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ブドウ酒醗酵中の酵母について

(第9報) 選択培地による野生酵母の分離 (その3)

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Dynamic Aspect of Yeast-flora during Vinous Fermentation

Part 9. Preferential Isolation of Wild Yeasts (Ⅲ)

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The frequency of occurrence of wild yeast flora during fermentation of white must containing 50 *ppm* (No. 1) and 150 *ppm* (No. 2) of sulfur dioxide were studied with a synthetic agar containing l-lysine as sole source of nitrogen and a malt agar containing 20 *ppm* of actidion. The representative 20 pure cultures out of 524 strains isolated at random, and the 14 cultures of 23 strains obtained collectively were identified according to the system of Lodder and Kreger-van Rij and classified in 14 groups (species) as follows :

Rhodotorula mucilaginosa (A), *Candida* spp. (B), *C. krusei* (C), *C. guilliermondii* var. *membranaefaciens* (D), *C. albicans* (E), *C. tropicalis* (F), *Torulopsis vinacea* (G), *T. bacillaris* (H), *Kloeckera apiculata* (I), *K. africana* (J), *Schizosaccharomyces pombe* (K), *Saccharomycodes ludwigii* (L), *Saccharomyces rosei* (M), *S. florentinus* (N).

It was shown that the initial populations of these yeasts were about 1.5×10^5 per *ml* of must No.1 and 0.5×10^5 per *ml* of No.2, and decreasing in numbers toward later. During the initial stages of fermentation C, G, H, I and M predominated in must No.1 whereas in No.2 only G and M were isolated prevalently. On the other hand, L developed during the intermediate phase while M disappeared (in must No. 2), however G servied, and a minute number of C and K was found until later. In this respect, it was reported that the occurrence of K was worth recognizing and also G was the one most prevalently isolated.

緒　　言

前報[2)3)]でリジンを唯一の窒素源とする合成培地 (SAL) およびアクチジオンを添加した麦芽寒天培地 (MAA) を野生酵母の選択培地としてブドウ酒果醪からどのような種類の酵母が分離されるかの問題について検討した結果，常用培地では分離が困難な酵母群が

分離され，特にMAAでは *Schizosaccharomyces pombe* が，SALでは *Saccharomycodes ludwigii* と *Saccharomyces bisporus* が選択的に分離され，SO_2 添加果醪には *Sc. ludwigii* が，酒精分の低い果醪では産膜性の酵母群が多いことなどのほか，新種として発表した *Torulopsis vinacea*[4] は両培地により初めて多数の菌株が分離されることを報告した。

それらの酵母のうち *T. vinacea* と *Sz. pombe* を除けば Domercq,[5] Brechot[6] らによっても分離され同時に醗酵中におけるそれらの酵母の消長が報告されているが，本邦産のブドウ酒果醪については醗酵中の野生酵母についての研究がほとんどないので本報ではこの両培地を使用し，ブドウ酒醗酵中どのような野生酵母が，どのように変動するかを知る目的で，分離酵母の同定と同定株の分離される頻度（分布状態）を求める実験をした。

実 験 の 部

1．供試白ブドウ酒果醪

山梨県産（1963年）の甲州種を原料とし，常法により果汁に補糖したのち協会1号ブドウ酒酵母（*S. cerevisiae* NJK-1）を酒母として斗瓶に 18*l* 宛仕込み，Table I のようにメタカリ（$K_2S_2O_5$）を SO_2 として 50 *ppm* 加えたもの（No.1）と 150 *ppm* のもの（No.2）について，それぞれ醗酵開始前（I，仕込後24時間），醗酵中期（II，仕込後6日または14日）および醗酵後了期（III，仕込後15日または30日）の3回，6区別に供試料を採取した。なお醗酵中は醗酵栓をつけ地下室に静置した。

Table I

供試果醪とその各醗酵段階における生菌数

Conditions of the Musts at the Period Sampled, and Yeast Populations Measured with the Two Selective Media

