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Genetic characterisation of New Zealand and Australian wine yeasts

Occurrence of killer systems and homothallism

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Abstract. Twenty five culture wine yeast strains from New Zealand and Australia were examined for killer capability or sensitivity. Eight yeast strains were K_2^+ killers, six of the K_2^+ R_1^- R_3^+ phenotype and two of the K_2^+ R_1^- R_3^- phenotype. The seventeen sensitive strains were separated into four phenotype classes. The homothallic life cycle was detected in twenty-one strains and one further strain is probably triploid.

INTRODUCTION

Investigations of the genetics of wine yeasts has revealed that they are amenable to modifications of their winemaking characteristics by genetic techniques, e.g. selective hybridisation (for review see Thornton 1983). Many New Zealand wine yeasts have a homothallic life cycle which initially poses a problem in selective hybridisation but which can be overcome by spore-cell mating (Thornton & Eschenbruch 1976). Wine yeasts which are tetraploid (Naumov & Kondrateva 1979) and triploid (Takahashi 1978) have also been reported.

Strains of *Saccharomyces cerevisiae* which produce a protein toxin lethal to sensitive yeast strains are described as 'killer' (Makower & Bevan 1963). Three different types of killer yeast strains have been identified, i.e. K_1^+, K_2^+ and K_3^+. The K_1^+ and K_2^+ strain are sensitive to each others toxins but K_2^+ and K_3^+ are reported to be cross-immune (for review see Wickner 1981). The occurrence of K_1^+ killer strains is widespread in laboratory yeasts whereas many wine yeasts are K_2^+ killers (Naumova & Naumov 1973; Naumov et al. 1973). The killer capability is a potentially valuable character in wine yeasts since it could aid in the elimination of sensitive wild wine yeast strains from grape juice, thereby reducing the need for sulphur dioxide addition. Also, killer yeasts apparently prevent the growth of pseudo-film-forming yeasts in stored bulk wine (Hara et al. 1980).

The paper reports the occurrence of homothallism and the distribution of

killer capability or sensitivity characters in twenty-five wine yeasts from New Zealand and Australia.

MATERIALS AND METHODS

Wine yeast strains. All the wine yeasts strains are, or have been, used commercially. They include the following:

Nine strains from the Australian Wine Research Institute, Adelaide, South Australia, courtesy of Dr P. Monk, AWRI (prefix) 3A, 58, 62, 79, 80, 89, 93 and 105.

Thirteen wine yeast strains from Te Kauwhata Viticultural and Oenological Research Station, DSIR Wine Research, Private Bag, Te Kauwhata, New Zealand, courtesy of Dr R. Eschenbruch; R (prefix) 28, 93, 102, 103, 104, 107, 108, 109, 170, 171, 176, 177 and 179.

Two wine yeast strains, GW8021 and AR2, from Australian wine companies and one, MD26, from Montana Wines, New Zealand.

Killer yeast strains. The killer yeast strains A364A (K_1^+) and 1384 (K_2^+) and the killer sensitive strain 5X47 were received from Dr R. B. Wickner, National Institute of Health, Bethesda, MD 20025, USA. Killer yeast strain $K_3GR_5(K_3^+)$ was isolated by Dr A. L. Extremera, Facultad de Ciencias, Universidad de Granada, Spain.

Killer capability or sensitivity. Killer capability or sensitivity was determined by a variation of the technique of Makower (1964). Plates of complete agar medium buffered to pH 4.8 were spread with a lawn of yeast and incubated for 2–3 hours at 20 °C. Master plates of yeast strains were replica plated onto the spread plates and incubated at 25 °C for 2–3 days. Killer capability or sensitivity was detected by a zone clearing in the lawn. All the wine yeast strains were tested in this way against the reference killer and sensitive strains and against each other.

Sporulation and ascus dissection. Routine methods for sporulation and ascus dissection were those described previously (Thornton & Eschenbruch 1976).

