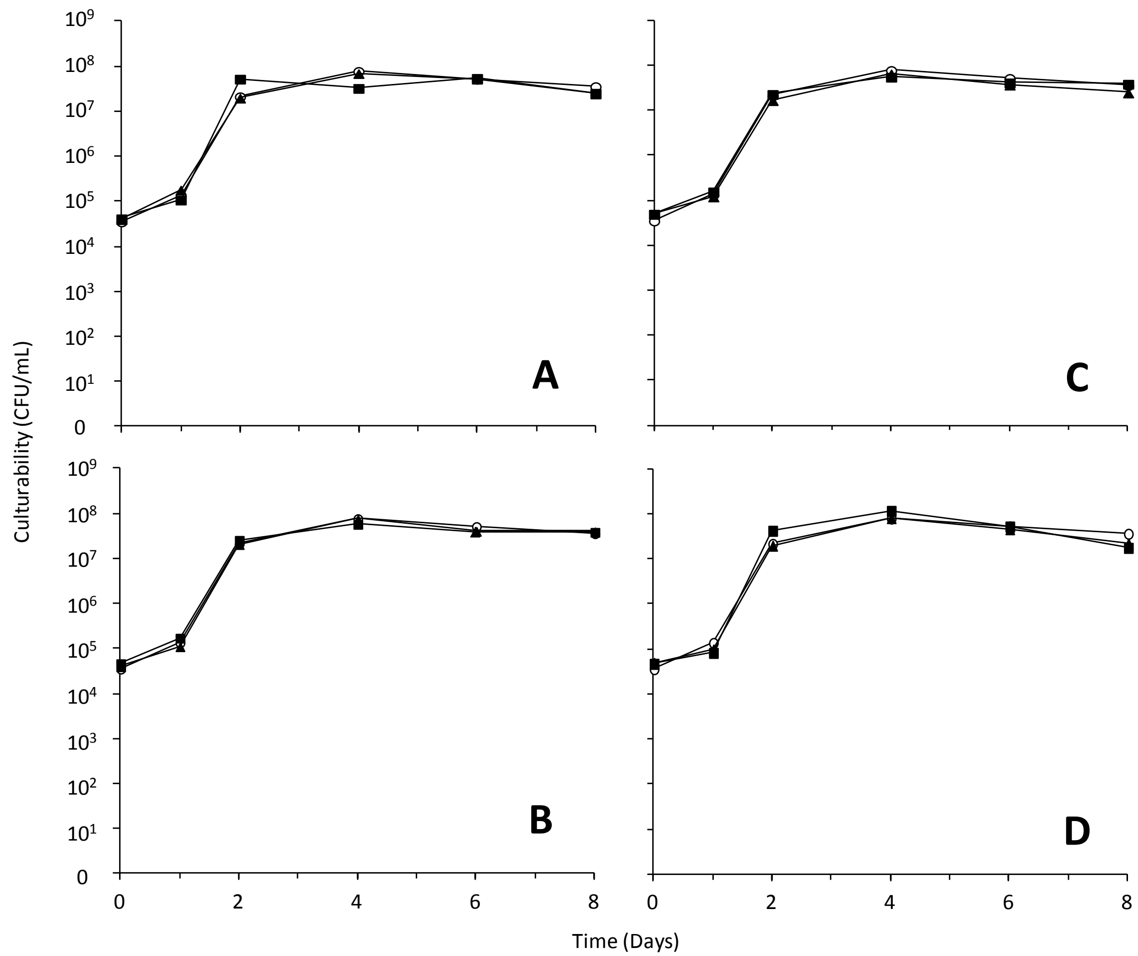

Figure 2. Culturabilities of *A. aceti* B7E in 5% YEPE medium with 0 (○), 4 (▲), or 10 g/hL (■) of chitosan preparations (A) Kiofine-B, (B) #1, (C) #2, or (D) #3.

