Seasonal Variation of Indigenous *Saccharomyces cerevisiae* Strains Isolated from Vineyards of the Western Cape in South Africa

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There is strong support for the use of naturally-occurring *Saccharomyces cerevisiae* strains that improve the sensory quality of wines and reflect the characteristics of a given region. Contrary to popular belief, *S. cerevisiae* is found at very low numbers on healthy, undamaged grapes and is rarely isolated from intact berries. The majority of studies on the population kinetics and geographic distribution of indigenous *S. cerevisiae* strains have not adequately focused on the variation in their numbers over a longer period of time. This paper discusses the results obtained in the first phase of a comprehensive research programme aimed at assessing how the natural population dynamics of *S. cerevisiae* are affected over the long term by abiotic factors. Indigenous strains of *S. cerevisiae* were aseptically isolated from eight sites in four areas in the coastal regions of the Western Cape, South Africa, during 1995 through 1998. Thirty colonies per site were isolated and the *S. cerevisiae* strains were characterised by electrophoretic karyotyping. Strain numbers per site varied over the four-year study period. Weather conditions resulting in severe fungal infestations and heavy applications of chemical sprays dramatically reduced the numbers of *S. cerevisiae* strains recovered during 1997. A return to normal weather patterns in 1998 resulted in a gradual recovery of the indigenous population. Indications are that some of the strains isolated are widespread in the study area and may represent yeasts typical of the area. Commercial wine yeast strains were recovered in only a few instances and the likelihood that commercial yeasts will eventually replace the natural yeast microflora in vineyards seems remote.

In a previous paper we reported on efforts to isolate strains of *Saccharomyces cerevisiae* from wine grapes at 13 sites in five areas within the coastal region of the Western Cape in South Africa (Van der Westhuizen, Augustyn & Pretorius, 2000). The sought-after yeasts were recovered from eight sites, while grapes from five sites contained no *S. cerevisiae* at all. As the grapes had been harvested and juices prepared under aseptic conditions, the yeasts recovered could only have come from the vineyards sampled.

The presence or absence of *S. cerevisiae* on grapes is the subject of some debate. While some studies, where grapes were harvested under aseptic conditions, indicated that strains of *S. cerevisiae* were present on grapes (Van Zyl & Du Plessis, 1961; Parish & Carrol, 1985; Török *et al.*, 1996, Van der Westhuizen *et al.*, 2000), others do not (Barnett *et al.*, 1972; Rosini, 1982, 1984). After examining the evidence at hand, Vaughan-Martini & Martini (1995) concluded that "we must exclude a natural origin for *S. cerevisiae*". However, Török *et al.* (1996) refuted this claim and concluded that the vineyard does, in fact, represent the primary source of these yeasts.

It has become abundantly clear that *S. cerevisiae* is not as plentiful in nature as was thought earlier (Peynaud & Domercq, 1959; Van Zyl & Du Plessis, 1961; Benda, 1964). It has also become clear the *S. cerevisiae* strains resident on surfaces in the winery are much more numerous than those present in the vineyard. These are, in all probability, the yeasts that will dominate spontaneous fermentations (Peynaud & Domercq, 1959; Rosini, 1984) and also play a role in other fermentations inoculated with pure yeast cultures (Rosini, 1984; Constanti *et al.*, 1997).

Some authors have presented evidence that specific strains of *S. cerevisiae* are widely distributed in particular areas (Vézinhet *et al.*, 1992; Versavaud *et al.*, 1995). We did not find such dominance in the coastal regions of the Western Cape (Van der Westhuizen *et al.*, 2000). Nevertheless, the 46 different karyotypes identified in that study represent a valuable natural resource (Pretorius, Van der Westhuizen & Augustyn, 1999). The aim of this study was to determine the natural population dynamics of *S. cerevisiae* strains in the same vineyards over a four-year period.

