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Brettanomyces bruxellensis biofilms: a lifestyle to withstand environmental stresses?

Sourced from the research article "New advances on the *Brettanomyces bruxellensis* biofilm mode of life" (International Journal of Food Microbiology 2020)[1].

>>> The ability to form biofilms is a potential resistance strategy, although so far it has not been explored much for the wine spoilage yeast, *Brettanomyces bruxellensis*. The capacity of two strains to adhere to and form biofilms on stainless steel chips in wine was studied. Using electron microscopy, particular cell structures, such as filamentous cells or chlamydospore-like structure, potentially involved in *B. bruxellensis* resistance, were identified. Moreover, free cells, released from the biofilms, were observed and hypothesised to be the source of recurrent spoilage in wine. <<<

Biofilm can be defined as a community of microorganisms that are irreversibly attached to a surface, bound together, and embedded in a self-produced matrix. This growth strategy, which is widespread among microorganisms, contributes to their persistence in various environments through increased stress resistance and colonisation of surfaces[2]. The capacity of the wine spoilage yeast, *Brettanomyces bruxellensis*, to form biofilms is poorly described in literature. If the adhesion onto winemaking equipment surfaces and the biofilm development abilities of *B. bruxellensis* were proved, this would allow the development of appropriate strategies for its efficient removal. In this context, the purpose of this work was firstly to evidence the biofilm lifestyle of *B. bruxellensis*; secondly, to visualise its various cell structures using microscopic observations; and thirdly, to study the impact of wine on the established biofilms produced by two *B. bruxellensis* strains.

■ Biofilm formation and structure in wine

The cells of two selected strains (named "11" and "14" and previously isolated from winemaking equipment) were adapted to red wine conditions (alcohol concentration and pH) as described by Longin *et al.*, 2016[3]. Moreover, before biofilm formation, starter cultures ensured cells were in the same physiological state corresponding to the end of exponential phase, allowing the accurate comparison of the formation of biofilm by different strains in the wine. The biofilm formation of *B. bruxellensis* in wine was studied on stainless steel chips using a protocol adapted from previous studies[4]. The 25 mm × 25 mm stainless-steel chips were immersed in 15 mL of red wine inoculated with 5.0 × 105 CFU/mL cells. After incubation for 7 and 14 days at 28 °C, cells were detached by sonication, cultivable cell populations on biofilm were determined by plating on YPD medium, and the biofilm structure was observed by Scanning Electron Microscopy (SEM).

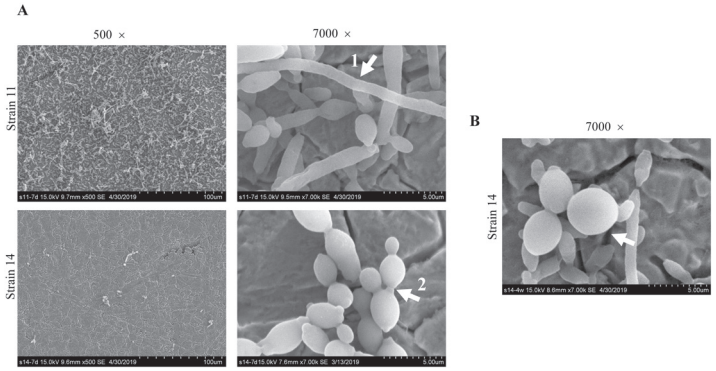

Figure 1. A) SEM observations of 7-day microcolonies of strains 11 and 14 developed in red wine on stainless steel chips. Magnifications were performed at 500 x (development of the microcolonies on the stainless-steel surface) and at 7000 x (filamentous cells and EPS indicated by white arrows n°1 and n°2 respectively). B) SEM observations of 14-day microcolonies of strain 14 developed in red wine on stainless steel chips. Magnifications were performed at 7000 x (chlamydospore like structure indicated by a white arrow).