No.	Must Stage(Time)[a]	Lot	SO_2 Added	Alcohol Content	Yesat Count per ml [b] SAL	MAA	Total
	days	*l*	*ppm*	*vol.%*	$\times 10^4$	$\times 10^4$	
1	I (1)	18	50	0	16.0	13.4	29.4
	II (6)	18	50	7	13.0	8.8	21.8
	III (15)	18	50	14	5.9	2.4	8.3
2	I (1)	18	150	0	5.8	1.6	7.4
	II (14)	18	150	7	2.0	1.2	3.2
	III (30)	18	150	14	0.9	0.2	1.1

a) I, II, III, initial, intermediate stage and after completion of the fermentation respectively.

b) Measured from the count of colonies appeared on the plating medium. SAL, modified Wickerham's synthetic agar medium containing 1g l-lysine-HCl as the sole source of nitrogen ; MAA, malt extract agar medium containing 20 *ppm* of actidions.

2．野生酵母の分離と供試代表菌株の選択

前報[2] に使用したものと同様の培地SAL, MAA（アクチジオン 20 *ppm*）を使用し，稀釈平板法により各供試料毎に無作為抽出法で約40株宛 合計524株のほか稀に出現する特殊菌株23株を分離した（Table II）.

これらはなお稀釈平板法を操返したのち培養的性質および形態学的性質が完全に一致する株毎に分群し，各群別にその代表的菌株1～4株を抽出し合計34株を供試菌株として分類試験に供した。

TABLE II

分離した野生酵母の群別菌株数とその代表菌株の番号

Grouping and Designation of the Representatives of the Isolates Obtained from Successive Stages of Fermentation

Group	Representative Strain No.	Plating Medium[a]	No. of the Isolates obtained[b] Must No.1 I	Must No.1 II	Must No.1 III	Must No.2 I	Must No.2 II	Must No.2 III	Total[c] at Random	Total[c] Collectively
A	R-4	SAL				1*				1(1)
B	K-42	MAA	1*			1*				2(1)
C	K-43～45	SAL	20	2	1*	1*	1*	1*	22(3)	4(0)
D	K-46；49	SAL	1*	1*		1*				3(2)
	K-47；48	MAA	1*	1*		1*				3(2)
E	K-50	MAA				1*				1(1)
F	K-51	MAA				1*				1(1)
G	O-73；76	SAL	6	21	11	13	11	7	69(2)	
	O-74；77	MAA	14	26	38	35	57	42	212(2)	
H	T-13；14	SAL	4	11		1	5		21(2)	
I	A-20	SAL		11					11(1)	
	A-21～23	MAA	20	21		1	1*		42(2)	1(1)
J	A-24	SAL					1*			1(1)
K	O-77；78	MAA		1*	1*		1*	1*		4(2)
L	O-79～81	SAL			17		27	27	71(3)	
M	O-82～84	SAL	10	25	16	23			74(3)	
N	O-85～88	MAA			1*	1*	2		2(2)	2(2)
Total	Obtained at random	SAL	40	70	44	37	43	34	268(14)	
		MAA	34	47	38	36	59	42	256(6)	
	Obtained collectively	SAL	1	1	1	3	2	1		9(4)
		MAA	2	2	2	5	2	1		14(10)

a) No isolate was obtained with the medium omitted.
b) An asterisk (*) attached to a number indicated that the isolates were obtained collectively.
c) Numbers of the strains studied taxonomically are given in the parentheses. For the abbreviation see TABLE I.

3. 供試代表菌株の分類

LODDER ら[7]の方法による分類試験の結果はTABLEIII～VIに示すように14群 (A～N) に分類され供試代表菌株25株は，次の7属11種に，D群の4株は *Candida guilliermondii* の変種に同定されるほかB群の1株は同定されるものがなく *Candida* spp. とし，N群の4株は *S. florentinus* の類縁菌とした。