MATERIALS AND METHODS

Areas sampled: Yeast strains were isolated from grapes sampled in the same vineyards at eight different sites in the Constantia, Stellenbosch, Somerset West and Hermanus areas of the Western Cape, South Africa, during the 1996, 1997 and 1998 seasons.

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Collection and isolation procedures were done according to the methods described by Van der Westhuizen *et al.* (2000).

In this study yeasts were isolated from the same vineyards at the same sites and areas as noted by Van der Westhuizen *et al.* (2000). These data were combined with those generated in this study to give a four-year observation period.

Meteorological data: The monthly rainfall and average monthly maximum temperature (1994 through 1998) for the Constantia, Stellenbosch and Somerset West areas were recorded by automatic weather stations. These data are presented in Tables 1 and 2, respectively.

Preparation of intact chromosomal DNA and pulse field gel electrophoresis: Samples were prepared according to the embedded agarose procedure of Carle & Olson (1985). The methods applied were as in Van der Westhuizen, Augustyn & Pretorius (1999).

Randomly amplified polymorphic DNA (RAPD) analysis: Yeast cells were cultured and the DNA isolation was performed using the method as described by Van der Westhuizen & Pretorius (1992). Polymerase chain reactions (PCR) were performed using primer OPC-09 (5'-CTCACCGTCC-3') as applied by Van der Westhuizen *et al.* (1999).

RESULTS AND DISCUSSION

Yeast isolation: During 1995 only eight of the original 13 sampling sites contained strains of *S. cerevisiae* (Van der Westhuizen *et al.*, 2000). These eight sampling sites formed the basis of this study over the next three years. Fermentation rates in the aseptically prepared musts again differed dramatically (data not shown) with some samples not fermenting at all. This not only happened in a particular year when considering all sites sampled, but also over years at a particular site. These observations indicated that while all sites had contained *S. cerevisiae* during 1995, this was not necessarily so over the next three years. This observation may indicate that external factors (climate, application of different numbers of chemical spray) had affected the natural *S. cerevisiae* population. On the other hand, it may simply be due to the fact that the low numbers of naturally occurring *S. cerevisiae* are normally distributed in a rather haphazard manner (Török *et al.*, 1996).

TABLE 1
Monthly rainfall (mm) in the Constantia, Stellenbosch and Somerset West areas (1994 to 1998).

Area/	Month*											
Year	1	2	3	4	5	6	7	8	9	10	11	12
Constantia												
LTA†	**26.3**	**23.9**	**34.5**	**78.0**	**137.9**	**187.6**	**177.3**	**155.6**	**108.0**	**60.1**	**42.3**	**40.2**
1994	18.8	3.7	2.9	53.7	80.9	513.3	159.2	45.5	74.1	19.5	17.5	8.0
1995	34.2	5.0	8.0	20.6	93.5	120.5	**295.0**	156.1	45.2	**112.8**	20.5	29.8
1996	2.0	**54.0**	39.5	56.0	70.8	225.6	196.8	143.7	**305.7**	**85.4**	**64.7**	**62.7**
1997	18.7	3.3	15.4	62.9	111.3	198.4	51.0	270.6	18.5	24.2	**93.5**	22.4
1998	19.5	0.8	15.5	71.0	250.7	134.6	163.2	78.4	73.3	33.9	65.3	35.9
Stellenbosch												
LTA†	**19.2**	**21.1**	**30.5**	**76.8**	**107.8**	**126.6**	**116.3**	**84.1**	**55.8**	**44.9**	**27.2**	**25.2**
1994	42.3	3.3	15.9	58.6	47.4	278.9	96.0	39.0	45.0	27.5	13.2	16.0
1995	17.1	7.8	12.8	22.5	95.1	136.6	**146.8**	123.6	24.8	**89.3**	12.2	**51.3**
1996	5.3	**56.1**	**41.9**	49.1	57.8	194.9	90.5	128.4	136.7	**106.5**	**60.3**	**55.3**
1997	12.5	1.5	4.6	59.4	91.3	167.9	31.3	91.3	15.2	15.7	**106.3**	11.7
1998	22.4	0.0	27.2	40.6	260.4	79.8	103.6	61.2	35.7	25.6	72.4	36.2
Somerset West												
LTA†	**10.2**	**11.6**	**16.1**	**43.7**	**91.6**	**196.8**	**101.0**	**69.9**	**45.5**	**43.4**	**58.6**	**45.5**
1994	22.8	3.8	8.2	41.0	59.4	296.8	98.2	37.0	67.8	30.4	17.4	27.0
1995	5.6	3.0	21.8	25.9	105.0	147.6	94.4	**116.8**	8.4	25.6	25.8	91.6
1996	4.4	**55.8**	33.4	1.2	69.4	194.8	**144.6**	67.0	**98.2**	**120.0**	**74.6**	**53.6**
1997	19.6	3.8	14.2	64.6	94.8	243.0	44.2	37.8	7.4	27.4	**98.6**	28.0
1998	3.6	0.0	18.2	53.2	193.2	101.6	123.8	91.0	45.8	13.4	76.4	55.2