The adhesion and biofilm formation of both strains in wine were monitored on the chip after 2 h, 24 h and 7 and 14 days of incubation using the previously described protocol. The yeast cultures were initially inoculated at around 106 CFU/mL (Table 1). Strain 14 had a weak adhesion rate of 0.69 % after 2 h in the wine compared to strain 11 (5.69 %). This difference in population was maintained throughout the 7 days, but not after 14 days of incubation, even though no additional biofilm growth was observed for either strains in the wine (Table 1). This suggests that *B. bruxellensis* is able to adhere to and form biofilm in harsh environments like wine, but biofilm growth is poor after 14 days of incubation.

In parallel, observations of strains 11 and 14 after 7 days incubation on stainless steel chips revealed the presence of microcolonies containing cells embedded in extracellular polymeric substances (EPS) (Figure 1A). In addition, it is important to note that numerous filamentous cells were observed within the microcolonies which are are hypothesised to play a structural role within the biofilm (Figure 1A). Microscopic observations also revealed the presence of structures larger than a usual yeast cell, with thick walls and derived from filamentous cells (Figure 1B). Such features are characteristic of chlamydospore-like structures, as previously reported for *C. albicans*[5].

Table 1. Microcolony growth on stainless steel chip in wine for strains 11 and 14 at 2 and 24 hours and 7 and 14 days. The values represent the average of three independent biological replicates, assigned with standard deviation (grey values). The sign* indicates significant difference (ANOVA, p-value ≤ 0.05) between the 2 strains for a time point.

Strain	Initial population in wine CFU/mL	Population on chip (CFU/cm²)			
	0 hour	2 hours*	24 hours*	7 days*	14 days
11	$5.44 \times 10^5 \pm 2.59 \times 10^5$	$3.22 \times 10^4 \pm 8.75 \times 10^3$	$5.26 \times 10^4 \pm 8.55 \times 10^3$	$1.06 \times 10^5 \pm 5.53 \times 10^4$	$1.79 \times 10^4 \pm 5.37 \times 10^3$
14	$1.33 \times 10^6 \pm 4.67 \times 10^5$	$9.53 \times 10^3 \pm 7.04 \times 10^3$	$1.50 \times 10^4 \pm 9.05 \times 10^3$	$4.78 \times 10^3 \pm 6.11 \times 10^3$	$5.29 \times 10^3 \pm 7.15 \times 10^3$

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■ Impact of wine on *B. bruxellensis* biofilm

Firstly, we produced biofilms on stainless steel chips in a YPD medium (as previously described), and after 7 days of incubation, chips with adhered cells in biofilms were transferred to wine to study the impact of wine on 7-day-old *B. bruxellensis* biofilms. Two types of cells were enumerated: (i) those that remained within the biofilm on the chips, and (ii) those that were released into the wine (Figure 2). For both strains, the amount of cells within the biofilms significantly decreased after 24 h, but remained stable thereafter for up to 14 days (Figures 2A and 2B). As previously described, strain 14 was more negatively affected by the wine than strain 11. In addition, an important phenomenon of cell release from the biofilm was observed as early as 2 h after inoculation in the wine (at around 106 CFU/mL) for both strains (Figures 2A and 2B). Thereafter, for strain 14, a decrease in the number of released cells was observed for 24 hours before remaining stable for up to 7 days. This was followed by an increase in cell numbers between day 7 and day 14. The same behaviour was observed for strain 11, but to a lesser extent.