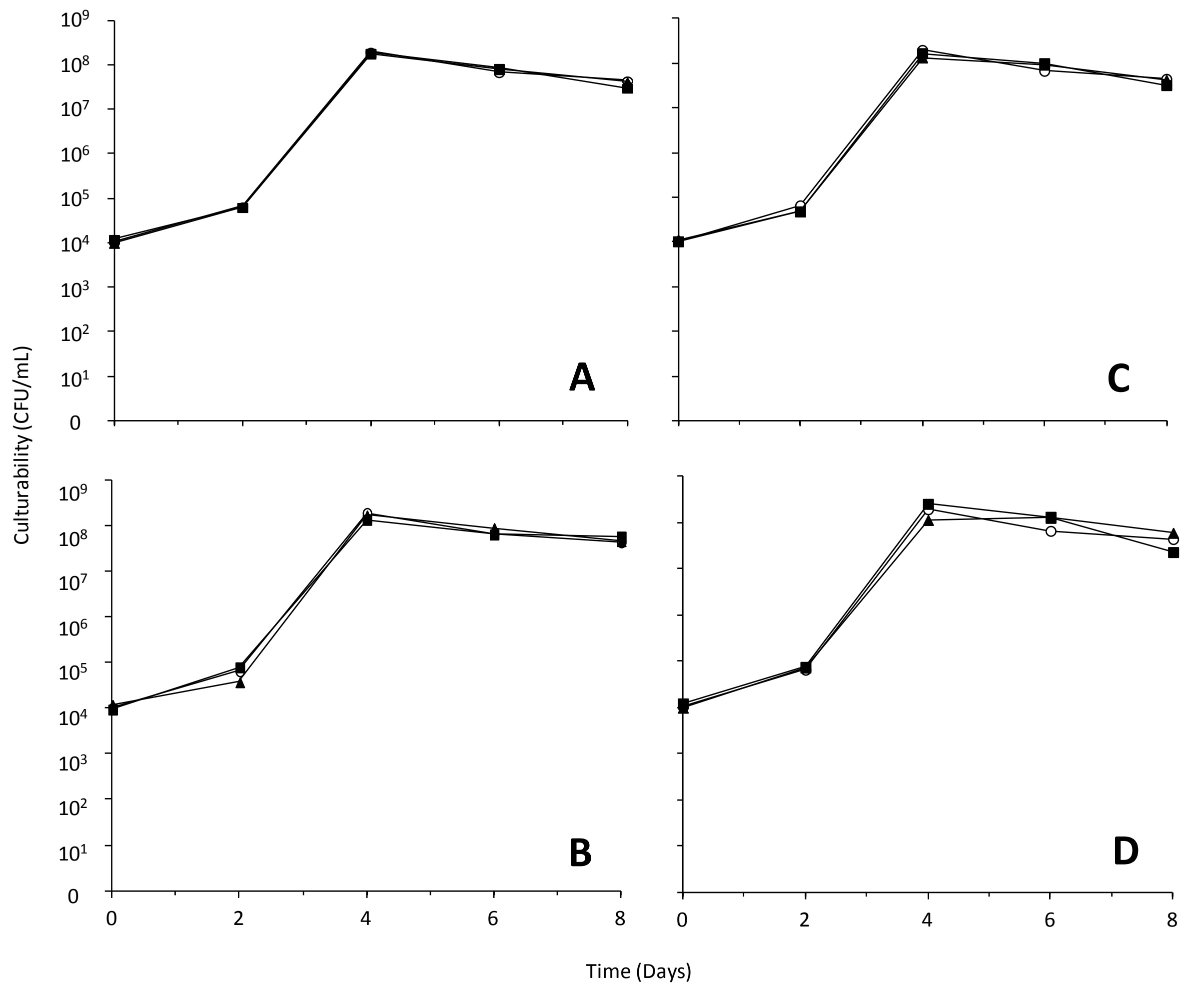

Figure 3. Culturabilities of *A. aceti* N6B1 in Merlot wine with 0 (○), 4 (▲), or 10 g/hL (■) of chitosan preparations Kiofine-B (A), #1 (B), #2 (C), or #3 (D).

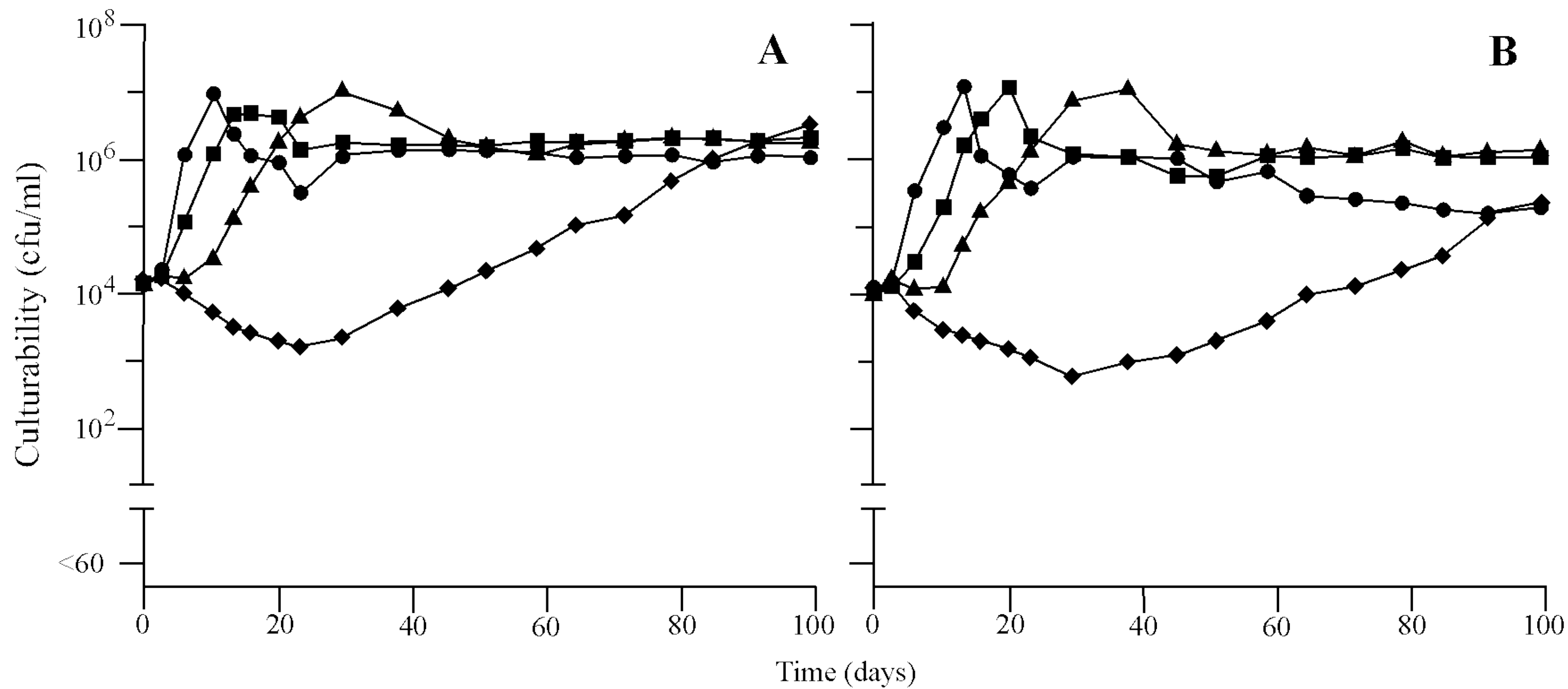

Figure 4. Culturability of *B. Bruxellensis* I1a (A) or F3 (B) in 12% v/v ethanol wine maintained at 21° (●), 18° (■), 15° (▲), or 12°C (◆).