RESULTS

Nineteen wine yeast strains with potentially valuable winemaking characters, e.g. efficient fermentation, relatively high glycerol production, etc., were examined for their ability to sporulate and for spore viability (Table 1). Strain

Table 1. Sporulation and spore viability of yeast strains

Strain number	Sporulation	% Spore viability[a]	Sporulation of spore cultures[c]	% Spore viability of sporulating spore cultures
AWRI				
3A	+	12.5[b]	10/10	95.0
58	+	0	–	–
62	+	45.0[b]	7/10	75.0
63	+	25.0[b]	7/10	80.0
79	+	12.5[b]	8/10	75.0
80	+	70.8	8/8	87.5
89	+	79.2	8/8	79.2
93	+	75.0	8/8	91.6
105	–	–	–	–
Ruakura				
28	+	33.3	8/10	80.0
108	+	75.0	8/8	75.0
109	+	75.0	8/8	100.0
170	+	25.0	8/10	87.5
171	+	40.0	7/10	87.5
176	+	87.5	8/8	100.0
177	+	45.5	0/8	–
179	+	100.0	8/8	100.0
Wine Companies				
AR2	+	58.3	8/8	95.0
GW8021	+	6.0[b]	0/6	–

[a] Six asci were dissected

[b] More than six asci were dissected

[c] Eight spore cultures from two tetrads or ten random spore cultures from strains not producing viable tetrads.

AWRI105 failed to sporulate, and no viable spores were recovered from strain AWRI58. The majority of strains produced asci containing four viable ascospores; the exceptions were AWRI strains 62, 63 and 79; Ruakura strains 28, 170 and 171; and strain GW8021. Since strain R28 sporulated with very low frequency, a technique described previously (Thornton & Eschenbruch 1981) was employed to enhance the number of asci. Spore cultures from all strains were cross-streaked with standard mating tester strains, and zygote formation was not observed in eighteen of them. This result suggested that the spore cultures of these eighteen strains were not haploid.

The exception was strain GW8021 in which three of the six spore isolates mated with the tester strains. Two of these were *a* mating type and one was α mating type, indicating that they were haploid or aneuploid. The very low spore viability of this strain, the observation of mating, and the lack of sporula-

Table 2. Characterisation of killer capability or sensitivity of yeast strains

Phenotypes	Yeast strains		
	AWRI	Ruakura	Wine Companies
Killer			
$K_2^+ R_1^- R_3^+$	63, 79	108, 179	AR2, MD26
$K_2^+ R_1^- R_3^-$	3A	107	
Sensitive			
$R_1^- R_2^+ R_3^+$			GW8021
$R_1^- R_2^+ R_3^-$		109, 177	
$R_1^- R_2^- R_3^+$	58, 62, 80, 89, 105	28, 102, 104, 170, 171	
$R_1^- R_2^- R_3^-$	93	93, 103, 176	

tion of non-mating spore cultures suggested that GW8021 was triploid.

Eight spore cultures (two tetrads) from each strain were subjected to induction of sporulation. Ten random spore cultures were used in the case of strains which did not produce viable tetrads. Ascus formation was observed in the majority of these cultures (Table 1). Six asci were dissected from sporulating spore cultures of each strain. Five random sporulating spore cultures were dissected or, if available, four cultures derived from a single tetrad. Zygote formation was not observed when spore cultures were cross-streaked with haploid mating tester strains. This result suggested that they were not haploid and that the ploidy of the original strains was greater than tetraploid.

One tetrad from each of the sporulating spore cultures was subjected to induction of sporulation, and, in nearly every instance, sporulation was observed. This result suggested that the parent strains are either octaploid of homothallic. Strain R177 was an exception to these observations in that it produced viable ascospores, but the spore cultures neither sporulated nor mated with haploid mating tester strains.

The nineteen wine yeast strains examined above and six wine yeast strains in which homothallism had been established previously, R93, R102, R104 and R107 (Thornton & Eschenbruch 1976); R103 and MD26 (Thornton 1982), were examined for killer capability or sensitivity. Six phenotypic classes of killer capability or sensitivity were established by cross-testing the twenty-five wine yeast strains against standard killer and sensitive strains and against each other (Table 2). Eight strains were found to be killers of the K_2^+ phenotype, and of these, six displayed sensitivity to K_1^+ killer strains (R_1^-) and resistance to K_3^+ killer strains (R_3^+). The other two strains were sensitive to both K_1 and K_3 killer strains ($R_1^- R_3^-$). The seventeen non-killer strains could be divided into four phenotypic groups of killer sensitivity. No other typer of killer resistance or sensitivity was detected when the wine yeast strains were tested against each other.

DISCUSSION

The occurrence of sporulation and the absence of mating with haploid mating tester strains in the spore cultures of fifteen of the nineteen wine yeast strains examined suggest that they may be polyploid or homothallic. Polyploidy is an unlikely explanation because when the asci from spore cultures were dissected, sporulation was observed in the new spore cultures. Furthermore, when these asci were dissected, sporulation was again observed in the spore cultures. These observations would demand that these wine yeast strains are octaploid. While tetraploid yeasts are relatively common in nature, and pentaploid and hexaploid strains can be synthesised (Mortimer & Hawthorne 1969), very few naturally occurring octaploid yeast strains have been observed. The occurrence of a homothallic life cycle in wine yeast is consistent with previous reports (Thornton & Eschenbruch 1976; Benitez et al. 1983) and has been confirmed in strains AWRI3A and AR2 (Thornton, unpublished results). These observations, while not conclusive, do suggest that all fifteen strains are homothallic.