TABLE III

培養上の標徴 *Cultural Characteristics*

Group	Streak Culture on Malt Agar after One Month at 25° C	Surface Growth on Malt Extract at 25° C
A	Red, shining, smooth, mucous	Ring
B	White powdery, delicately folded	Thick, slimy[a]
C	Yellowish brown, dull, almost flat, wrinkled	Thin, dry[a]
D	Yellowish brown, partly hirsute, entierly wrinkled	Thin, fragile[b]
E	Yellowish brown with a greenish tinge, pimpled	Islets, ring
F	Yellowish gray, partly hirsute, strongly folded	Thick, slimy[a]
G	Pale, grayish white, glistening-dull, almost smooth	None
H	Pale, grayish white, shining, thin	None
I	Pale, grayish white, glistening, flat, almost smooth	Islets
J	Yellowish brown, pimpled all over the surface	Islets
K	Pale brown, glistening, slightly raised, smooth	None
L	Pale, yellowish brown, glistening, almost smooth	Thin ring
M	Pale, yellowish gray, shining, flat, warty	None
N	Cream-colored, glistening, partly warted, raised	Thin ring

a) Creeping up.
b) After one month the tube is filled with a membranous yellowish mass.

TABLE IV

増殖法の標徴 *Characteristics of Reproduction*

Group	Budding	Pseudomycelium Formation on Potato Agar	Ascospore Formation per Ascus
A	Multilateral	None	None
B	Multilateral[a]	Abundant, "Mycocandida" type. True mycelium is produced.	None
C	Multilateral	Abundant, "Mycotoruloides" or "Candida" type	None
D	Multilateral[a]	Abundant, "Mycotoruloides" type. "Blastodendrion" extend into the air.	None
E	Multilateral	Rich, "Mycotorula" type. Chlamydospores are formed. True mycelium is produced.	None
F	Multilateral	Abundant, "Mycotorula" type. True Mycelium is produced	None
G	Multilateral	None	None
H	Multilateral	None	None
I	Bipolar	Primitive	None
J	Bipolar	Primitive, "Mycocandida" type	None
K	Fission	None	1-4 round or oval[b]
L	Bipolar	Fairly well	2-4 round[c]
M	Multilateral	None	1-4 round[d]
N	Multilateral	Fairly well, "Candida" type	1-3 round[b]

a) Occasionally shoulder budding. b) Many asci are conjugated. c) Spores are conjugated in pairs in 4-spored-ascus. Str. O-79 seldom produce 4 spored-asci.
d) Protuberances are present.

TABLE V

細胞の形と大きさ *Shape and Size of the Cells*

Group	Shape	Size (μ) after 3 days at 25°C In Malt Extract	On Malt Agar
A	Short oval to oval	(2 −3.5)×(3.5− 6.5)	(3 −5)×(4.5− 7.5)
B	Long oval to cylindrical[a]	(2 −4)×(3.5−17)	(2 −5)×(2.5−14)
C	Long oval to cylindrical	(3 −5.5)×(5.5−11)	(3 −5.5)×(4 −10)
D	Long oval to elongated[a]	(1 −4)×(3.5−13)	(1.5−4)×(2.5−13)
E	Round to short oval	(3.5−7.5)×(4 − 8)	(3 −6)×(3.5− 8)
F	Oval	(4 −8)×(5 − 9)	(3 −5)×(4 − 7.5)
G	Round to slightly oval	(3.5−5)×(3.5− 5.5)	(2.5−4)×(2.5− 4.5)
H	Short oval to oval	(2 −3.5)×(3 − 5)	(2 −3.5)×(3 − 5.5)
I	Apiculate or oval	(2 −5.5)×(5 − 9)	(2.5−5.5)×(5 − 9)
J	Lemon-shaped	(3 −5)×(4.5−17)	(3.5−5.5)×(4.5−15.5)
K	Short cylindrical[b]	(4 −5)×(5.5− 7.5)	(4 −5)×(5.5−11)
	Long cylindrical[c]	(2 −3.5)×(6 −16)	(2 −3.5)×(5.5−11)
L	Lemon-shaped	(5 −6)×(8.5−23)	(5 −7.5)×(7.5−23)
M	Round to short oval	(4 −6.5)×(5 − 7)	(3.5−7)×(4.5− 7.5)
N	Round to short oval	(4.5−5.5)×(4.5− 7)	(3.5−5)×(3.5− 6)

a) Occasionally long pseudomycelial cells are present.
b) Strain O-77. c) Strain O-78.

