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Microecology of yeasts and yeast-like organisms associated with an English vineyard

by

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Mikroökologie der Hefen und hefenähnlichen Organismen aus einem englischen Weinberg

Zusammenfassung. — Mit Hilfe verschiedener Schätzmethoden wurde die quantitative Verteilung der Hefen und hefenähnlichen Organismen im Weinberg von Long Ashton ermittelt. Die Probenahme erfolgte in allen Stadien der Rebenentwicklung. Hefen und ähnliche Organismen fanden sich an sämtlichen Teilen der Rebe, im Boden, in der Luft, an anderen Pflanzen sowie an tierischen Überträgern dieses Biotops. Es traten viele potentielle Urheber von Fehlgärungen, z. B. Kahmhefen, auf, während Weinhefen (*Saccharomyces* spp.) nur selten festgestellt wurden. Unter den isolierten Mikroorganismen befanden sich aus Weinbergen bisher nicht bekannte Arten der Gattungen *Trichosporonoides, Saccharomycopsis* (syn. *Endomycopsis), Sporobolomyces, Sporidiobolus, Rhodosporidium, Candida, Nadsonia* und *Schizosaccharomyces.* Des weiteren wurden Species von *Aureobasidium, Trichosporon, Sporobolomyces, Rhodotorula, Cryptoccus, Metschnikowia, Torulopsis, Candida, Pichia, Hansenula, Kluyveromyces, Saccharomyces* und *Kloeckera* identifiziert.

Introduction

All aspects of viticulture and oenology have been investigated extensively with the ultimate objective of economically producing the most successful wines (Amerine and Singleton 1971). Reviews on the yeast flora of wine grapes have been published (e. g. Amerine and Kunkee 1968; Kunkee and Amerine 1970). However, most studies have been on mature grapes or fresh must, with the exception of some investigators who also examined unripe berries as well as other parts of the vine, and other vineyard sources (Van Zyl and du Plessis 1961, Minárik 1965, Parle and di Menna 1966, Svejcar 1967). Davenport (1969 a, 1970 a, 1970 b, 1971, 1972, 1973) reported on a project in which a systematic study was made of an English vineyard with particular reference to yeasts and yeast-like organisms. This study was different from all others in that he used the developing grape vine stages as a biological calendar in sampling and monitoring numerous vineyard environments for yeasts. Presented here is a summary of a three year survey of the yeasts and yeast-like organisms isolated and identified from vines and other vineyard sources.

Materials and Methods

The vineyard was examined as an ecosystem and all sampling and monitoring programmes were related to grape vine development stages, from dormant bud to mature fruit, with emphasis on the microecology of yeasts (Davenport 1973). Samples were taken aseptically and processed immediately using the methods based on those of Beech and Davenport (1971) and Davenport (1973).

The samples were weighed and sufficient chilled sterile glass distilled water was added to give a 10-fold dilution slurry when homogenised in a Waring blender. Direct microscopic observation of this slurry was used to determine the potential yeast flora and as a guide for subsequent plating out on several agar media of vary-

Table 1

The incidence of yeasts and yeast-like organisms from various environments sampled at different periods of grape vine development
Verteilung der Hefen und hefenähnlichen Organismen aus verschiedenem Milieu. Probenahme zu unterschiedlichen Zeiten der Rebenentwicklung

Environment	Sampling times[1])	Range of viable counts X 10^{-3}/g of sample Yeast groups[2])							
		I	II	III	IV	V	VI	VII	VIII
Soil	FF	0 > 5000	0—2	—[3])	0—200	0—170	—	—	—
Rootlets	FF	0—100	0—2000	—	0—6000	0—400	—	0—10	—
Compost	ES	0—1600	60—400	0—500	0—80	0—500	—	—	—
Vine leaf litter	ES	2—260	0—7600	0—40	0—40	0—4000	—	—	—
Vine bark	ES	2000—9000	20—4000	20—1400	0—21	—	—	—	—
Vine leaves	ES	0 > 2000	0—200	0—20	0—10	0—0.2	—	0—0.2	—
Vine flowers	FF	12 > 2000	0—810	1—31	0—122	1—5	0—7	EN[4])	—
Immature grapes	IM	0 > 2000	0 > 2000	EN	0—10	0—300	0—30	EN	—
Mature grapes	MF	0—400	0—200	0—650	0—60	0—60	0—465	0—172	—
Mummified grapes	ES	0—8	0—50	EN	—	—	EN	TM[6])	—
Dormant buds	DB	3—TM	0—TM	—	0—200	0—100	0—680	—	—
Wild flowers	ES	7—100	0.1—26	0.1—100	1—60	1—16	1—7	EN	—
Carabus violaceus[5]) (invertebrate vector)	IM	0—0.3	—	0—0.1	—	0—1	—	0—0.5	—