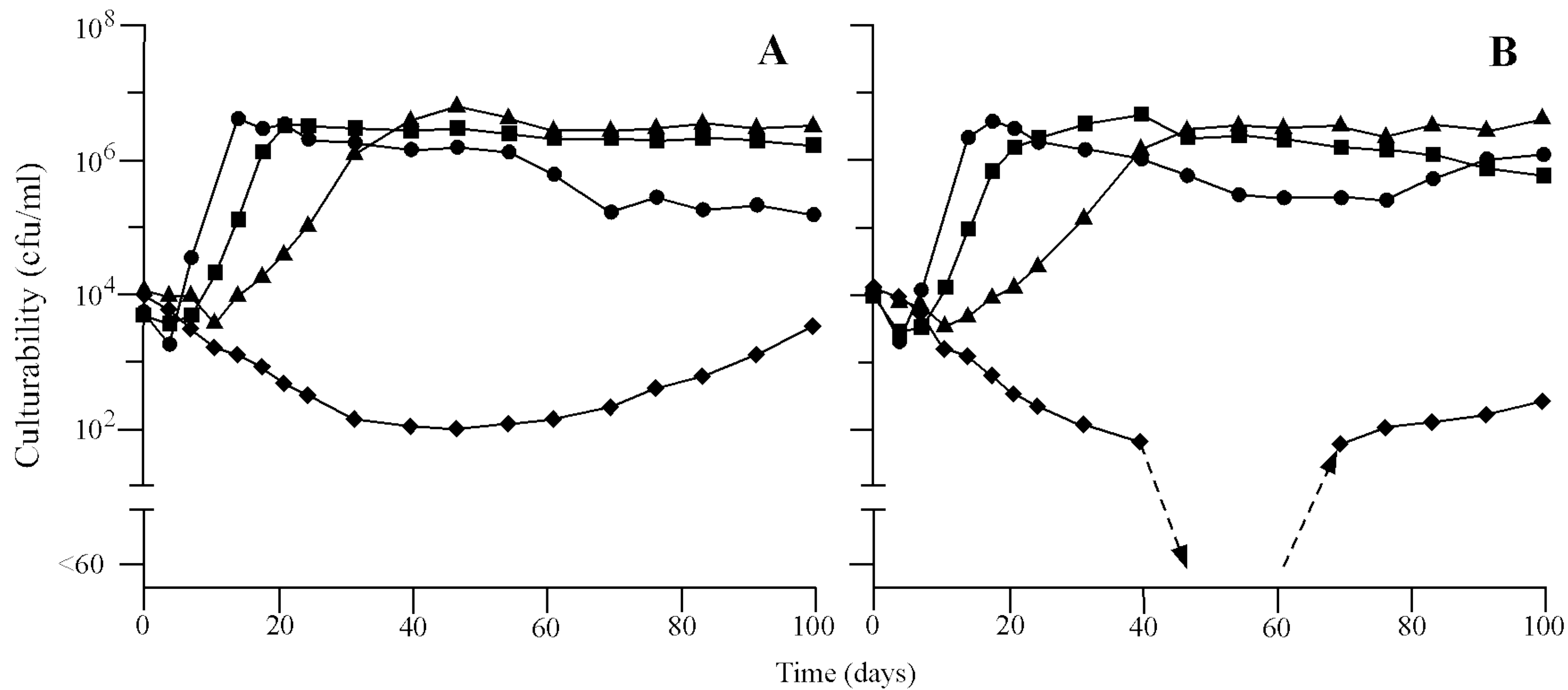

Figure 5. Culturability of *B. Bruxellensis* I1a (A) or F3 (B) in 13% v/v ethanol wine maintained at 21° (●), 18° (■), 15° (▲), or 12°C (◆).

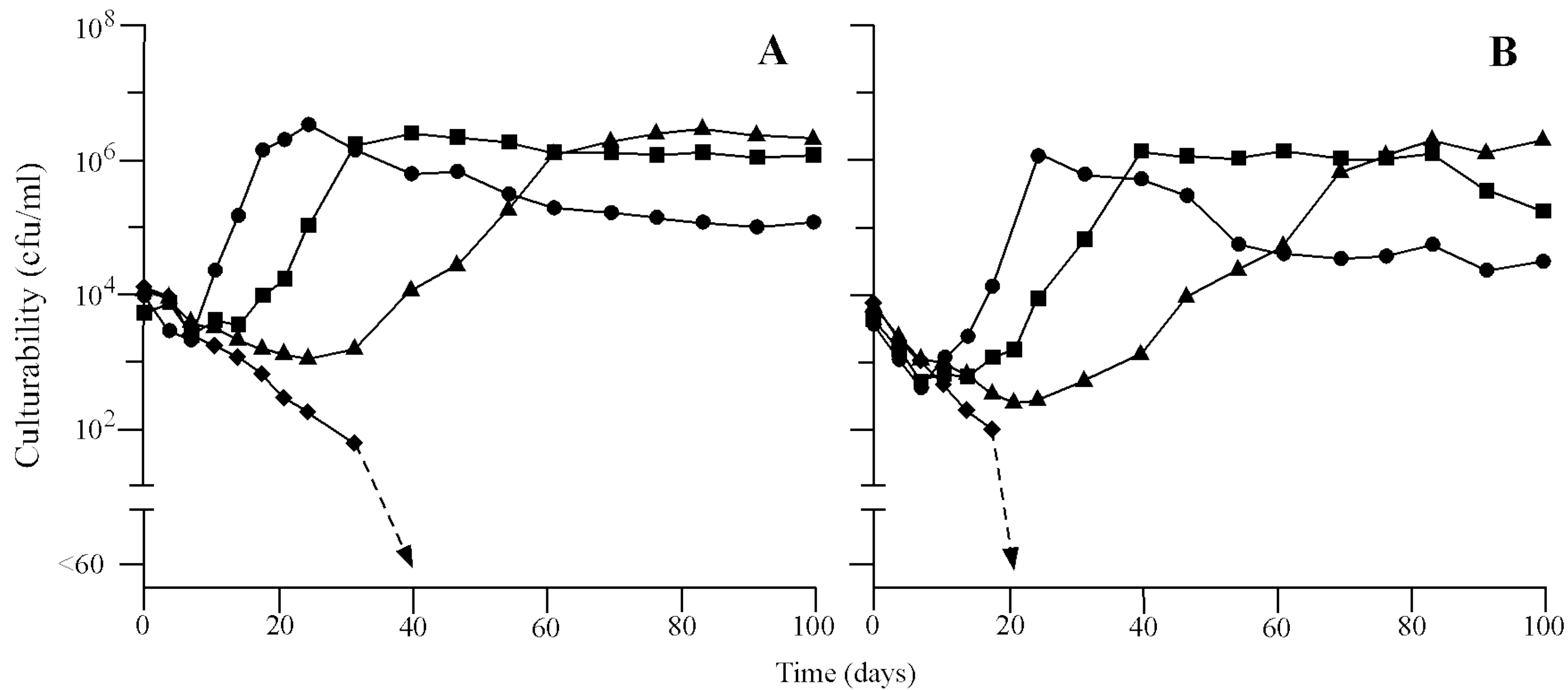

Figure 6. Culturability of *B. Bruxellensis* I1a (A) or F3 (B) in 14% v/v ethanol wine maintained at 21° (●), 18° (■), 15° (▲), or 12°C (◆).