TABLE VI

生理的性質 *Physiological Properties*

Group	Fermentation								Assimilation								Arbutin Split.	Litmus Milk	Fat. Split.
	D	G	S	M	L	R	Me	T	D	G	S	M	L	T	KNO_3	EtOH			
A	−	−	−	−	−				+	+w	+	+	−		−	+	+w	Coag.	
B	−	−	−	−	−	−			+	−	−	−	−		−	+ P	−	Blue	−
C	+[a]	−	−	−	−				+	−	−	−	−		−	+ P	−	Blue	
D	+	+w	+	±[b]	−	+	−	+w	+	+	+	+	−	+	−	+ P[e]	+[c]	Coag.[f]	+[f]
E	+	+w	−	+	−	−	−	+w	+	+	+	+	−	+	−	+ P	−	Coag.	+
F	+	+	+	+	−	−	−	+w	+	+	+	+	−	+	−	+ P	+	Blue	+
G	+w[b]	−	−	−	−		−		+	+[d]	−	−	−	+	−	−			
H	+	−	+	−	−	+	−		+	−	+	−	−		−	−			
I	+	−	−	−	−				+	−	−	−	−		−	−			
J	+	−	−	−	−				+	−	+w	+	−		−	−	−		
K	+	−	+	+	−	+	−		+	−	+	+	−		−	−			
L	+	−	+	−	−	+	−		+	−	+	−	−		−	−			
M	+	−	+	−	−	+	−		+	−	+	−	−		−	−			
N	+	+	+	+	−	+	+	+	+	+	+	+	−	+	−	−	−		

D, glucose; G, galactose; S, saccharose; M, maltose; R, raffinose; Me, melibiose; T, trehalose; w, weak ; P, pellicle.
a) Occasionally rather slow. b) Occasionally negative. c) Occasionally weak or negative. d) Adaptively possitive or negative by the liquid method.
e) The color of medium changes to yellowish after 2 weeks. f) Strong.

RHODOTORULA MUCILAGINOSA (JÖRG.) HARRISON,
Trans. Roy. Soc. Canada V, 22 : 187 (1928) ;
Lodder and K.-van Rij, The Yeasts, Amsterdam, 656 (1952) ;
Ohara and Nonomura, Bull. Res. Inst. Ferm. Yamanashi Univ., No.2, 6 (1956)

One isolate was identified : R-4 (Group A). It was obtained from sulphurized white wine must of Koshu variety (No. 2) at initial stage of fermentation with a synthetic agar medium (SAL).

CANDIDA KRUSEI (CAST.) BERKHAUT,
Schimmelgesl. Monilia, Oidium, Oospora en Torula,
Diss., Utrecht, 43 (1923) ; Group II Lodder and K.-van Rij, l, c.,
492 (1952) ; Ohara and Nonomura, l, c., No. 3, 3 (1956)

Three isolates were identified : K-43, K-44, K-45 (Group C). It was one of the most aboundant yeasts isolated from the must No. 1 at early stage of fermentation with a synthetic medium (SAL). However, it was obtained from both musts throughout the experiments.

CANDIDA GUILLIERMONDII (CAST.) LANG. *ET* GUERRA VAR.
MEMBRANAEFACIENS LODDER *ET* VAN RIJ, l. c., 518 (1952) ;
Ohara et al., l. c., No. 7, 4 (1960)

Four isolates were identified : K-46, K-47, K-48, K-49 (Group D). It was obtained from the musts at early stage of fermentation with both medium (SAL, MAA) used.

There was a strong positive reaction for spliting of fat and gave a positive reaction to a weak trehalose fermentation. LODDER and K.-VAN RIJ studied two strains labeled *C. chalmersi* 813 and 814 obtained from MRAK ('42) who had isolated these strains from figs and dates. However, it has not been reported that these yeast had been isolated from grape must and wine.

CANDIDA ALBICANS (ROBIN) BERKHOUT, l. c. (1923) ;
Lodder and K.-van Rij, l, c., 466 (1962)

One isolate was studied : K-50 (Group E). It was obtained from sulphurized must (No.2) at initial stage with a malt agar (MAA). It was considered that this strain identical to R-type have so far been observed a pimpled profile of streak culture, but a pellicle formation was observed when ethanol was used as the sole carbon source and gave a positive reaction to a trehalose fermentation.