[1]) Sampling times based on the biological calendar method (DAVENPORT 1970 and 1973):
DB = dormant bud FF = flowering-fruit set MF = mature fruit
ES = early shoots IM = immature fruit

[2]) Yeast groups:
Pigmented colonies I = dark pigmented and/or yeast-like fungi; II = carotenoid; III = pulcherrimin.
Non-pigmented colonies IV = mucoid; V = smooth; VI = rough and/or smooth.
Cell shape and/or reproduction VII = apiculate, bipolar buding; VIII = fission.

[3]) — = Not detected.

[4]) EN = Enrichment culture.

[5]) = Yeasts per specimen.

[6]) TM = Too many to count.

ing nutritional status which were incubated at 5, 15 and 37 °C. Some of the remaining slurry was mixed with liquid grape juice yeast extract (1%), pH 4.5, and placed in a medical flat bottle containing an inverted Durham tube to show gas production. These enrichment cultures were incubated at 25 °C for up to one month, after which some of the contents were streaked out on grape juice yeast extract agar. This test was specifically for detecting fermenting yeasts (*Saccharomyces* spp.) Yeast colony counts were made on all but the enrichment cultures, and representative types of each morphological form were purified and subsequently identified using the methods of Lodder (1970) and Davenport (1973).

Results and Discussion

Table 1 gives some examples of yeast counts obtained from various vineyard sources. There are very few selective and differential media for isolating yeasts (Beech and Davenport 1971) hence several cultural techniques were used, including the use of many kinds of media each of different nutritional status (Davenport 1973). These methods influenced the apparent incidence of each kind of yeast isolated, therefore the range of yeast counts is given for each morphological group from which examples of individual species were subsequently identified. Table 2 lists those yeasts of microecological significance, while Table 3 gives those organisms of potential industrial significance. Dark and carotenoid pigmented yeasts, and sometimes rough yeasts, were isolated from inside dormant buds, leaves, immature and mature grapes (Davenport 1970 a); since these organisms were also associated with similar environments in cider apple orchards it is concluded that these groups are part of a resident microflora of fruit organs. Other yeasts were also considered part of the resident cider apple orchard flora (Davenport 1968, 1969 b) and some of the species given in Table 2 would also be part of the vineyard resident microflora; this topic will be published later.

In general, the yeasts isolated in this survey were the same type as reported by Kunkee and Amerine (1970). However, there were other species, *Trichosporonoides* spp., *Sporidiobolus* spp., *Rhodosporidium* spp., *Leucosporidium* spp. and *Candida* spp. which have not been reported as vineyard yeasts. The most likely reason why these species have not been isolated before is that they were all isolated from plates incubated at 5 °C and in most cases did not grow at temperatures above 15 °C. *Saccharomyces* spp. were isolated, by enrichment cultures only, from acid soil, mushroom compost and vine flowers but not on mature fruit. This is different from the results of Relan and Vyas (1971) on ripe grapes. It is worth noting that Barnett *et al.* (1972) also reported the absence of *Saccharomyces* spp. on wine grapes of Bordeaux. This is different from the results of Van Zyl and du Plessis (1961), Parle and di Menna (1966) and Domercq (1956), all of whom found *Saccharomyces oviformis* (now *Sacch. bayanus* — Lodder 1970) on ripe grapes. The yeasts found on vine flowers by Van Zyl and du Plessis (1961) only included *Cryptococcus* spp. and *Torulopsis* spp. whereas in this survey, species of dark and carotenoid pigmented yeasts, sporing strains of *Metschnikowia pulcherrima* and *Metschnikowia reukaufii* were found together with similar yeasts reported by former authors.