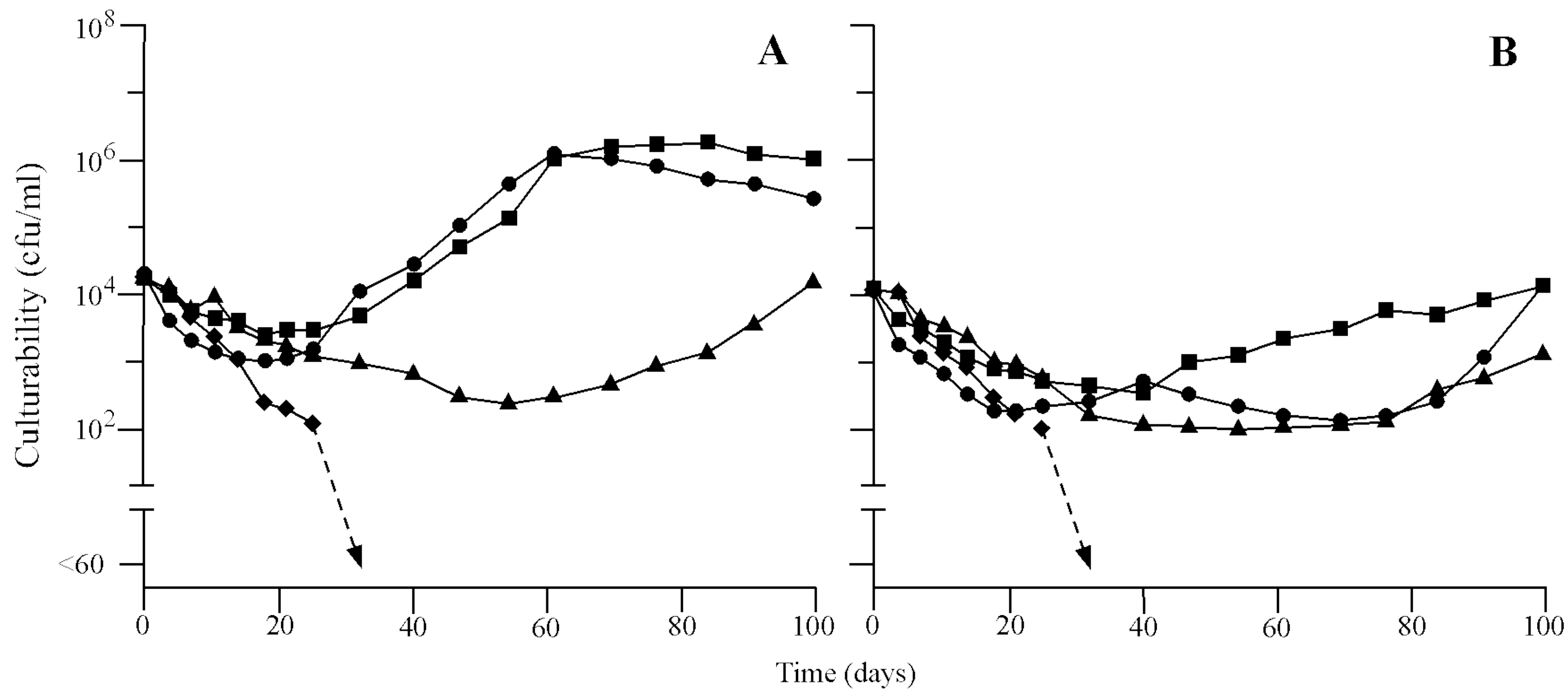

Figure 7. Culturability of *B. Bruxellensis* I1a (A) or F3 (B) in 15% v/v ethanol wine maintained at 21° (●), 18° (■), 15° (▲), or 12°C (◆).