CANDIDA TROPICALIS (CAST.) BERKHOUT,
l. c., 43(1923) ; Lodder and K.-van Rij, l. c., 502 (1952) ;
Soneda, Nagaoa, 6 : 9 (1959)

One isolate was studied : K-51 (Group F). It was obtained from the must (No.2) at initial stage with a malt agar (MAA).

Characteristics of this strain differs from those of the standard description of LODDER and K.-VAN RIJ in its thick slimy pellicle formation on malt extract. There was also a positive reaction to a splitting of fat and a weak trehalose fermentation, and to the assimilation of kerosene. However, this strain considered to be identical to the isolate described by SONEDA.

TORULOPSIS VINACEA OHARA ET AL.
J. Gen. Appl. Microbiol., 10: 77 (1964)

Four isolates were identified : O-73, O-74, O-75, O-76 (Group G). It was isolated most frequently throughout the experiments, especially from the must after the completion of fermentation with a malt agar(MAA).

TORULOPSIS BACILLARIS (KR. *ET* KRUMB.) LODDER,
Cent. Bakt. II, 86 : 277 (1963) ; Lodder and K.-van Rij, l. c., 423 (1952) ; Ohara et al., l. c., No. 3, 3 (1956) ; No. 6, 17 (1959)

Two isolates were identified : T-13, T-14 (Group H). It was isolated from both musts examined at intermediate stage of fermentation with a synthetic medium (SAL).

KLOECKERA APICULATA (REESS *EMEND.* KLÖCKER)
JANKE, Cent. Bakt. II, 76 : 161 (1928)
Lodder and K.-van Rij, l. c., 596 (1952) ; Ohara et al., l.c., No. 3, 4 (1956) ; No. 6, 15 (1959) ;

Four isolates were identified : A-20, A-21, A-22, A-23 (Group I). It was isolated most frequently from the must No.1 at early stage of fermentation with a malt agar (MAA).

KLOECKERA AFRICANA (KLÖCKER) JANKE,
l. c., 76 : 161 (1928): Lodder and K.-van Rij, l.c., 600 (1952) ; Ohara and Nonomura, l. c., No. 3, 5 (1956)

One isolate was identified : A-24 (Group J). It was obtained from sulphurized must (No.2) at intermediate stage with a synthetic agar (SAL).

SCHIZOSACCHAROMYCES POMBE LINDNER,
Woch. Brau., 10 : 1298 (1893) ; Lodder and K.-van Rij, l. c., 82 (1952) ; Ohara and Nonomura, l. c., No. 8, 7 (1961); Peynaud et al., Arch. Mikrobiol., 48 : 150 (1964) ; Peynaud and Sudraud, Ann. Tech. agr., 13 : 312 (1964)

Syn. *SCHIZOSACCHAROMYCES ACIDODEVORATUS* CHALENKO,
Arb. Zentr. Wiss. Önolog. Lab. Moskou-Leningrad, 1 (1941) ;
Kudrjawzew, Die Systematik der Hefen, Berlin, 245 (1960)

Two isolates were studied : O-77, O-78 (Group K). It was obtained from both

musts at later stage of fermentation with a malt agar (MAA).

Sporulation of the strain O-78 isolated collectively from the must No. 2 after completion of the fermentation was scarcely observed in prolonged culture on malt agar.

SACCHAROMYCODES LUDWIGII HANSEN,

Cent. Bakt. II, 12; 538 (1904) ; Lodder and K.-van Rij, l. c., 303 (1952) ; Ohara et al., l. c., No. 9, 17 (1962)

Three isolates were identified : O-79, O-80, O-81 (Group L). It was isolated most frequently from the must No.2 at later stage and rather abundantly from the must No.1 after completion of the fermentation, with a synthetic agar (SAL).

There was usually 2 spores per ascus, seldom 4-spored-ascus present in the strain O-79.

SACCHAROMYCES ROSEI (GUILL.) LODDER *ET* VAN RIJ,

l. c., 187 (1952) ; Ohara et al., l. c., No. 6, 14 (1959)

Syn. *DEBARYOMYCES ROSEI* KUDRJAWZEW, l. c., 218 (1960)

Three isolates were identified : O-82, O-83, O-84 (Group M). It was isolated frequently from the must No. 1 during and after fermentation, and from the must No.2 at initial stage with a synthetic agar (SAL).