*1 to 12: January through December.

†LTA: Long-term average.

TABLE 2
Average monthly maximum temperature (°C) in the Constantia, Stellenbosch and Somerset West areas (1994 to 1998).

Area/ Year	Month*											
	1	2	3	4	5	6	7	8	9	10	11	12
Constantia												
LTA†	**24.8**	**25.3**	**24.4**	**22.1**	**19.6**	**17.6**	**16.9**	**17.1**	**18.3**	**20.2**	**22.1**	**23.6**
1994	24.9	26.0	24.9	23.3	18.6	16.6	17.1	17.3	19.2	21.6	22.5	24.5
1995	25.5	26.0	25.2	21.0	20.6	18.0	15.2	17.1	18.7	**19.2**	22.6	24.7
1996	25.3	25.6	23.5	23.6	20.9	18.4	16.0	16.3	16.5	**19.3**	**19.3**	**23.0**
1997	24.5	23.4	23.6	21.3	20.4	15.8	18.0	16.9	21.0	22.7	**20.9**	23.5
1998	24.2	26.6	23.5	22.5	19.4	17.6	16.5	18.0	18.0	20.6	21.7	24.3
Stellenbosch												
LTA†	**27.7**	**28.1**	**26.6**	**22.9**	**20.1**	**17.6**	**17.1**	**18.0**	**20.0**	**22.8**	**25.2**	**26.5**
1994	28.6	30.3	27.6	25.3	19.7	16.6	17.4	18.2	20.6	24.5	24.2	27.7
1995	28.7	30.4	28.1	23.8	22.0	18.1	15.5	17.5	20.0	20.8	24.9	27.4
1996	29.0	**28.5**	**25.8**	25.3	21.8	18.1	16.2	17.0	17.8	**21.2**	**21.3**	**24.8**
1997	27.3	27.0	26.2	22.6	21.8	16.5	18.8	17.6	22.7	25.6	**23.6**	26.7
1998	26.8	29.9	25.9	24.8	19.9	17.7	17.2	18.8	19.7	23.3	24.1	27.4
Somerset West												
LTA†	**28.0**	**29.1**	**27.2**	**24.4**	**21.3**	**18.6**	**17.7**	**18.5**	**20.8**	**23.3**	**23.7**	**26.1**
1994	28.5	29.4	27.4	25.8	20.2	17.6	18.4	18.5	20.8	23.5	23.4	27.0
1995	28.1	30.4	27.8	22.8	22.3	19.1	15.0	17.8	20.2	23.1	24.7	**27.0**
1996	28.5	**29.0**	25.7	27.2	22.4	19.9	16.9	17.9	18.6	**21.4**	**21.0**	**25.7**
1997	27.8	26.9	26.4	23.1	22.1	17.4	19.8	16.9	23.7	25.1	**23.1**	Nd+
1998	26.2	30.0	26.6	25.3	19.9	18.9	18.3	19.7	19.8	22.8	23.2	26.4

*1 to 12: January through December.
†LTA: Long-term average.
+Not determined.