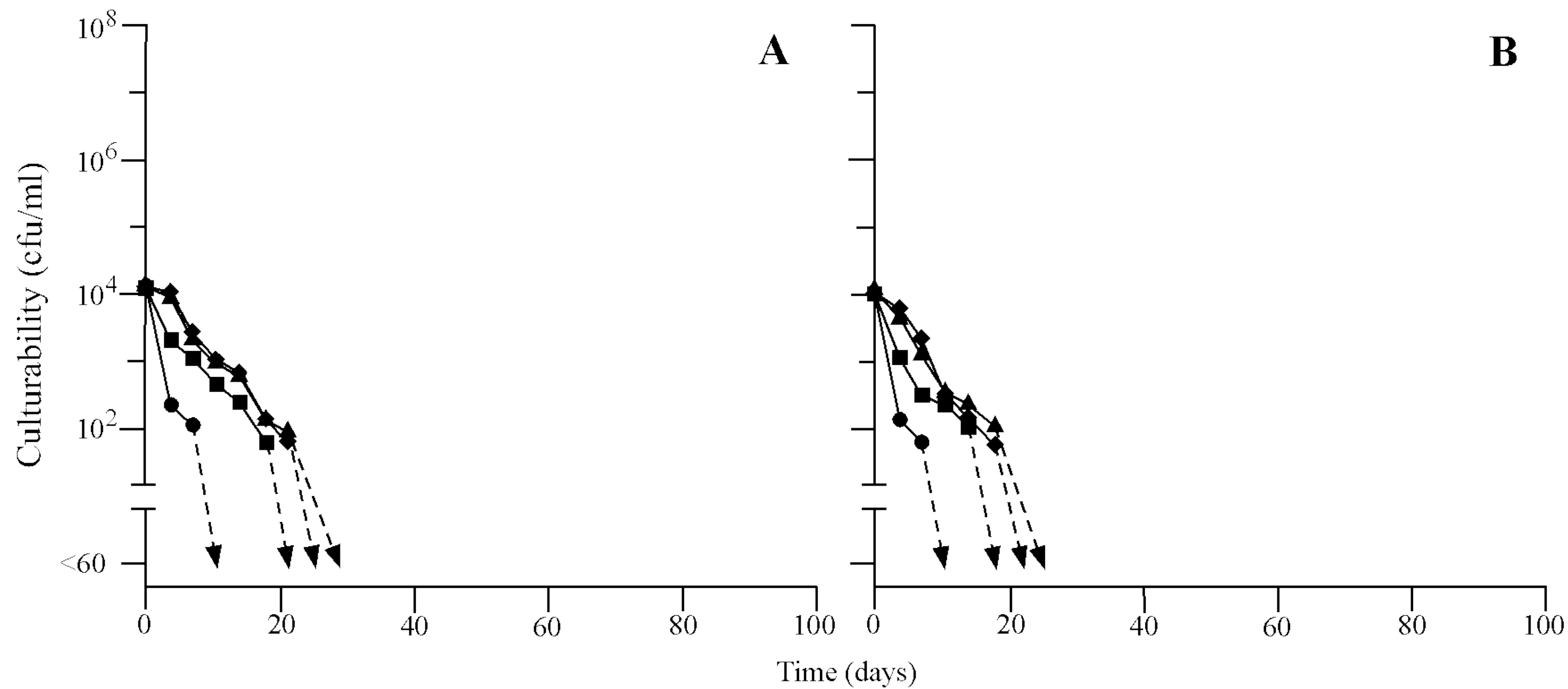

Figure 8. Culturability of *B. Bruxellensis* I1a (A) or F3 (B) in 16% v/v ethanol wine maintained at 21° (●), 18° (■), 15° (▲), or 12°C (◆).

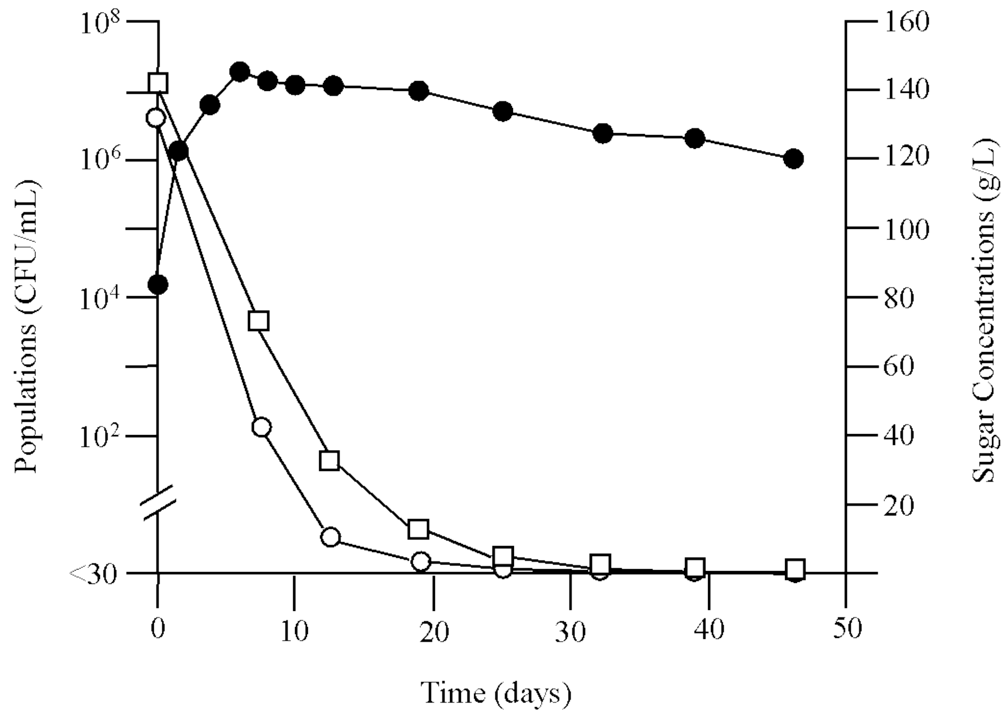

Figure 9. Culturabilities (●) and concentrations of glucose (○) or fructose (□) of Chardonnay musts inoculated with *S. cerevisiae*.