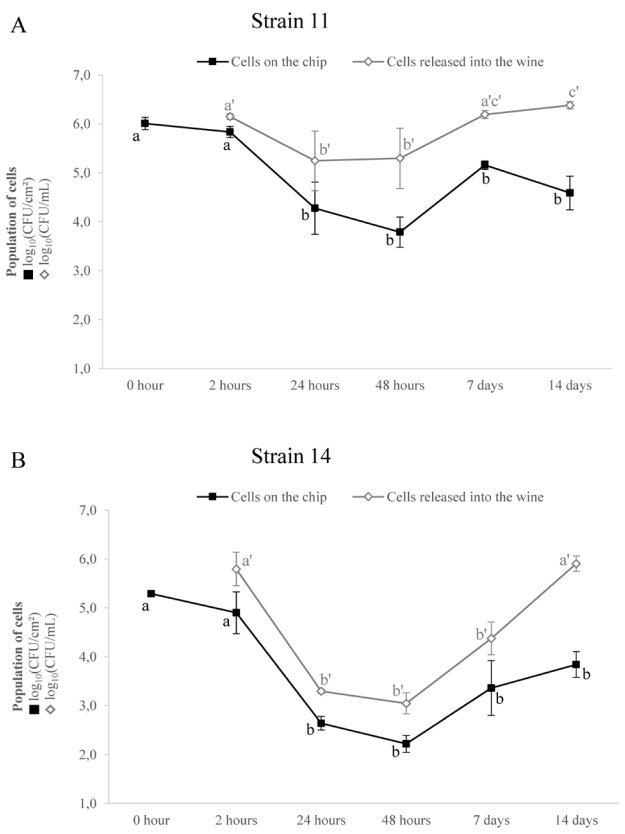

Figure 2. Microcolony behaviour in wine for (A) strain 11 and (B) strain 14. The cell population on the chip is expressed in log10 (CFU/cm²) and the cell population released in the wine in log10 (CFU/mL). Error bars represent the standard deviation between three independent replicates. A different letter indicates a significant difference (ANOVA, p-value ≤ 0.05).

■ Conclusion

In this study, the ability of the spoilage yeast *B. bruxellensis* to form biofilm on stainless steel was demonstrated in wine. As described for other microorganisms[6], the biofilm lifestyle may allow *B. bruxellensis* to persist in wine and wine-related environments and, furthermore, to be an important pathway for the contamination of the wine. This type of development must be taken into account when cleaning tanks or pipes, because once a biofilm has formed, the cleaning process could be less effective, as the biofilm lifestyle allows for better resistance to stressful conditions. Moreover, SEM observations revealed "chlamydospore-like" structures that have never been reported for *B. bruxellensis*. Chlamydospores have been described as forms of resistance in some fungi[7], but in yeasts their role has never been clearly identified. Future work should focus on determining the actual nature and role of these "chlamydospore-like" structures in *B. bruxellensis*, as well as the role of this structure in the persistence of spoilage yeast in wine cellar. ■

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1 Lebleux, M., Abdo, H., Coelho, C., Basmaciyan, L., Albertin, W., Maupeu, J., Laurent, J., Roullier-Gall., Alexandre, H. Guilloux-Benatier, M., Weidmann, & S. Rousseaux, S. (2020). "New Advances on the *Brettanomyces bruxellensis* Biofilm Mode of Life." *International Journal of Food Microbiology*, vol. 318, p. 108464., doi:10.1016/j.ijfoodmicro.2019.108464.
2 Davey, M.E., & O'toole, GA. (2000). Microbial Biofilms: from Ecology to Molecular Genetics. *Microbiol Mol Biol Rev*, vol. 64, p. 847-867.
3 Longin, C., Degueurce, C., Julliat, F., Guilloux-Benatier, M., Rousseaux, S., & Alexandre, H. (2016). Efficiency of population-dependent sulfite against *Brettanomyces bruxellensis* in red wine. *Food Res Int*, vol. 89, p. 620-630.
4 Bastard, A., Coelho, C., Briandet, R., Canette, A., Gougeon, R., Alexandre, H., Guzzo, J., & Weidmann, S. (2016). Effect of biofilm formation by Oenococcus oeni on malolactic fermentation and the release of aromatic compounds in wine. *Front Microbiol*, vol. 7, p. 613.
5 Martin, S.W., Douglas, L.M., & Konopka, J.B. (2005). Cell cycle dynamics and quorum sensing in Candida albicans chlamydospores are distinct from budding and hyphal growth. *Eukaryot Cell*, vol. 4, p. 1191-202.
6 Davey, M.E., & O'toole, GA. (2000). Microbial Biofilms: from Ecology to Molecular Genetics. *Microbiol Mol Biol Rev*, vol. 64, p. 847-867.
7 Ojeda-Robertos NF, Torres-Acosta JF, Ayala-Burgos AJ, Sandoval-Castro CA, Valero-Coss RO, Mendoza-de-Gives P. 2009. Digestibility of Duddingtonia flagrans chlamydospores in ruminants: in vitro and in vivo studies. *BMC Vet Res* 5:46.