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RESEARCH

The Yeasts in a Riesling Must From the Niagara Grape-Growing Region of Ontario

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Abstract

The yeasts in the must from grapes grown in the Niagara Peninsula have been identified and enumerated. The persistence of these indigenous or wild yeasts throughout the fermentation was examined. The number and diversity of yeast species are similar to those reported in other wine districts of the world. The most numerous indigenous species in fresh must were an unknown species of *Candida, Hanseniaspora uvarum, Candida diversa* and *Aureobasidium pullulans.*

Saccharomyces cerevisiae was not found in the initial 5,000 colony forming units analysed. The first *S. cerevisiae* was isolated when the alcohol concentration reached 10g/L. *S. cerevisiae* then increased in numbers throughout the fermentation. At the end of the fermentation only *S. cerevisiae* was isolated.

Résumé

Les levures du moût de raisins dans la Péninsule du Niagara ont été identifiées et énumérée. La persistance de ces levures indigènes ou sauvages fut étudiée au cours de la fermentation. Le nombre et la diversité des espèces de levures furent semblables à celles rapportées dans d'autres régions vinicoles du monde. Les espèces indigènes les plus nombreuses dans le moût frais furent une espèce inconnue de *Candida, Hanseniaspore uvarum, Candida diversa* et *Aureobasidium pullalans. Sacchariomyces cerevisiae* ne fut pas trouvé dans les premières 5,000 unités de colonies analysées. Le premier *S. cerevisiae* fut isolé lorsque la concentration alcoolique atteignit 10g/L. *S. cerevisiae* augmenta ensuite en nombre tout au cours de la fermentation, *S. cerevisiae* fut le seul à être isolé.

Introduction

The "wild yeasts" of the grape bloom are reported to contribute variously to "off flavors" and complexity of a wine (Schulle, 1953; Sapis-Domercq, 1969; Benda, 1970; Sponholz and Dittrich, 1974). In an effort to understand and control the influence of the wild yeasts, most wine districts of the world have undertaken some taxonomic surveys cataloging the indigenous microfungi of grapes, musts and wines (Mrak and McClung, 1940; Peynaud and Domercq, 1953; Yokotsuka, 1954; Castelli, 1955; Castelli and DelGuidice, 1955; Domercq, 1957; Inigo Leal *et al.*, 1963; Benda, 1964; Minarik, 1964; Sapis-Domercq and Guittard, 1976; Fleet *et. al.*, 1984). No such studies have been performed in the Ontario wine districts.

The present work is a report of a taxonomic survey of the yeasts and yeast-like molds from the Niagara Peninsula. The report also describes the relative persistence of these yeasts throughout the fermentation.

Materials and Methods

Riesling grapes used in the study were grown in the vineyards of a commercial winery in the Niagara grape growing region. Random samples of grapes were pooled at harvest on September 27, 1988. The juice was taken directly from the press with no addition of SO_2. The juice measured 17.9° Brix with a pH of 3.1. To examine the succession of yeast populations during a natural fermentation, juice was put into sterile 4-L glass carboys fitted with fermentation locks. A small sample, taken in a sterile 250 mL plastic bottle, was kept on ice until plating (within 4 h) to examine the initial microbial population. Fermentation was carried out at room temperature for six days. Samples were taken for plate counts, residual sugar and ethanol determinations. Microbiological examination was done using Malt Extract Agar (MEA), (Difco Laboratories, Detroit, MI) and Lysine Agar (LA). LA supports the growth of most yeast species with the notable

exception of *Saccharomyces* species (Taylor and Marsh, 1984; Heard and Fleet, 1986). LA is Yeast Carbon Base and 15 g/L Noble Agar (Difco) supplemented with 0.9 g/L L-lysine (Fisher Scientific, Ottawa, ON). Both media were adjusted to pH 3.6. The initial sample was plated out onto 100 MEA plates. Samples taken during the fermentation were diluted serially and plated onto 10 plates of both LA and MEA. Plates were incubated at room temperature for five days before the optimal dilution (100 to 200 cfu/plate) was identified for counting.

Isolates were grouped according to colony and cellular morphology for enumeration. Representatives of each type were then pure cultured on Yeast Extract-Peptone Dextrose Agar (YEPD, components from Difco). Cultures were stored on YEPD slants at 4°C. Identification was done at the University of Western Ontario, Department of Plant Sciences, by the tests and classification schemes described by van der Walt and Yarrow (1984).