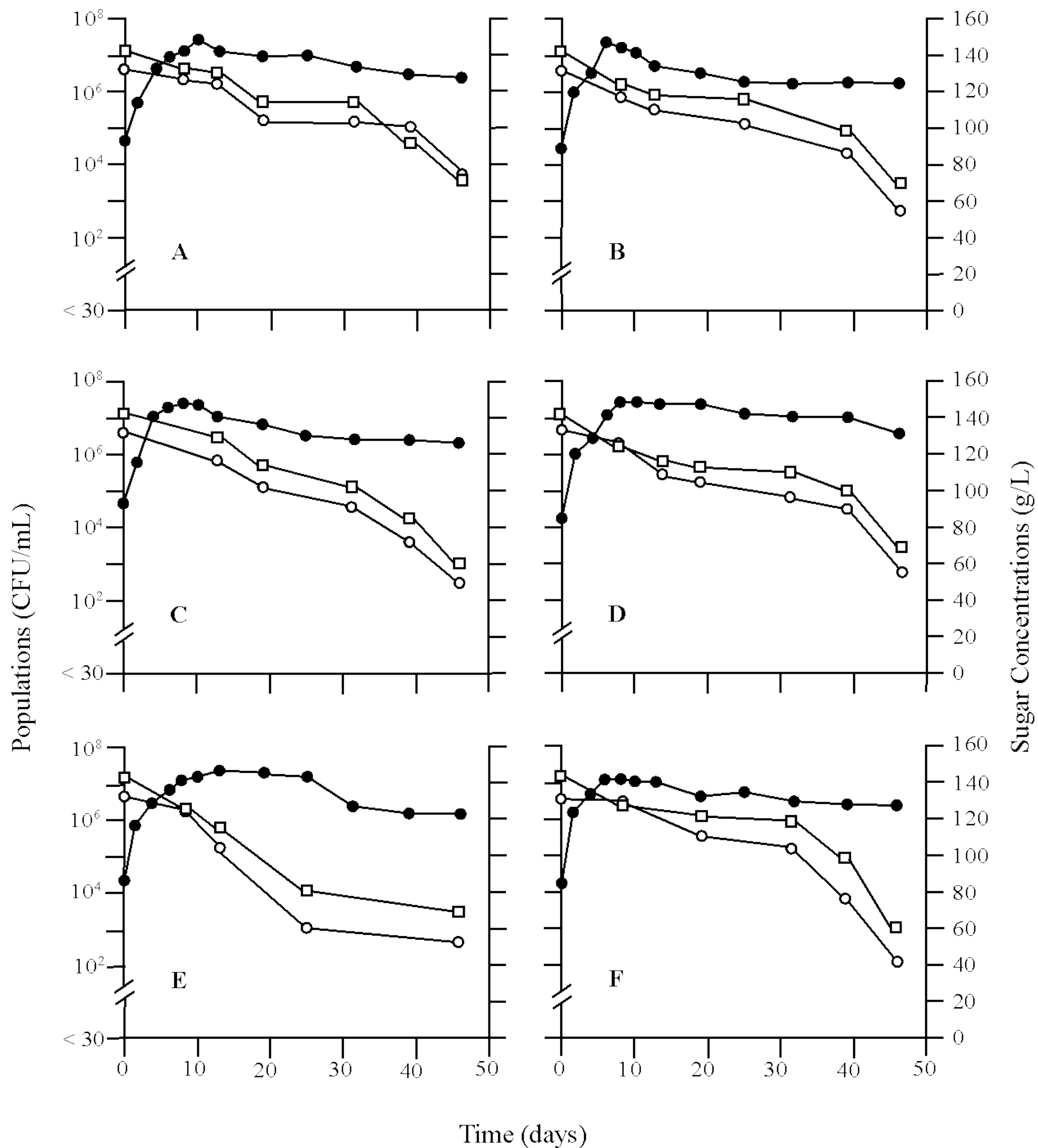

Figure 10. Changes in culturabilities (●) and concentrations of glucose (○) or fructose (□) of Chardonnay musts inoculated with (A) *C. californica*, (B) *C. oleophila*, (C) *Mt. pulcherrima*, (D) *My. caribbica*, (E) *My. guillermondii*, or (F) *W. anomalus*.

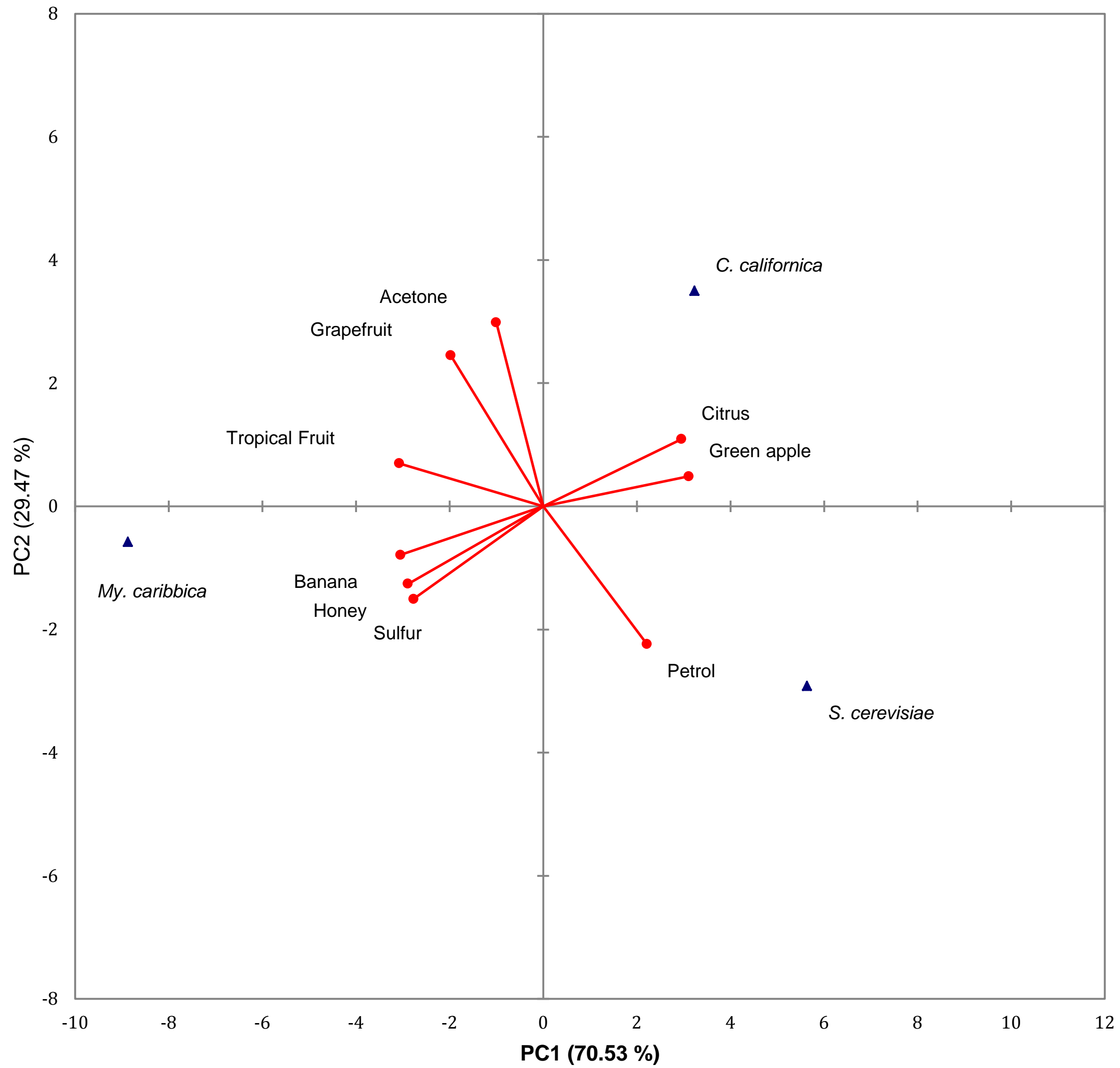

Figure 11. Principal component analysis of aromatic attributes (●) in Chardonnay wine as affected by *C. californica, My. caribbica*, or *S. cerevisiae* (▲).