Electrophoretic karyotyping: CHEF-DNA analysis was run on 30 isolates per sampling site. Only one sample contained a mixed culture of *S. cerevisiae* and non-*Saccharomyces* yeasts. The remaining samples contained either non-*Saccharomyces* or *S. cerevisiae*, as was also the case in our previous study (Van der Westhuizen *et al.*, 2000). The *S. cerevisiae* strains recovered during 1996 to 1998 are listed in Table 3. These data are summarised in Table 4, which lists the number of unique karyotypes per site per year and includes data for 1995 taken from Van der Westhuizen *et al.* (2000). Data in Tables 3 and 4 do not take into account that a particular yeast may be present at more than one site, or occur in more than one year. Comparison of karyotypes between sites and over a period of years indicated that a number of yeasts were present at more than one site or re-occurred over years (Table 5). RAPD-PCR analysis confirmed that yeasts indicated to be the same by means of electrophoretic karyotyping were in fact the same (data not shown). The number of commercial yeasts recovered was small and none were found during 1998. As noted before (Van der Westhuizen *et al.*, 2000), the likelihood that commercial yeasts will eventually replace the natural yeast microflora in vineyards seems remote. This finding should appease those people who are concerned about possible detrimental effects of uncontrolled spreading of commercial yeast cultures in the environment.

Seasonal variation in the *S. cerevisiae* population: Data presented in Table 3 clearly indicate that the number of *S. cerevisiae* strains per site varied dramatically between 1996 and 1998. This fact is emphasised when perusing the summarised data (1995 - 1998) presented in Table 4.

It is well known that grape yeast microflora vary from area to area and from vintage (year) to vintage (Benda, 1964; Frezier & Dubourdieu, 1992; Vézinhet *et al.*, 1992; Schütz & Gafner, 1994). However, authors rarely comment on possible factors that affect yeast populations. Benda (1964), who studied the yeast microflora in Franken during 1959 and 1960, reported July to October 1959 to be a relatively dry and sunny period in contrast to the wet and more overcast 1960. She concluded that these climatic differences had affected the yeast microflora present on the grapes.

TABLE 3
Distribution and frequency of S. cerevisiae strains per sampling site between 1996 and 1998.

Area[1]	Sampling site[2]	*S. cerevisiae* strains per sampling site								
		1996 strain = number[3] (%)			1997 strain = number (%)			1998 strain = number (%)		
A	Groot Constantia (1)	C6-1	30	(100)	None	-	-	C8-1	24	(80)
	Buitenverwachting (2)	B6-1	1	(3)	None	-	-	B8-1	non-*cerevisiae*	
		B6-2	2	(7)				B8-2	1	(3)
		B6-3	9	(30)				B8-3	3	(10)
		B6-4	4	(13)				B8-4	15	(50)
		B6-5	14	(47)				B8-5	5	(17)
								B8-6	3	(10)
								B8-7	1	(3)
B	Jordan (3)	J6-1 (N96)	21	(71)	J7-1 (VIN13)	30	(100)	J8-1	10	(34)
		J6-2	1	(3)				J8-2	1	(3)
		J6-3	1	(3)				J8-3	4	(13)
		J6-4	3	(10)				J8-4	12	(40)
		J6-5	1	(3)				J8-5	1	(3)
		J6-6	3	(10)				J8-6	1	(3)
								J8-7	1	(3)
	Lievland (4)	None	-	-	None	-	-	L8-1	27	(90)
								L8-2	3	(10)
	Mont Fleur (5)	M6-1	16	(54)	None	-	-	M8-1	30	(100)
		M6-2	1	(3)						
		M6-3	1	(3)						
		M6-4	3	(10)						
		M6-5	3	(10)						
		M6-6	3	(10)						
		M6-7	2	(7)						
		M6-8	1	(3)						
C	Vergelegen (7)	V6-1 (VIN13)	17	(57)	V7-1	30	(100)	V8-1	23	(77)
		V6-2	4	(13)				V8-2	7	(23)
		V6-3	5	(17)						
		V6-4	3	(10)						
		V6-5	1	(3)						
D	Bouchard Finlayson (12)	F6-1	30	(100)	F7-1	13	(43)	F8-1	4	(14)
					F7-2 (VIN13)	16	(54)	F8-2	5	(17)
					F7-3	1	(3)	F8-3	5	(17)
								F8-4	7	(23)
								F8-5	6	(20)
								F8-6	1	(3)
								F8-7	1	(3)
								F8-8	1	(3)
	Hamilton Russell (13)	None	-	-	None	-	-	HR8-1	15	(50)
								HR8-2	4	(13)
								HR8-3	6	(20)
								HR8-4	2	(7)
								HR8-5	3	(10)