Triploid wine yeasts have been reported (Takahashi 1978), and the observations in respect to strain GW8021 suggest strongly that it also is triploid. Moreover, crosses of mating spore cultures of strain GW8021 with haploid heterothallic strains indicate that strain GW8021 is heterothallic and that its chromosomes function in an orthodox manner (Thornton, unpublished results). Further investigation is required to explain the lack of sporulation and mating in spore cultures of strain R177, the lack of spore viability in strain AWRI58 and the lack of sporulation in strain AWRI105.

Six of the eight killer wine yeasts have a $K_2^+ R_1^- R_3^+$ phenotype similar to that observed in other killer wine yeast strains (Naumova & Naumov 1973; Naumov et al. 1973). The other two killer strains are unusual as they displayed sensitivity to K_3^+ strains, and it has been claimed that K_2^+ and K_3^+ strains are cross-immune to each other's toxins (Wickner 1981). The zone of inhibition, or degree of killing, around colonies of K_3^+ strains on lawns of these two wine yeasts, although smaller than those usually found on R_3^- lawns around K_3^+ strains, was quite distinct.

The seventeen killer sensitive wine yeast strains can be separated into four phenotypic classes. One class, $R_1^- R_2^+ R_3^+$, corresponds to the pattern of the majority of killer wine yeast strains, i.e. $K_2^+ R_1^- R_2^+ R_3^+$, but which have lost the killer (K_2^+) capability, i.e. strain GW8021 could have arisen from the killer class to which strains AWRI63, AWRI79, R108, R179, AR2 and MD26 belong (Table 2). A second class $R_1^- R_2^+ R_3^-$, corresponds to the pattern of those killer wine yeasts which have lost the killer (K_2^+) capability and which are not cross-immune to killer strains of the K_3^+ type, i.e. R109 and R177 could have arisen from the killer class to which strains AWRI3A and R107 belong (Table 2). The killer sensitive class $R_1^+ R_2^- R_3^+$ was not observed, nor was

the neutral or resistant class $R_1^+ R_2^+ R_3^+$.

The presence of K_2^+ killing ability in eight of the wine yeast strains examined and the sensitivity of all twenty-five strains to K_1^+ killer toxin is a similar pattern to that reported in wine yeast strains isolated in other winemaking regions of the world (Naumova & Naumov 1973; Naumov et al. 1973). This pattern is apparently representative of the *Saccharomyces cerevisiae* flora of grape skins since the use of wine yeast strains with K_1^+ killing ability eliminates sensitive yeasts from fermenting grape juice (Hara et al. 1980; Hara 1984). Killer yeast strains have also been applied to winemaking in France by P. Barre (Montpellier) and C. Cuinier (Côte du Rhone) who have isolated K_1^+ wine yeast strains (personal communication) which are now produced by bulk yeast manufacturers. A further refinement of the *Saccharomyces cerevisiae* killing system for winemaking would be the incorporation of resistance in K_1^+ strains to the K_2^+ killer strains found in the grape juice. However, toxin production and resistance to that toxin reside on the same double stranded RNA plasmid in the cell cytoplasm and the plasmid carrying the $K_1^+ R_1^+$ factors excludes the plasmid carrying the R_2^+ factor from the cell (Wickner 1983).

A further development might be the incorporation into K_1^+ killer wine yeast strains of the killer activity displayed by other genera of yeasts commonly found among the grapeskin flora, e.g. *Pichia*. This would be technically more difficult since the genetic source of the killer activity of these yeasts is chromosomal rather than cytoplasmic (Young & Yagiu 1978). However, the resulting yeast with multi-killer properties would eliminate virutally all the yeast flora of grape juice, thereby reducing the need for the addition of sulphur dioxide which is currently used to suppress their growth.

The widespread occurrence of homothallism in wine yeasts is not a barrier to the development by selective hybridisation of wine yeasts with improved winemaking characteristics (Thornton 1983). Triploid wine yeasts capable of sporulation can also be utilised for this purpose when stable haploid offspring can be isolated. The killer capability is potentially valuable in wine yeasts, and investigations are proceeding to develop strains which are not only killer but also have resistance to the toxins of others types of killer strains.

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