Table 1. Concentrations of volatile acidity (g/100 mL), 4-ethylphenol (µg/L), and 4-ethylguaiacol (µg/L) in Merlot wines containing 12, 13, 14, 15, or 16% v/v ethanol and incubated at 12°, 15°, 18°, or 21°C for 100 days after inoculation of *B. bruxellensis* I1a.

Ethanol (% v/v)	Temperature (°C)	Volatile Acidity (g/100 mL)	4-Ethylphenol (µg/L)	4-Ethylguiacol (µg/L)
12	12	0.065^{ghi}	151.7^{d}	37.67^{c}
	15	$0.079^{ef,}$	2403^{b}	381.3^{a}
	18	0.103^{bcd}	2368^{b}	397.7^{a}
	21	0.128^{a}	2589^{ab}	384.0^{a}
13	12	0.055^{i}	11.67^{d}	nd
	15	0.078^{fg}	2494^{ab}	346.3^{ab}
	18	0.104^{bcd}	2452^{ab}	368.7^{a}
	21	$0.117^{a,b}$	2727^{a}	365.0^{a}
14	12	0.057^{hi}	10.33^{d}	nd
	15	0.072^{fgh}	1813^{c}	263.7^{b}
	18	0.095^{cde}	2365^{b}	361.3^{a}
	21	0.110^{abc}	2325^{b}	383.0^{a}
15	12	0.053^{i}	11.33^{d}	nd
	15	0.055^{i}	10.50^{d}	nd
	18	0.080^{efg}	2453^{ab}	331.0^{ab}
	21	0.089^{def}	2359^{b}	353.7^{a}
16	12	0.053^{i}	10.67^{d}	nd
	15	0.054^{i}	19.00^{d}	10.00^{c}
	18	0.056^{hi}	17.67^{d}	8.000^{c}
	21	0.055^{hi}	9.000^{d}	nd

[a to i] Means within a column with different superscripts are significant at $p \leq 0.05$.

nd: not detected (4-ethylguaiacol ≤4 µg/L)

Table 2. Concentrations of volatile acidity, 4-ethylphenol, and 4-ethylguaiacol in Merlot wines containing 12, 13, 14, 15, or 16% v/v ethanol and incubated at 12°, 15°, 18°, or 21°C for 100 days after inoculation of *B. bruxellensis* F3.

Ethanol (% v/v)	Temperature (°C)	Volatile Acidity (g/100 mL)	4-Ethylphenol (µg/L)	4-Ethylguaiacol (µg/L)
12	12	0.053^{h}	12.33^{e}	nd
	15	0.077^{ef}	2216^{bc}	321.3^{a}
	18	0.094^{cd}	2382^{ab}	317.7^{a}
	21	0.104^{bc}	2593^{a}	322.7^{a}
13	12	0.055^{h}	12.00^{e}	nd
	15	0.080^{def}	2074^{c}	273.3^{a}
	18	0.114^{b}	2569^{a}	360.0^{a}
	21	0.136^{a}	2584^{a}	344.0^{a}
14	12	0.055^{h}	15.00^{e}	nd
	15	0.072^{fg}	1289^{d}	154.7^{b}
	18	0.092^{cde}	2432^{ab}	331.0^{a}
	21	0.102^{bc}	2251^{bc}	274.0^{a}
15	12	0.054^{h}	10.67^{e}	nd
	15	0.054^{h}	10.50^{e}	nd
	18	0.055^{h}	32.67^{e}	13.00^{c}
	21	0.056^{gh}	25.33^{e}	10.33^{c}
16	12	0.053^{h}	16.67^{e}	nd
	15	0.054^{h}	19.33^{e}	nd
	18	0.055^{h}	12.33^{e}	nd
	21	0.057^{gh}	11.67^{e}	nd

$^{a\ to\ h}$ Means within a column with different superscripts are significant at $p \leq 0.05$.

nd: not detected (4-ethylguaiacol ≤4 µg/L)

Table 3. Concentrations of residual sugars (glucose + fructose) and volatile acidities after inoculation of *S. cerevisiae* or other yeasts into a Chardonnay grape must.

Species	Residual Sugar (g/L)	Volatile Acidity (g/L)
S. cerevisiae	<2	0.38 ± 0.02^{cd}
C. californica	155 ± 2.6^{a}	0.28 ± 0.03^{d}
C. oleophilia	122 ± 2.8^{bc}	0.83 ± 0.04^{a}
Mt. pulcherrima	110 ± 1.4^{c}	0.31 ± 0.005^{d}
My. caribbica	127 ± 1.1^{b}	0.49 ± 0.01^{bc}
My. guilliermondii	128 ± 3.6^{b}	0.58 ± 0.001^{b}
W. anomalus	124 ± 5.6^{b}	0.76 ± 0.04^{a}

$^{a\ to\ d}$ Means within a column with different superscripts are significant at $p \leq 0.05$.

Acknowledgements:

Appreciation is sincerely extended to Washington Wine Advisory Committee and the many wineries for participating in the *Brettanomyces* research project. We also thank Ste Michelle Wine Estates (Paterson, WA) for supplying red wines and analytical support, R. DeScenzo (ETS Laboratories, St. Helena, CA) for analytical support, and G. Specht (Lallemand, Montréal, Quebec, Canada) for various yeast strains and other support.

Outside Presentations of Research:

Publications (graduate students indicated by "*")

Umiker*, N.L., R. DeScenzo, J. Lee, and C.G. Edwards. Removal of *Brettanomyces bruxellensis* from red wine using membrane filtration. *J. Food Proc. Pres.* 37: 799-805 (2013).

Sturgeon*, J.Q., J.C. Bohlscheid, and C.G. Edwards. Effect of nitrogen source on yeast metabolism and H_2S formation. *J. Wine Res.* 24: 182-194 (2013).

Zuehlke*, J.M. and C.G. Edwards. Impact of sulfur dioxide and temperature on culturability and viability of *Brettanomyces* in wine. *J. Food Prot.* (in press, 2013).