SACCHAROMYCES FLORENTINUS (CAST.) LODDER *ET* VAN RIJ, l. c., 213 (1952)

Four isolates were studied : O-85, O-86, O-87, O-88 (Group N). It was obtained from the must No. 1 after completing of the fermentation and from the must No. 2 at early stage with a malt agar (MAA).

These strains gave a positive reaction to a fairly good trehalose fermentation, and to fermentation of yeast-extract, and no growth was observed at 32° C. Therefore, it was considered that these strains was not identical, but closely related to the species. Only one CASTELLI's strain had been studied by LODDER and K.-VAN RIJ.[7]. However, DOMERCQ[5] isolated six strains of these yeasts from various musts.

CANDIDA SPP.

One isolate was studied : K-42 (Group B). It was obtained from both musts examined at initial stage of fermentation with a malt agar (MAA).

This strain resembles *C. lipolytica* (Harrison) Diddens et Lodder, *C. mycoderma* (Reess) Lodder et K.-van Rij and *C. zeylanoides* (Cast.) Lang. et Guerra in its inability to ferment sugars and to assimilating glucose only, however it differs from *C. lipolytica* in its poor physiological activity towards protein and fat ; from *C. mycoderma* in its morphological properties of forming true mycelium and from *C. zeylanoides* in its thick slimy pellicle formation on malt extract.

However there are some questions whether the isolate is a yeast or not.

4．果醪中における各酵母群の分離頻度と分布状態

SALおよびMAA両培地で各試料毎に稀釈平板法により得られた集落数より，果醪 1*ml* 当りの生菌数を算出した結果は TABLE I に示した通りで，供試代表菌株を選択したA～N群の各群に属する全菌株は，それぞれの代表菌株と全く同一の性質を有するものと仮定すれば，各果醪における各時期別に分離された菌株数（TABLE II）からA～N群別の分離頻度は TABLE VII のようになり，TABLE I に示した各果醪の各時期における生菌数中でA～N群の野生酵母が占める割合を計算すると Fig. 1 のように図示することができる。

TABLE VII

分離酵母の群別分離頻度

Frequency with which Each of the Wild Yeast Groups were Found during the Successive Stages (I, II, III) *and Throughout the Fermentation Period* (ϕ)

Group of Isolates		Medium[a] used	The Frequency of Isolation(%)[b]								
			Must No.1				Must No.2				Total
			I	II	III	ϕ	I	II	III	ϕ	
A	*R.mucilaginosa*	SAL					+			+	+
B	*Candida sp.*	MAA	+			+	+			+	+
C	*C.krusei*	SAL	50	3	+	14	+	+	+	+	8
D	*C.guilliermondii* var. *membran.*	SAL	+	+		+	+			+	+
		MAA	+	+		+	+			+	+
E	*C.albicans*	MAA					+			+	+
F	*C.tropicalis*	MAA					+			+	+
G	*T.vinacea*	SAL	15	30	25	25	35	26	21	27	26
		MAA	41	55	100	65	97	97	100	99.8	83
H	*T.bacillaris*	SAL	10	16		10	3	11		5	8
I	*K.apiculata*	SAL	?	16		7					4
		MAA	59	45		35	3	+		0.1	16
J	*K.africana*	SAL						+		+	+
K	*Sz.pombe*	MAA		+	+	+		+	+	+	+
L	*Sc.ludwigii*	SAL			39	11		63	79	48	26
M	*S.rosei*	SAL	25	35	36	33	62			20	28
N	*S.florentinus*	MAA			+	+	+	3		0.1	1
Total		SAL	100	100	100	100	100	100	100	100	100
		MAA	100	100	100	100	100	100	100	100	100

a) No isolate was obtained with the medium omitted.

b) The proportions of isolates obtained at random to the sum total.
+, indicates unestimated occurrence ; ?, indicates probable occurrence.
For the abbreviations of medium see TABLE I.