Ethanol was measured by HPLC using a Waters system equipped with an R401 optical refractometer (Waters Associates, Mississauga, ON) and a BioRad HPX-87H column (RioRad Laboratories, Mississauga, ON). Reducing sugar was determined according to Amerine and Ough (1980).

Results

Notwithstanding the unique climate of the Niagara Peninsula, the total number and the species diversity of the indigenous yeasts in this must and wine does not differ greatly from reports from wine districts in other parts of the world (Benda, 1964; Minarik, 1964; Davenport, 1974; Rosini *et al.*, 1982; Fleet *et al.*, 1984). With the possible exception of the *Candida* species, the must and fermentation population was similar to those found elsewhere.

The concentration of the total initial microbial population in the juice from Riesling grapes harvested from the Niagara Peninsula was log 6.9 cfu/mL. Yeast accounted for 89% of the 5017 cfu's identified. Thirteen species of yeast were identified (see Table 1). Identification of yeast species using morphological (Figure 1), and physiological tests was straightforward with the exception of the most numerous yeast, an unknown species of *Candida* which assimilated only glucose, 2-keto-gluconate, and tannin among the carbon sources tested and lysine (slowly) and cadaverine among the nitrogen sources tested. The unknown yeast fermented glucose vigorously and had a unique profile in the test battery (van der Walt and Yarrow, 1984). Although it bears some resemblance to *Candida stellata*, the unknown fails to assimilate sucrose or raffinose. Unlike the unknown, *Candida stellata* isolates tend to grow poorly on amino acid free media (Lachance, unpublished). Work is presently in progress to determine whether the unknown *Candida* sp is a novel species. Consistent with reports from other parts of the world, (van Zyl and du Plessis, 1961; Relan and Vyas, 1971; Rosini *et al.*, 1982; Parish and Carroll, 1985), the apiculate yeast *Hanseniaspora uvarum* (Figure 1a), was a dominant yeast species in the initial microbial population. *H. uvarum* and the multilateral budding yeast *Candida* sp (Figure 1b) accounted for more than 60% of the total yeast population. About 13.3% of the cfu's were large pale pink colonies that turned green then black when mature. They were identified as the mold-like yeast *Aureobasidium pullulans* (Figure 1c). Other species included *Candida diversa, Cryptococcus laurentii, Cryptococcus albidus, Pichia kluyveri, Issatchenkia terricola, Metschnikowia pulcherrima, Saccharomycopsis crataegensis*, and a single isolate of *Candida steatolytica.*

Table 1. Identity and numbers of yeast found in the initial must sample.

Species	Total Counts	Frequency log cfu/mL	cfu as % of total
Yeasts			
Candida sp	1420	5.38	32.1
Hanseniaspora uvarum	1336	5.34	30.2
Aureobasidium pullulans	589	4.99	13.3
Candida diversa	432	4.85	9.8
Pichia kluyveri	296	4.69	6.7
Rhodotorula glutinis	204	4.53	4.6
Cryptococcus laurentii	102	4.23	2.3
Cryptococcus albidus	20	3.51	0.5
Issatchenkia terricola	16	3.43	0.4
Metschnikowia pulcherrima	9	3.17	0.2
Saccharomycopsis crataegensis	2	ns[1]	ns
Sporobolomyces roseus	1	ns	ns
Candida steatolytica	1	ns	ns
Bacteria			
all species	590	4.99	ni[2]
Total cfu's identified	5017		

[1]ns = not statistically significant
[2]ni = Bacteria not included in % calculations

