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Growth of Natural Yeast Flora during the Fermentation of Inoculated Wines

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The growth of yeasts that occur naturally in grape juice was quantitatively examined during the fermentation of four wines that had been inoculated with *Saccharomyces cerevisiae*. Although *S. cerevisiae* dominated the wine fermentations, there was significant growth of the natural species *Kloeckera apiculata*, *Candida stellata*, *Candida colliculosa*, *Candida pulcherrima*, and *Hansenula anomala*.

Traditionally, wine has been produced by the natural fermentation of grape juice by yeasts that originate from the grapes and winery equipment. Yeasts of the genera *Kloeckera*, *Hansensiaspora*, *Candida*, *Pichia*, and, sometimes, *Hansenula* grow during the early stages of fermentation but eventually die off, leaving *Saccharomyces cerevisiae* as the dominant species to complete the fermentation (1, 3, 6, 7). Collectively, these species contribute to the final quality of the wine. In the newer wine-producing countries such as the United States, South Africa, and Australia, where a desirable natural flora may not be established in the vineyard and winery, there is a reluctance to rely on natural fermentation, and selected yeast cultures are inoculated into the grape must to induce fermentation (6, 7). The main advantages of inoculated wine fermentations are a more rapid and even rate of fermentation and wine of more consistent quality (5, 6, 9). It is believed that the inoculated species, generally a single strain of *S. cerevisiae*, dominate the fermentation and rapidly suppress the growth of unwanted natural yeast species (1, 5, 9). However, this assumption has not been examined in quantitative detail, and the possibility remains that natural yeasts still undergo significant growth and thereby contribute to the final quality of the wine. In this note, we examine the growth of natural yeast flora in wines produced by inoculation with *S. cerevisiae*.

The wines were produced from grapes harvested during the 1984 vintage in Australia. Wine A, a white Riesling, and wine C, a red Malbec, were produced in 20-liter volumes in the laboratory from grapes obtained from a winery in South Australia. Wine B, a white Semillon, and wine D, a red Hermitage, were fermented under commercial conditions in 20,000-liter tanks at two wineries in the Hunter Valley district of New South Wales, Australia. Sodium *meta*-bisulfite was added to each grape must (pH 3.0 to 3.3) to give 50 mg of total sulfur dioxide per liter. The musts were then inoculated with starter cultures of commercial strains of *S. cerevisiae* (inoculated at 2% of the wine volume) and fermented at 20°C for white wines and 25 to 30°C for red wines. Daily samples were taken during fermentation for the isolation and enumeration of yeasts by spread inoculation onto plates of malt extract agar (Oxoid Ltd.) and lysine agar (Oxoid). After the plates were incubated for 5 days at 20 to 25°C, yeasts were enumerated, and representative colonies were isolated and subcultured onto malt extract agar for subsequent identification by the tests and classification schemes of Kreger van Rij (4).

The growth of individual yeast species during fermentation of each of the four wines is shown in Fig. 1. As expected after inoculation, *S. cerevisiae* dominated the fermentation of all wines. This species was readily enumerated by plating wine samples onto malt extract agar, but its dominance on this medium masked the presence of colonies of other yeast species. These non-*Saccharomyces* species were best enumerated on lysine agar on which the growth of *S. cerevisiae* was selectively inhibited (8). Using this medium, we showed that there were major contributions by the natural yeast flora, several species of which showed significant growth during the first 24 to 48 h of fermentation. *Kloeckera apiculata* occurred in all fermentations at populations of 10^6 to 10^7 cells per ml, and, in the case of the two white wines (Fig. 1A and B), it survived 9 and 6 days, respectively, into the fermentation. *Candida stellata* was isolated from all the wines and was present in highest numbers in the two white wines. Isolation of other yeasts varied between wines. For example, *Candida pulcherrima* contributed to the fermentation of the white and red wines prepared from South Australian grapes (Fig. 1A and C), and *Hansenula anomala* and *Candida colliculosa* were present during the early stages of red wine fermentation at a Hunter Valley winery (Fig. 1D).