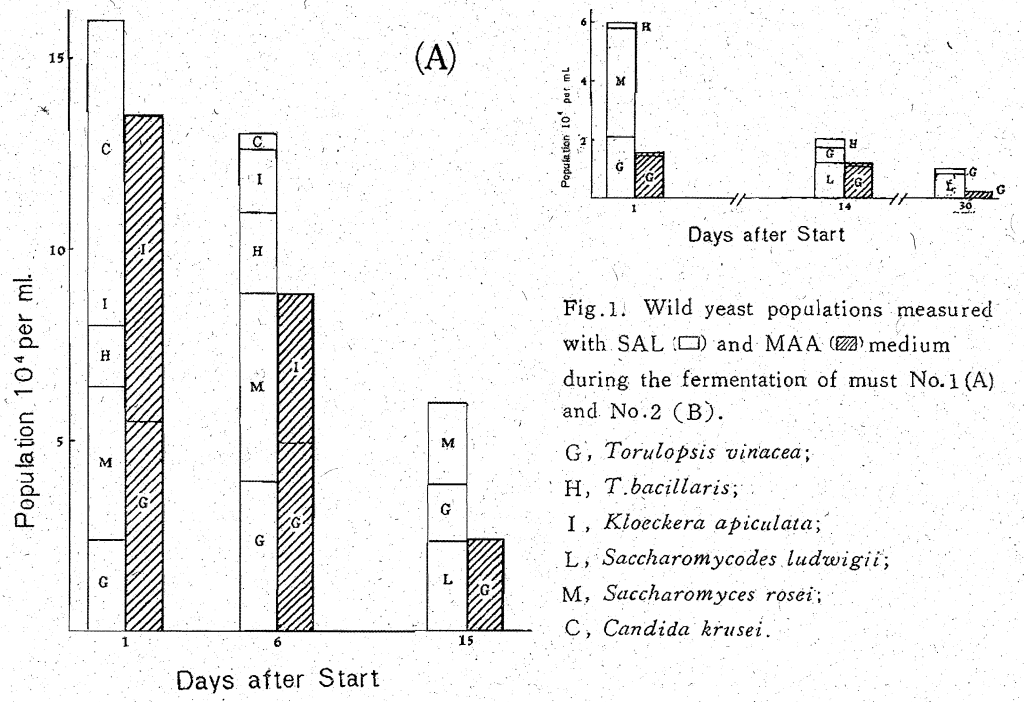

Fig.1. Wild yeast populations measured with SAL (□) and MAA (▨) medium during the fermentation of must No.1 (A) and No.2 (B).

G, *Torulopsis vinacea*;
H, *T. bacillaris*;
I, *Kloeckera apiculata*;
L, *Saccharomycodes ludwigii*;
M, *Saccharomyces rosei*;
C, *Candida krusei*.

考　　察

SAL と MAA 両培地を使用して測定した生菌数を比較すると，SAL を用いた場合が常に MAA によるものより多くなっている。これは SAL には生育するが，MAA には生育しない酵母即ち *C. krusei*, *T. bacillaris*, *S. rosei*, *Sc. ludwigii* 等（但し *T. bacillaris* は MAA でもアクチジオンが 10 *ppm* のときやや生育する）[2] があるのに対し，MAA に生育するが SAL に生育しない酵母即ち *Sz. pombe*, *S. florentinus* 等が前者に比較してはるかに劣勢（分離頻度が低い）であるためと考えられる。

醗酵中これら野生酵母の生菌数は醗酵の段階（Ⅰ，Ⅱ，Ⅲ）が進むに従って明らかに減少し，また SO_2 を多く使用した場合（No.2）は当初（Ⅰ）から著しく生菌数が少なく，特に MAA では *Sc. ludwigii* が生育しない[2] ため一層生菌数が少なくなっている（Table Ⅰ, Fig. 1）

しかし果醪にはこれらの野生酵母とは別に *S. oviformis* 等の野生酵母が非常に多いことは既報[1)2)] のほか，Minarik[9] らによっても明らかにされているばかりでなく，SAL および MAA に比較的よく生育する *T. vinacea* と *K. apiculata* も別にこれらの培地に稀釈平板培養したとき出現する集落数は接種菌数とかなり差のあることや，前報[2)3)] で比較的高い分離頻度を示した *S. bisporus*, *H. anomala*, *T. famata* 等が分離されなかったこと，また *T. bacillaris* あるいは *K. apiculata* などの生育がこれら両培地で必ずしも適当であるといえない点なども考慮すれば，供試果醪中の野生酵母の生菌数は実際は Table Ⅰ の測定値よりはるかに多いものと考えなければならない。