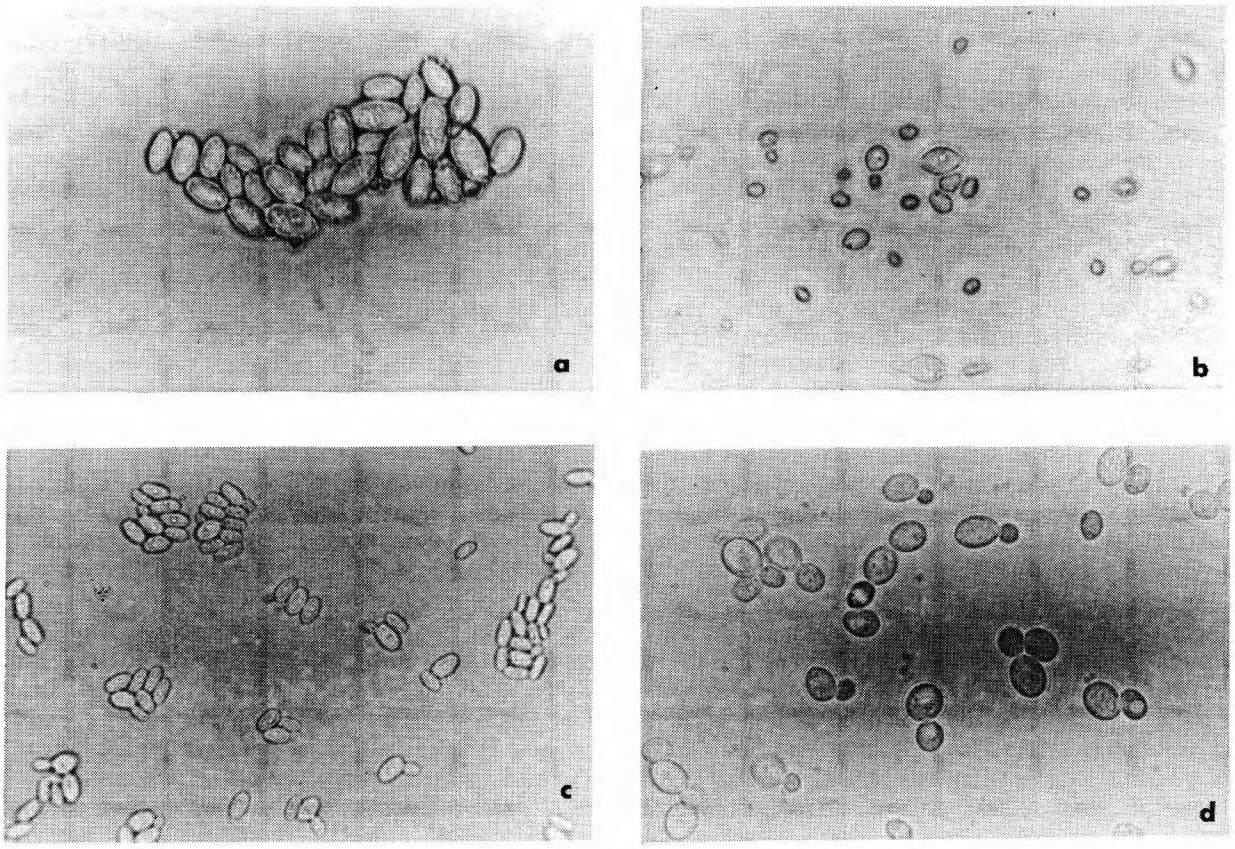

Figure 1. Yeasts Found in a Must From the Niagara Peninsula.
a) *Aureobasidium pullulans* b) *Hanseniaspora uvarum,* c) *Candida* sp, d) *Rhodotorula glutinis*

Pink- or orange-pigmented yeasts varied greatly according to the site of collection (data not presented). The most frequent was *Rhodotorula glutinis* (Figure 1d) with only a single colony of *Sporobolomyces roseus.*

Studies on grape and must microflora (Rosini *et al.*, 1982) have shown maximum diversity of yeast species occurs on the mature grape and during must preparation. As the fermentation proceeded, the total number of yeast cells increased to log 7.82 cfu/mL (Figure 2) and the number of yeast species present decreased (Figure 3). No isolates of the moderately fermentative (*Pichia* and *Metschnikowia*) yeasts were found after 28 h fermentation and the population of *Aureobasidium, Rhodotorula* and *Cryptococcus* had declined markedly (Figure 2). After 47 h, only the fermentative yeasts were isolated. After 71 h the population of *Hanseniaspora uvarum* had increased to a maximum of log 6.84 cfu/mL and the ethanol concentration reached 24 g/L. From this point the population of *H. uvarum* declined until it was undetectable (less than log 2 cfu/mL) by the end of the fermentation.

The *Candida* sp population showed an initial increase to log 5.84 cfu/mL then declined slowly to less than log 2 cfu/mL by 130 h. Under microaerophilic conditions other *Candida* species (*C. krusei, C. vini, C. fermentans*) can tolerate more than 10% (v/v) levels of ethanol (Gao and Fleet, 1988). Although *Candida* has been reported in most studies, its persistence as shown in Figure 3 is unusual. The proportion of *Candida* sp varied from 10 to 32% of the initial must population depending on the geographic location of the sample (data not shown). The population dynamics of *Candida* sp paralleled that of *S. cerevisiae* until the end of the fermentation when *Candida* sp could no longer be detected. In addition to 80 g/L ethanol, a pure culture of the *Candida* sp isolated in this study (isolation no. R-7), produced more than 240 mg/L 2-methyl-1-propanol which is well above the taste threshold (Rankine, 1967; Holloway and Subden, 1990). If not controlled, indigenous *C. stellata* can negatively affect the quality of wine (Sponholz and Dittrich, 1974; Minarik and Hanicova, 1982).