The results indicate that the rhizosphere, embracing root surfaces of vine and of other vegetation, the soil and its macrofauna and microflora (Davenport 1970 a, 1973), contained the largest number of yeast species (72). Less than half this number of species were found in the phyllosphere (31) and in the atmosphere (17). It was also observed that most of the yeasts from the atmosphere were from airborne propagules, e. g. seed heads and flying insects, whereas the air itself contained

Table 2

Yeasts and yeast-like organisms isolated and identified from environments within an English vineyard.
A. Organisms of microecological significance only
Die aus verschiedenen Zonen eines englischen Weinbergs isolierten und identifizierten Hefen und hefenähnlichen Organismen.
A. Nur mikroökologisch bedeutsame Organismen

Yeast groups[1])	Species identified	Environments[2])		
		Atmosphere	Phyllosphere	Rhizosphere
I	*Aureobasidium pullulans*	U	U	U
	Cladosporium spp.		vl	
	Trichosporon capitatum			L.v
	Tr. capitatum var. A^+			
	Tr. cutaneum		b	S.v
	Tr. fermentans			S.M.v
	Tr. pullulans		b	S.M
	Tr. variable/Endomycopsis burtonii			M.v
	Trichosporonoides sp. A^{++}			v
	Trichosporonoides sp. B^{++}			v
	Trichosporonoides sp. C^{++}			b
	Endomycopsis sp. A^{++}			L
	Endomycopsis sp. B^{++}			M.v
	E. bispora			L
	E. vini			v
	Oosporidium margitiferum			W.S
II	*Sporobolomyces* spp.	v		
	Sporobolomyces sp. A^{++}	v		
	Sp. odorus	a	b	S
	Sp. pararoseus	U	U	U
	Sp. roseus	U	U	U
	Sporidiobolus spp.	v		
	Rhodosporidium sp.			v
	Rhodotorula glutinis var. *glutinis*	U	U	U
	Cryptococcus flavus			v
	Cr. laurentii var. *laurentii*	wf	wf.ff	S.M
III	*Metschnikowia pulcherrima*	wf	U	S.M.v
IV	*Cr. albidus* var. *albidus*	a	b.wf	v
	Cr. gastricus var. A^+			L
	Bullera spp.			M
	Bullera sp. A^{++}			M
	Leucosporidium spp.	v		v
	Leu. gelidum/Candida gelidum		wf	r
	Lipomyces starkeyii			S
	Torulopsis molischiana/ Hansenula capsulata var. A			v

(Continued from table 2)

Yeast groups	Species identified	Environments		
		Atmosphere	Phyllosphere	Rhizosphere
V	*T. inconspicua*		vl.ff	M
	T. ernobii var. A+			M
	T. ernobii var. B+			v
	T. apis			v
	T. fujisanensis			v
	T. colliculosa	v		
	T. gropengiesseri		wf	
VI	*Candida* spp.			L
	Candida sp. A			v
	Candida sp. B		wf	
	C. melinii			L
	C. muscorum			S
	C. humicola			L
	C. mesenterica			L
	C. borgoniensis	v		
	C. brumptii			v
	C. tenuis			L.v
	C. langeronii			v
	C. maritima			S
	C. stellatoidea var. A+			v
	C. norvegensis			S
	Pichia sp.			L
	Hansenula californica			S
	Kluveromyces phaffii			v
	Metschnikowia reukaufii		wf.ff	L

Codes for Tables 2 and 3

[1]) Yeast groups I—VIII — see below Table 1

\+ = new varieties slightly difference from species described by Lodder (1970)

++ = new species — identification pattern different from any known organisms

[2]) Ecological distribution:

C = compost
F = mature grapes
L = leaf litter
M = mummified fruit
P = pears
S = soil
U = ubiquitous
W = willows
V = vines
a = air
b = vine bark
ff = vine flowers
vl = vine leaves
r = vine rootlets
v = invertebrate vectors
wf = wild flowers, blossoms or seed heads

Industrial significance:

F = film former
H = haze former
O = osmophile
T = taints produced
LY = lipolysis

very few yeasts (in 56 litres) unless the air sampling apparatus was sited near vegetation, in which case the yeast counts were sometimes × 15 higher.