次に醗酵の各時期にどのような野生酵母が増殖，あるいは消滅するかについてみると，Fig. 1から醗酵開始期（Ⅰ）の主要酵母はSO_2の少ない果醪（No. 1）では *C. krusei* (C), *K. apiculata* (I), *T. bacillaris* (H), *S. rosei* (M), *T. vinacea* (G) の5群でSO_2の多い果醪（No.2）では M, G の2群であり，それらは醗酵の経過と共に C, I, H 群の順序で次第に消滅し，M と G 群が生き残るが，果醪 No. 1 では醗酵終了期に，果醪 No. 2 では中期から *Sc. ludwigii* が増殖した。この酵母は既報[3]のようにSO_2耐性があるため果醪 No.2 では *S. rosei* に代って生菌数が多くなっている。しかし他方 *C. krusei* は完全に消滅したのではなく生菌数は少ないが最後まで分離されているので，この種の酵母（*C. mycoderma*, *Pichia membranaefaciens* 等の産膜酵母）は生育の条件さえよくなれば，醗酵終了後においても再び増殖してくる危険のあることが考えられる。

なお前報[3]で指摘したように MAA により *Sz. pombe* が選択的に分離され，本報の供試果醪からも，中期（Ⅱ）および終了期（Ⅲ）に出現していることを知った。従ってこの種の酵母は Osterwalder（'24）により分離されて以来[7] ブドウ果醪からはごく稀にしか分離されない[10]とされているが，醗酵後期の果醪に生菌数こそ少ないがかなり普遍的に分布しているものと考えられる。しかもこの種の酵母はリンゴ酸を分解する力があるとされ，[11)~14)] ブドウ酒の醸造に応用することも試みられている[15]ので，その生態は今後特に注目すべきものがあると考える。また普通の培地では分離されないため従来全く知られていない *T. vinacea* が予想外に多く，しかもアルコールやSO_2に対しても耐性があることからこの酵母も醸造学的に案外大きな役割を果しているものと考える。

要　旨

1）前報に引続き SAL および MAA 両培地を野生酵母の選択培地として使用し，ブドウ酒果醪の醗酵中における野生酵母群の動態を知るため醗酵初期，中期および後期別に分離された酵母群別にその分布状態（生菌数の割合）を求めた。

2）分離した547株の代表菌株34株のうち29株は7属11種と1変種に同定されたが，4株は *S. florentinus* の類縁菌とし，1株は同定されるものがなく *Candida* spp. とした。それらのうちにはブドウ果醪から分離された報告のない *C. guilliermondii* var. *membranaefaciens*（4株）と稀にしか分離されない *Schizosaccharomyces pombe*（2株），および別に新種として発表した *Torulopsis vinacea*（4株）があるほかには特に珍らしいものはない。

3）両培地で測定した野生酵母の生菌数は初め $10^5/ml$ 程度であったが，醗酵が進むに従って次第に減少する。特にSO_2を多く（150 *ppm*）加えた場合はその傾向が著るしい。

4）これらの野生酵母を群別にみると *C. krusei*, *K. apiculata*, *T. bacillaris* は醗酵初期には多いが中期までにほとんど消滅したのに対し *T. vinacea* と *S. rosei* は終期まで多数に残存する。しかしSO_2の多い場合 *S. rosei* は中期までに減少し，それに代って *Sc. ludwigii* が多くなった。

5）生菌数は微量ではあるが醗酵終期まで *Sz. pombe* および *C. krusei* が分離されたほか *T. vinacea* は初期から終期まで最も優勢な野生酵母の一つであることを明らかにした。

終りに実験に協力された宮川政国，白上安佐両君に感謝の意を表します。

なお，本稿印刷中 *C. guilliermondii* var. *membranaefaciens* と同定した菌株は，CBS において YARROW らにより，胞子を造らないが *Pichia guilliermondii* Wickerham ('65) とすべきであることを知らされた。

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