Our data demonstrated that yeasts naturally present in the grape must make a significant contribution to the fermentation even when the must is inoculated with 10^5 to 10^7 cells of *S. cerevisiae* per ml. The species that develop and their growth and survival characteristics are very similar to those which occur in natural, uninoculated fermentation (3). As discussed by Benda (1) and Fleet et al. (3), these species may have important influences on wine flavor. Thus, the assumption that inoculated *S. cerevisiae* suppresses significant development of natural yeasts during wine fermentations is not strictly correct.

Although *S. cerevisiae* dominated all four wine fermentations, we are unable to state that the dominant strain was the same one which was inoculated into the must. There is the possibility that the dominant *S. cerevisiae* originated from the natural flora. Using electrophoretic methods to differentiate *S. cerevisiae* strains, Bouix et al. (2) showed that in some cases the strain inoculated may not be the dominant strain at the end of fermentation. Moreover, it has been suggested (3) that different *S. cerevisiae* strains may develop at different stages during natural fermentations. Because the *S. cerevisiae* strain can have significant effects on wine quality (1), it is becoming increasingly important to have

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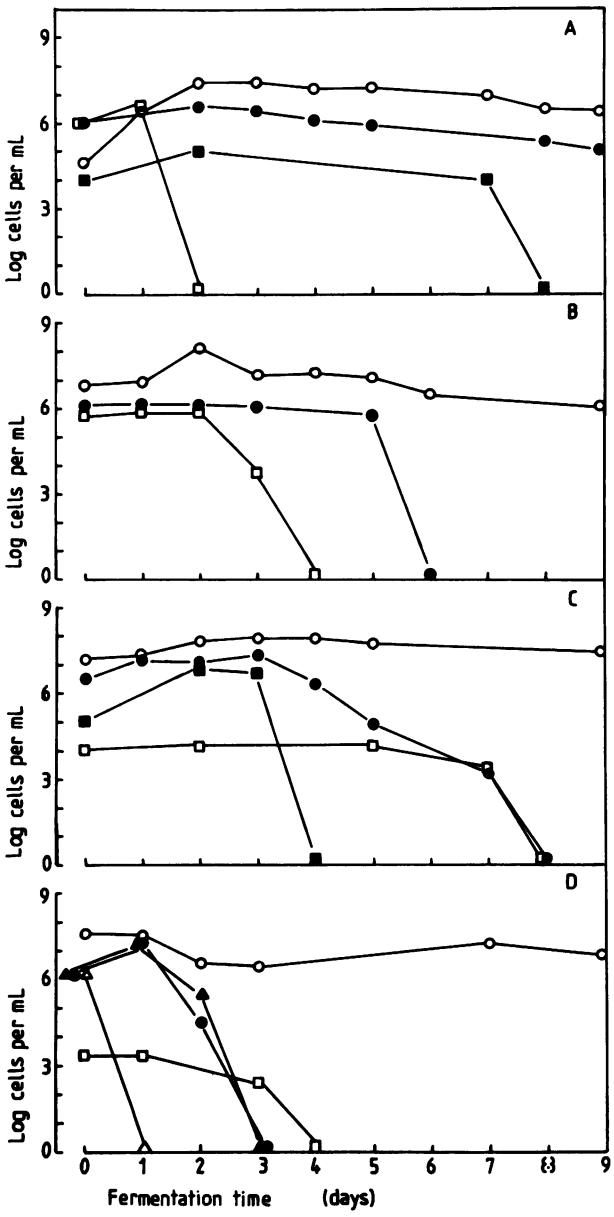

FIG. 1. Growth of yeasts during inoculated fermentation of white wines A and B and red wines C and D. Symbols: ○, *S. cerevisiae*; ●, *K. apiculata*; □, *C. stellata*; ■, *C. pulcherrima*; ▲, *C. colliculosa*; △, *H. anomala*. *S. cerevisiae* was enumerated on malt extract agar; the other species were enumerated on lysine agar.

better knowledge of how these might vary during wine fermentation. It may be that, in induced fermentations, the main effect of inoculated *S. cerevisiae* is to influence the development of the *Saccharomyces* strains rather than to inhibit the growth of non-*Saccharomyces* yeasts.

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