[1] A = Constantia; B = Stellenbosch; C = Somerset West; D = Hermanus.
[2] Sampling site number according to Van der Westhuizen *et al.* (2000).
[3] Number out of 30.

TABLE 4
Summary: Number of *S. cerevisiae* strains per site per year.

Area	Site	1995*	1996	1997	1998
Constantia	1	4	1	0	1
	2	15	5	0	6
Stellenbosch	3	4	6	1	7
	4	3	0	0	2
	5	9	8	0	1
Somerset West	7	2	5	1	2
Hermanus	12	4	1	3	8
	13	10	0	0	5

*Data from Van der Westhuizen et al. (2000).

TABLE 5
Yeast strains present at more than one site or at the same site in different years*.

Group no.	Equivalent strains			
	1995	1996	1997	1998
1	B5-3:**		-	B8-7
2	B5-5 : HR5-7			F8-7
3	B5-6 : C5-3 : F5-3	F6-1 : M6-1 :		B8-3
4	B5-13 :			B8-4 : V8-2
5	B5-14 :	B6-1		
6		B6-5 : V6-3		
7		C6-1 :		C8-1
8				J8-1 : L8-1
9	M5-2 :	M6-8		
10	M5-4 :	M6-7		
11	M5-6 :	M6-4 :	V7-1	
12	V5-1 : HR5-10			
13	F5-1 : HR5-4			
14	F5-2 :			F8-1
15			F7-3 :	F8-3
16				F8-2 : HR8-2
17	HR5-9 :			B8-1 : HR8-1

*Does not include commercial yeast strains: VIN13 = J5-1 : L5-2 : V6-1 : J7-1 : F7-2; N96 = L5-3 : J6-1.

**Site abbreviations as in Table 3: B = Buitenverwachting; C = Constantia; J = Jordan; L = Lievland; M = Mont Fleur; V = Vergelegen; F = Bouchard Finlayson; HR = Hamilton Russell.

Perusal of the data in Table 4 reveals a number of trends, some coupled to area/site, while others are more general. Briefly, from 1995 to 1996, the numbers of *S. cerevisiae* strains recovered at Constantia and Hermanus declined dramatically while numbers actually increased, or stayed approximately the same, at three of the four remaining sites. During 1997 a total of five *S. cerevisiae* strains were detected at only three sites. Strain count increased at all sites during 1998. Consideration of the meteorological data in Tables 1 and 2 helps to explain some of these trends. In the Western Cape vines typically flower from the end of October to November depending on factors such as grape cultivar, *terroir*, general growth area (*e.g.* warmer inland/cooler coastal), specific vintage (*e.g.* cooler/warmer or wetter/dryer than usual). Harvest takes place from February to April subject to the same factors as mentioned above. Table 1 clearly indicates that late 1995, early 1996 and particularly late 1996 had periods in which unusually high volumes of rain were recorded. This high level of moisture coupled to temperatures (Table 2) that, although generally lower than the long-term averages, were still conducive to fungal growth resulted in particularly severe outbreaks of disease. Consequently, on average, 2- to 2.5-fold more rounds of fungicide sprays were applied during these unusually wet periods. Clearly the higher rainfall and concomitant much-increased application of anti-fungal sprays severely affected the naturally occuring yeasts as reflected in the extremely low recovery recorded during 1997. Data in Table 4 represent mean values for an entire area and may differ from that relevant for a particular site (terrain). This fact will help explain the apparent contradiction between the 1995/1996 rainfall patterns (Table 1) and numbers of *S. cerevisiae* strains present in the Somerset Wes/Stellenbosch areas (Table 4). Meteorological data for the Hermanus area were not available, but consultations with winemakers confirmed a situation similar to that recorded for Constantia (Table 1), a fact reflected in the declining strain counts between 1995 and 1997 (Table 4). Indigenous yeast populations will clearly always be affected by application of fungicides, even in those "normal" years when producers follow standard spraying programmes.