S. cerevisiae was not detected in the must until

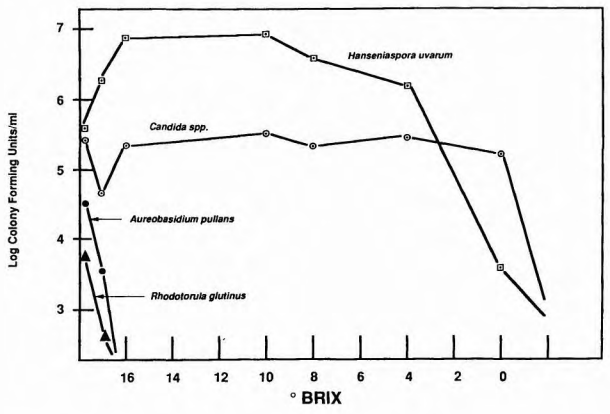

Fig. 2. Population Changes of Selected Species.

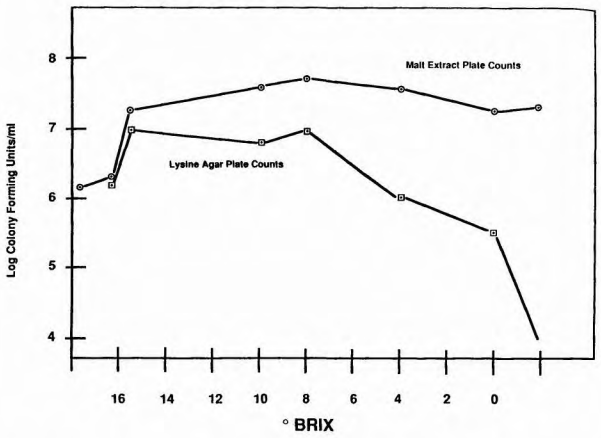

Fig. 3. Yeast Population Succession During Fermentation. The total numbers of yeast colony forming units are shown on the malt extract plate counts. The lysine plate counts represent the non-*Saccharomyces* species.

47 h of fermentation. At this time the total yeast population was log 6.9 cfu/mL and it was the numerically dominant species present. The *S. cerevisiae* population rose to log 7.77 cfu/mL by 71 h and remained constant until the end of fermentation. After 130 h the fermentation ceased (74 g/L ethanol, 0.1% reducing sugar) and *S. cerevisiae* (including *S. cerevisiae* var *bayanus*) was the only yeast that could be isolated. Whether this was a wild type *S. cerevisiae* or a commercial wine yeast present as a minor contaminant introduced into the juice during its passage through the press or other winery equipment is not known.

In all studies the apiculate yeasts dominate the initial microbial populations in grape musts. There are however, differences in the ratio of perfect (mostly *Hanseniaspora*) to imperfect (mostly *Kloeckera*) isolates. In Niagara, Northern Italy (Rosini *et al.*, 1982) and Arkansas (Moore *et al.*, 1988), the perfect form predominates in contrast to the predominating imperfect forms in Bordeaux (Domercq, 1957), Majorca (Mora *et al.*, 1988), and Southern Italy (Castelli, 1954). The contribution of only a few of the "wild" yeasts to wine composition is described elsewhere (Soles *et al.*, 1982).

Conclusions

The thirteen species of yeast found in the musts of the Niagara Peninsula were similar to the yeast microflora found in most of the wine regions of the world. *Saccharomyces cerevisiae* was not detectable in the initial must sample but appeared in samples after the fermentation had produced 10g/L ethanol. Presumably, *Saccharomyces cerevisiae* (and other species), were present in the initial must but at frequencies less than 1/5000 yeast colony forming units.

With the exception of *Candida* sp, the species succession in the fermenting must was similar to those in other parts of the winemaking world. *Candida* sp persisted in the fermenting must until the ethanol reached 74g/L. In wine fermentations the *Candida* sp bears some resemblance both morphologically and metabolically to *Candida stellata* which is ethanol and SO tolerant (Sponholz and Dittrich, 1974) and is of some concern as it has been reported to produce 2-methyl-1-propanol at concentrations above the sensory threshold in wines.

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