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Adhesion and biofilm production by wine isolates of *Brettanomyces bruxellensis*

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• Bacteria in nature are either in a *planktonic* state ("free-swimming"), or *adhered* to surfaces. Those that adhere to surfaces do so after they aggregate on a desirable surface and start producing an extracellular glue-like matrix that protects them and attaches them irreversibly to that surface. At this point, the resultant community –often a heterogeneous group of organisms- is called a *biofilm*. Even though bacteria are the most frequent organisms present in a biofilm, fungi, algae, and protozoa can also be a part of it.

• You may not be familiar with biofilms, but they are all around us. Examples of biofilms are the plaque that forms on our teeth, the gunk that clogs the drains, the slippery rocks in a stream, or the coating that grows on the filter surface of wastewater treatment facilities. Even though this latter case is an example of desirable biofilms (when waste water passes over the filters, bacteria in the coatings on the filters digest the organic compounds), biofilm formation in the winery is clearly undesirable as it can become a serious source of contamination.

• Given the significance of *Brettanomyces bruxellensis* as a spoilage organism in the winery, knowledge of its biofilm-forming properties could help us understand how it spreads, and therefore, how to control it. In this study, the authors tested the ability of different isolates of *B. bruxellensis* to 1) adhere to plastic surfaces and to 2) form biofilms (the next step up, but with drastic consequences for sanitation). They also tested the effectiveness of different cleaning solutions on preventing adherence and biofilm formation.

• To test for biofilm formation, the authors used microtiter plates, using an artificial juice medium (50% grape juice, 45% water, 5% alcohol), and allowing 14-day incubation. To test for adhesion ability, they used a low-sugar medium (low sugar levels are known to enhance adherence), and incubated for only 6 hr (long enough to allow cells to adhere, but not to form a biofilm). After rinsing and staining, the cells were measured using an ELISA plate reader. The authors repeated the tests at different pHs, to see how pH affected adhesion and biofilm formation.

• **Ability of Brett isolates to adhere and form biofilms**. Of the 40 pure cultures tested, 38 were able to adhere to the surface of the microtiter plate. Additionally, 15 of them could also form biofilms. These biofilm-forming isolates had originated in many different countries, showing that this Brett characteristic has a broad geographic distribution. The authors found that adherence and biofilm formation increased with increasing pH. Since earlier studies have shown that there is no difference in Brett growth at pH values from 2.0 to 4.5, the authors suggest that the differences they found are not due to greater biomass at higher pH values, but that pH may fundamentally affect cell surface properties involved in adhesion. As the authors state, this provides one more reason for winemakers to try to keep wine pH in the lower pH range.

• **Effectiveness of cleaning agents**. When six commercial cleaning agents (Acryl Aquaclean, Biocidad ZF, caustic soda, Oakite 62, Sanibac, and soda ash) were compared with deionized water for their ability to
remove adherent cells and biofilms, only caustic soda was able to remove all the cells and resultant biofilms. Biocidal ZF was the most effective of the remaining detergents. The chlorine-based Sanibac did not show a good ability to remove biofilms, but instead killed the yeast in the biofilm.

In summary, organisms forming biofilms are known to resist sanitation practices. The authors showed that 15 (37%) of the Brett isolates tested were able to form biofilms. Caustic soda is not appropriate for cleaning oak barrels. Among the remaining agents tested, the quaternary ammonium-based cleaning agent and the alkaline detergent were more successful in removing adhering cells than the ketone-based detergent, the chlorine-based sanitizer, and soda ash. The authors suggest that some of the strategies to prevent biofilm formation on critical winery surfaces include: the correct choice of detergents and disinfectants, the use of physical cleaning methods, a hygienic plant layout and equipment design, and the right choice of equipment materials –i.e. biofilms may form on all types of surfaces but Teflon and nylon are easier to clean than stainless steel and aluminum.

Agent	Category	Active ingredients
Acryl Aquaclean	Detergent	ketones, surfactants
Biocidal ZF	Detergent	quaternary ammonia, surfactants
Caustic soda		sodium hydroxide
Soda ash		sodium carbonate
Oakite 62	Alkaline detergent	sodium hydroxide, surfactants
Sanibac	Sanitizer	chlorine

Table by authors

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