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"Genetic and physiological characterization of *Brettanomyces bruxellensis* strains isolated from wines"

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In this paper the authors try to put some order within the species *Brettanomyces bruxellensis*, recognized for the barnyard/burnt plastic/Band-Aid® off-characters in wines. This paper was awarded the 2007 Best Enology Paper by the American Society for Enology and Viticulture.

• The authors conducted *genetic* characterization of 47 strains of *B. bruxellensis*. And they complemented this work with the *physiological* characterization of 35 of these strains. Their main goal was to determine if DNA sequencing could be linked to specific physiological traits important in winemaking. For example, they might be able to identify groups of *B. bruxellensis* that grew more rapidly, or were more resistant to SO2, and thus, had more potential to cause damage in wines.

• For the *genetic characterization* they used PCR (polymerase chain reaction) to multiply a portion of the yeast's highly conserved DNA (26S rDNA). The analysis of the resulting sequences allowed them to cluster the yeasts in similar groups based on the number of nucleotide changes. For the *physiological characterization*, the authors looked at the nutrient habits of the yeasts (what carbon source and nitrogen source they used), their temperature preferences, their alcohol-, SO_2-, and low-pH tolerances, and their production of ethyl phenols.

• **Genetic characterization results.** The authors were able to classify the 47 Brett strains into 4 main groups based on their DNA similarity. Group A corresponded mostly to strains of European origin. Group B, the largest, included mostly North American and South American strains. In group C, 64% of the strains were from United States and 27% from New Zealand. Finally, group D was a small group of 3 strains from Malta and California. So, even though the groupings based on DNA sequence seemed to cluster the strains according to geographic origin, there were exceptions. The authors proposed two hypotheses to explain their results: 1) either similar winemaking practices and wine types may select for similar strains, or else 2) cooperative winemaking practices and wine blending on a global level may be fueling a worldwide distribution of strains. To find out which hypothesis is correct, many more isolates would have to be analyzed.

• **Physiological characterization results.** The flexibility of most of the "Brett" strains in their ability to use different substrates as sources of carbon and nitrogen was impressive to the authors. Most of the isolates could grow on any of the monosaccharides *glucose*, *fructose*, and *galactose*, or the disaccharides *sucrose*, *maltose*, *cellobiose*, and *trehalose* tested. (The monosaccharide arabinose, the disaccharide lactose and the trisaccharide raffinose, on the other hand, did not support their growth). Approximately 25% of strains could grow on ethanol as the sole carbon source. Finally, all of the isolates could grow on ammonium, proline, and arginine as a nitrogen source.

• Additionally, the authors were able to see that all of the isolates were tolerant to 10% alcohol, and that all could grow at pH 2.5 (and 94% of them also grew at pH 2.0). One third of the isolates were able to grow at 10°C, and another third at 37°C. Finally, half of the isolates produced high levels of ethyl phenols (4-ethyl phenol and 4-ethyl guaiacol), the other half equally divided between moderate and low production. Overall, no two isolates were exactly the same in all the characteristics tested.

• As the authors point out, the above characteristics can have important implications for the winemaker. Although only 25% of the strains could use ethanol as a carbon source, this means that there are plenty of strains that can grow on the ethanol in the bottle, even if the wine is completely dry. Similarly, production of 4-ethyl phenol and 4-ethyl guaiacol has been used as the method to diagnose the presence of *Brettanomyces* in wine. But, as we can see, 25% of the strains studied produced only moderate levels (700-2000 µg/L), and another 25% produced extremely low levels (<4-60 µg/L). Thus, the presence of Bretanomyces will go undetected if we only measure these two compounds.

• **Comparison between genetics and physiology.** By comparing large sets of the genetic and physiological characteristics, the authors hoped to determine whether specific groups of *Brettanomyces* have different growth behaviors or flavor impacts. For example, the ability to identify a specific strain in the winery, coupled to the knowledge of its physiological needs, may allow the winemaker not only to track its spread, but also to devise strategies for effective control. The authors were able to show that the physiological parameters they studied were correlated to the groupingss they established when using a specific gene (26S rDNA). However, to be able to predict the growth potential and flavor impact of any given strain, they will need to refine their classification by including other genes, particularly those involved in the pathways of off-flavor production.

In summary, the authors were able to genetically classify a large number of *Brettanomyces bruxellensis* strains isolated throughout the world, and to associate each with a specific, unique physiological pattern. This is already very useful because it allows a better understanding of some of the strains we are likely to find in our cellars. Still, the final goal of the authors is to be able to predict the impact in the resultant wine of each individual strain (or its degree of mischievousness), and for that, more specific genes will need to be studied.

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