It was pointed out earlier that, with one exception, must/wine samples prepared from greapes harvested at the various sites contained either *S. cerevisiae* or non-*Saccharomyces* yeasts. Data in Tables 3 and 4 clearly indicate that the initially scarce *S. cerevisiae* (Peynaud & Domercq, 1959; Van Zyl & Du Plessis, 1961) were hard hit by the intensified spraying programmes mentioned above. By 1997 these yeasts had been almost completely eradicated in the vineyard sections under study. Alternatively, their numbers were reduced to such low levels that they could not maintain themselves in competition with the more numerous non-*Saccharomyces* yeast. It is therefore very interesting, though not unexpected, to note that as chemical spray programmes returned to normal (rainfall closer to the long-term average, Tabel 1), *S. cerevisiae* strains started reappearing, or increasing in number, at all sampling sites.

Distribution of yeasts between areas: Perusal of the data summarised in Table 5 indicates 17 groups of equivalent yeasts. Two additional groups representing recoveries of commercial yeast are presented as a footnote.

No single *S. cerevisiae* strain was present at one single site in all four years (Table 5). Groups 1, 5, 7, 9, 10, 14 and 15 represent six different yeasts present at the same respective site for two years. Sometimes these yeasts were recorded in successive years (*e.g.* groups 5, 9, 10 and 15), whilst at other sites the relevant yeast reappeared after one or two years (*e.g.* groups 1, 7 and 14). This cycle of presence, absence, reappearance was noted earlier during a study of *S. cerevisiae* naturally present on grapes in Champagne and the Loire Valley (Vézinhet *et al.*, 1992). The yeasts represented by groups 3 and 11, respectively, were the only ones to be recorded in three seasons. From Table 5 it is also clear that one of the yeasts was recorded at four different sites (group 3) and another at three sites (group 2). Of the remaining yeasts, seven were found at one site only, while eight more were recorded from two sites. The fact that many of these groups represent a yeast recovered from widely separated sites and in different years would seem to indicate the presence of an indigenous microflora typical of the general area under study. That these yeasts were not recovered annually could be ascribed to natural population fluctuations – fluctuations brought about by man-made external factors (chemical sprays), or an inadequate sampling technique that is unable to compensate for the normal haphazard distribution of *S. cerevisiae* (Török *et al.*, 1996) in the vineyard.

CONCLUSIONS

Data generated in this study clearly indicate that *S. cerevisiae* does occur in nature in numbers sufficient to conduct a successful, spontaneous fermentation. Grapes from sites which yielded no *S. cerevisiae* at a particular sampling will more than likely contain sufficient wine yeasts when the sample size is increased, for example, when harvesting the whole vineyard. Some of the *S. cerevisiae* strains recovered may be widespread in the area studied. It remains to be determined if these yeasts actually contribute to the character of the wines from these areas. Whatever the case, these indigenous wine yeasts represent a valuable natural resource and the best of them will be included in our extensive yeast breeding programme.

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