

How *Brettanomyces/Dekkera* spp. Can Affect Wines

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Introduction

Brettanomyces/Dekkera spp. can be potent spoilage organisms in wine and these yeasts produce high concentrations of volatile acids, phenolic compounds, of which the ethyl phenols are the most prominent. At low concentrations these compounds can add to wine complexity, but at high concentrations it is regarded as wine spoilage (“medicinal”, “creosote”, “burnt plastic”, “barnyard”, “mousey”, etc.). *Brettanomyces* spp. has also been isolated from sparkling wines where they cause haziness and gushing in bottle fermented sparkling wines. Once this yeast is established in wineries, it is difficult to eliminate. Spoilage of wines by *Brettanomyces* can be devastating and wineries have had to shut down to get rid of this contaminant.

Over the last three years we have sampled more than 490 barrels in 49 wineries in the Okanagan. Our aim was to isolate and identify *Brettanomyces/Dekkera* spp. from the wineries in the Okanagan Valley and to help wineries get rid of these yeasts or to minimize spreading of these contaminants. In addition, we were interested too in the ability of these yeasts to spoil wines. It has also been suggested that *Brettanomyces* can add to the complexity of red wines. However, this seems to be a topic of dispute. No systematic study on the occurrence and spoilage of wine by *Brettanomyces* in British Columbia wineries has been done prior to our study. Although the focus of this project was to identify *Brettanomyces/Dekkera* spp. in Okanagan wineries, we simultaneously analyzed the wine samples for the presence of lactic acid bacteria.

Objectives

- (i) To isolate, identify, enumerate and characterize the *Brettanomyces* spp. and strains present in wineries of the Okanagan Valley over a period of three years.
- (ii) To establish the levels of different *Brettanomyces* strains required to spoil red wine.
- (iii) To identify bacterial contaminants present in barrels.

Summary of results

We used genomic DNA isolated from strains tentatively identified as *Brettanomyces/Dekkera* and Nested PCR and RAPD-PCR methods to confirm their identity as *Brettanomyces/Dekkera* isolates. During the first year we found that 6/10 wineries were contaminated with Brett, during the second year 6/13 wineries tested positive for this spoilage yeast, and during the third year only 4/16 wineries tested had Brett contamination in their barrels. Wineries seem to have adopted more rigorous cleaning procedures, and isolated or discarded old and contaminated barrels. Furthermore, discussions with winemakers revealed that ozonation is currently the preferred treatment of *Brettanomyces*-infected barrels. This treatment seems effective, but some winemakers find a re-occurrence of *Brettanomyces* in previously infected barrels after ozonation. Other wineries “peroxi-clean” contaminated barrels by treating the barrels with a sodium peroxide solution. However, this practice strips the barrel of almost all its wood-contributing characteristics that add to the flavour and aroma of the wine; in essence the barrel becomes only a storage vessel after such a treatment. Recent research advocates that Velcorin (dimethyl dicarbonate or DMDC) treatment is effective against *Brettanomyces*. However, this treatment is expensive and many smaller wineries would struggle to justify using this expensive treatment. In an effort to find an effective, cost-efficient method to treat *Brettanomyces*-contaminated barrels, we will do fundamental studies on *Brettanomyces* and determine the decimal reduction time for this yeast in the presence of ethanol.

We also studied the spoilage of wine by *B. bruxellensis* strain EC121 and *D. intermedia* strain EC77. The yeast cells were harvested by centrifugation and washed several times with sterile water. *Brettanomyces* and *Dekkera* were inoculated into bottled Pinot Noir wines previously determined to be free of the *Brettanomyces* spoilage character. The following levels of contamination were used for both strains: 0 (control), 10, 100, 1,000, 10,000 and 1,000,000 cells/ml. Wines were incubated at room temperature for 80 days and analyzed by GC/MS. Surprisingly, we found that the two typical *Brettanomyces/Dekkera* strains isolated did not produce 4-ethyl phenol and 4-ethyl guaiacol in wine. Instead, the contaminated wines were found to be intensely bitter and showed distinct signs of corkiness. We were unable to identify the compound responsible for the bitterness by GC/MS or LC/MS analyses.

Lactobacillus and *Pediococcus* spp. were found to be prevalent in wineries in the Okanagan. These bacteria can be responsible for the spoilage of wines. During 2003 we found that 55 barrels in 9 of the 16 wineries sampled contained short bacilli; 30 of these barrels were contaminated with acid forming lactic acid bacteria. These bacteria were non-motile rod-shaped bacteria and are possibly of the genus *Lactobacillus*. The presence of these bacteria could lead to high volatile acids and spoilage of wines. Eight barrels from three wineries were contaminated with cocci. Six of these barrels were found in one winery. Stringent cleaning of barrels and sulphating of wines should prevent growth and proliferation of these spoilage organisms. Winemakers should take great care to ensure that the pHs of wines are stabilized at approximately 3.2. A high pH will render SO₂ less effective and promote growth of spoilage lactic acid bacteria. We found no bacterial contaminants in barrels in five wineries that have previously participated in our study; these wineries have adopted effective cleaning procedures for their barrels and/or are now maintaining SO₂ at sufficient high levels to prevent

growth and proliferation of spoilage bacteria. Spoilage bacteria present in wineries in the Okanagan could affect the quality of the wine and wineries will have to eliminate or minimize growth of these spoilage organisms to further improve the quality of their wines.

Industrial importance of our data

It is important that winemakers are now aware of the fact that *Brettanomyces/ Dekkera* spp. are indeed present in many wineries in the Okanagan. Hygiene in the winery is of the utmost importance to prevent the spread of this spoilage yeast. Many winemakers were surprised at how contaminated their barrels were, especially with lactic acid bacteria.

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