Schopp*, L.M., J. Lee, J.P. Osborne, S.C. Chescheir*, and C.G. Edwards. Metabolism of non-esterified and esterified hydroxycinnamic acids and esters in red wines by *Brettanomyces bruxellensis*. *J. Agric. Food Chem.* (accepted, 2013).

Zuehlke*, J.M., D.A. Glawe, and C.G. Edwards. Efficacy of dimethyl dicarbonate against yeasts associated with Washington State grapes and wines. *J. Food Proc. Pres.* (submitted, 2013).

Zuehlke*, J.M. and C.G. Edwards. Application of *Zygosaccharomyces bailli* to remove residual sugar from stuck wine fermentations. *Enz. Microbiol. Technol.* (in preparation, 2013).

Childs*, B.C. J.C. Bohlscheid and C.G. Edwards. Impact of sugar concentration on the nitrogen requirements of *Saccharomyces* and its implication for post-fermentation spoilage. *Am. J. Enol. Vitic.* (in preparation, 2013).

Book Chapters (refereed)

Zuehlke*, J., B. Petrova*, and C.G. Edwards. Advances in the control of wine spoilage by *Zygosaccharomyces* and *Dekkera/Brettanomyces. In:* Annual Review of Food Science and Technology. 4: 57-78. Annual Reviews, Palo Alto, CA (2013).

Popular Articles (refereed)

Edwards, C.G. Management of *Brettanomyces* or do we still have to burn down the winery? *Wines & Vines* 94: 78-80 (April 2013).

Extension and/or Experiment Station Bulletins (refereed)

Edwards, C.G. and B.A. Watson. Basic Microbiological and Chemical Analyses for Wine. 82 pp. Washington State University Cooperative Extension EM047, Pullman, WA (2013).

Abstracts and Papers Presented at Technical Meetings

Petrova*[†], B., G. Specht, and C.G. Edwards. Effectiveness of fungal chitosan against *Brettanomyces bruxellensis* in barreled red wine. Presented at Washington Association of Wine Grape Growers annual meeting, Kennewick, WA. February 5-8 (2013).

Schopp*[†], L.M., J. Lee, and C.G. Edwards. Differential metabolism of hydroxycinnamic acids and their tartaric acid esters by *Brettanomyces* and *Pediococcus* in red wines. Presented at Washington Association of Wine Grape Growers annual meeting, Kennewick, WA. February 5-8 (2013).

White*[†], K.P., J. Zuehlke*, and C.G. Edwards. The influence of non-*Saccharomyces* yeast strains on the flavor profile and metabolic behavior of fermentation in Chardonany wines. Presented at Washington Association of Wine Grape Growers annual meeting, Kennewick, WA. February 5-8 (2013).

Zuehlke*[†], J. and C.G. Edwards. Interaction between factors affecting *Zygosaccharomyces bailli* growth. Presented at Washington Association of Wine Grape Growers annual meeting, Kennewick, WA. February 5-8 (2013).

Schopp*, L.M., J. Lee, and C.G. Edwards[†]. Differential metabolism of hydroxycinnamic acids and their tartaric acid esters by *Brettanomyces* and *Pediococcus* in red wines. Presented at American Society for Enology and Viticulture, Monterey, CA. June 24-28 (2013).

Diako*, C., C.G. Edwards, and C.F. Ross[†]. Red wine quality assessment using the electronic tongue. Presented at American Society for Enology and Viticulture, Monterey, CA. June 24-28 (2013).

Invited Presentations

Edwards[†], C.G. Managing *Brettanomyces* in wines. Presented to food engineering faculty and students at the Middle East Technical University, Ankara, Turkey, May 23 (2013).

Edwards[†], C.G. Managing *Brettanomyces* in wines. Presented to agriculture faculty and winemakers at the Cyprus University of Technology, Lemesos, Cyprus, May 28 (2013).

Current Graduate Students (chair) and Funding Sources

Aplin IV, J. (M.S. in Food Science). Thesis research area: Quality impacts of non-*Saccharomyces* yeasts (2013-present).

- *Funding source (2013-14): Washington State University (School of Food Science).*
- *Proposed funding source (2014-2015): Washington Wine Commission.*

Cartwright, Z. (Ph.D. in Food Science). Dissertation research area: Factors affecting the survival of *Brettanomyces* (2013-present).

- *Funding source (2013-2014): Washington State University Distinguished Research Assistantship.*
- *Proposed funding source (2014-2015): Washington Wine Commission.*

Childs, B.C. (M.S. in Food Science). Thesis research area: Optimization of yeast nutrient additions based on must sugar concentrations and ramifications towards microbial spoilage (2010-present).

- *Funding source (2010-2013): NW Center for Small Fruit Research.*

Osborne, Taylor (M.S. in Food Science). Thesis research area: Influence of ethanol x temperature interactions on growth of *Brettanomyces* (2012-present).

- *Funding source (2013-2014): Washington Wine Commission.*
- *Proposed funding source (2014-2015): Washington State University (School of Food Science).*

Petrova, B. (Ph.D. in Food Science). Dissertation research area: Impact of chitosan on *Dekkera/Brettanomyces* (2010-present).

- *Funding source (2010-2014): Fulbright Student Program, Department of State, USA.*

White, K.P. (M.S. in Food Science). Thesis research area: Impact of non-*Saccharomyces* yeasts on wine quality (2011-present).

- *Funding source (2013-2014): None.*

Research Success Statements:

Based on several original research publications from our laboratory (Connell et al., 2002; Jensen et al., 2009; Morneau et al, 2011; Schopp et al., 2013; Umiker et al., 2013; Zuehlke and Edwards, 2013; Zuehlke et al., 2013), a "trade journal" article was written and published (Edwards, 2013) that provided specific recommendations that winemakers can use to limit infections due to *Brettanomyces*. The article was peer-reviewed by three winemakers and will hopefully help winemakers worldwide. Current research can provide recommendations for wine storage temperatures given the amount of ethanol present. Chitosan appears to be highly successful towards lowering populations of *Brettanomyces* but is not effective against *Acetobacter*. Research continuing with non-*Saccharomyces* yeasts found in Washington vineyards will test their potential for commercial use.

Funds Status:

Monies were encumbered for a nine-month assistantship (fall and spring semesters) as well as summer salary for the graduate student.