About one third of the total yeasts and yeast-like organisms isolated were of potential industrial significance (Table 3); of these about 50% formed taints in grape

Table 3

Yeasts and yeast-like organisms isolated and identified from environments within an English vineyard.
B. Organisms of potential industrial significance

Die aus verschiedenen Zonen eines englischen Weinbergs isolierten und identifizierten Hefen und hefenähnlichen Organismen.
B. Organismen von möglicher technologischer Bedeutung

Yeast groups	Species identified	Environments[1])			
		Atmo-sphere	Phyllo-sphere	Rhizo-sphere	Industrial importance
III	*Kluyveromyces lactics/ T. sphaerica*			WS[a]	T
	K. marxianus/ C. macedoniensis			Wv	T
IV	*Saccharomyces aceti*	Wv	Wv	M	T
	Sacch. bailii			M	TO
	Sacch. cerevisiae		ff	VS.C	H
	Sacch. montanus			WS	T
	Sacch. rouxii		wf	PS	O
	Torulopsis dattila			v	O
	T. magnoliae		wf		O
	T. candida/Debaryomyces hansenii	U	U	U	F
	T. castellii			r	F
	Candida valida/ P. membranaefaciens		M.v	M.v	F
	C. lipolytica var. *lipolytica*			v	LY
	C. vini			v	F
	C. zeylanoides			v	F
	Pichia kudriavzevii/ C. krusei		wf		F
	Hansenula anomala var. *anomala*		M	M.L	F.T
	H. subpelliculosa			L	O.T
	H. polymorpha			Sv	O
VII	*Hanseniaspora valbyensis/ Kloeckera apiculata*	v	U.M	Sv M.L	T
	Hanseniaspora uvarum		M	Sv M	T
	Kloeckera africana		M	L.M	T
	Kl. corticus/H'spora osmophilia			v	T
	Kl. javanica var. *lafari*			L	T
	Nadsonia sp. X		W.ff.F	VS	T
VIII	*Schizosaccharomyces* sp. A			S	T
	Schizosaccharomyces spp.			SL	O

[1]) Code given below Table 2.

juice, 20% were osmophilic (i. e. growth 60% w/w glucose yeast extract agar — Lodder 1970), 20% produced films, 5% showed haze formation in wine and a further 5% had a strong lipolysis reaction. In most cases invertebrate vectors were found to be the main agents of dispersal of these important strains.

An example of a group of potentially important industrial strains is the species of osmophilic yeasts found on mummified fruit. Mummified grapes left on the vines and mummfied pears found on the adjacent orchard ground presented a special ecological niche for the survival of some well known industrial contaminants (Davenport 1968, 1973). It was concluded that the high sugar content of the mummified fruit contributed to the survival of many spoilage yeasts and bacteria. Among the spoilage yeasts were osmopihlic strains identified as *Sacch. bailii* (formerly *Sacch. acidifaciens* — Lodder 1970) which can spoil many types of foods and *Sacch. rouxii*, strains of which are often concerned with spoilage of high sugar content products, e. g. syrups (Walker and Ayres 1970). They gave a comprehensive account of yeasts as spoilage organisms in which some examples were given of osmophilic yeasts in floral nectar and soil around apiaries. These osmophilic yeasts were the cause of fermenting honey which is one of many types of spoiled food of high sugar content. It can be seen from Table 3 that similar observations were confirmed by this survey. Hence one should consider or determine the possible effect of the crop environment on the distribution and significance of microorganisms before industrial processing procedures.

Summary

An extensive environmental study, with particular reference to yeasts and yeast-like organisms, was made of the vineyard at Long Ashton. The results of this microecological survey were obtained by using a variety of techniques in which a quantitative estimate was made of the yeasts and yeast-like organisms. Sampling was carried out at all stages of grape vine deveopment. Sources of yeasts and yeast-like organisms included all vine parts as well as the soil, air, other plants and animal vectors which frequented those sources. Many potential spoilage organisms, e. g. film yeasts, were present but fermenting yeasts (*Saccharomyces* spp.) were rarely found. Among the microorganisms isolated were new unreported species of *Trichosporonoides*, *Saccharomycopsis* (synonym of *Endomycopsis*), *Sporobolomyces*, *Sporidiobolus*, *Rhodosporidium*, *Candida*, *Nadsonia* and *Schizosaccharomyces*. Other isolates were identified as species within the genera *Aureobasidium*, *Trichosporon*, *Sporobolomyces*, *Rhodotorula*, *Cryptoccus*, *Metschnikowia*, *Torulopsis*, *Candida*, *Pichia*, *Hansenula*, *Kluyveromyces*, *Saccharomyces* and *Kloeckera*.

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