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Dynamic Aspect of Yeast-flora during vinous Fermentation.*

Supplement to Part 2. Characteristics of the Identified Strains.

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In preceding papers[1)2)], the developments of various yeast groups in the grape musts during fermentation were traced. Ten isolates belonging to wine yeasts, and twenty two isolates belonging to the others were selected for further studies. They were allocated to six genera and seventeen species according to the LODDER and KREGER van RIJ system.[3)]

In Part 2 of this paper,[2)] some oenological properties of the isolates were also reported but taxonomic were not, because of the restriction of pages. So in the present paper the authors describe the taxonomic characteristics of these thirty two isolates.

TABLE 1

PHYSIOLOGICAL PROPERTIES OF THE STRAINS IDENTIFIED

Species	Strain	Fermentation							Assimilation							Splitting of arbutin
		Gl	Ma	Sa	Ga	La	Ra	Me	Gl	Ma	Sa	Ga	La	MeOH	KNO_3	
1	S-1, S-3, S-4, S-5	++	+	+	+a)	−	⊥	−	+	+	+	+	−	+W	−	−
2	S-8, S-9	++	+	+	+a)	−	⊥	−	+	+	+	+	−	+W	−	+
3	S-12	+	⊥b)	+	−	−	⊥	−	+	+	+	−	−	+W	−	−
4	S-2	++	+	+	+	−	⊥	−	+	+	+	+	−	−	−	−
5	S-6, S-7	+	−	+	⊥	−	⊥	−	+	+	+	+	−	+W	−	+
6	d-1, d-2, d-3, d-4	+	−	−	−	−			+	−	−	−	−	++R	−	−
7	d-6, d-7	⊥	−	−	−	−			+	−	−	−	−	++R	−	−
8	d-5	−c)	−	−	−	−			+	−	−	−	−	++R	−	−
9	a-1, a-2, a-3	++	−	−	−	−			+	−	−	−	−	−	−	+W
10	a-4	++	−	−	−	−			+	+	−	−	−	−	−	+W
11	a-5	++	−	−	−	−			+	+	⊥	−	−	−	−	+W
12	S-10	+	−	−	−	−			+	−	−	−	−	+W	−	−
13	S-11	+	+	+	−	−	⊥	−	+	+	+	−	−	+W	−	−
14	T-1,T-2,T-3,T-4,T-5	++	−	+	−	−	⊥	−	+	−	+	−	−	−	−	−
15	R-1	+	−	−	−	−			+	+	+	+	−	+W	−	+
16	R-2	−	−	−	−	−			+	+	+	+	−	+R	−	−d)
17	R-3	−	−	−	−	−			+	+	+	+	−	+R	+	⊥e)

Gl : Glucose ; Ma : Maltose ; Sa : Saccharose ; Ga : Galactose ; La : Lactose ; Ra : Raffinose ; Me : Melibiose ; MeOH : Methanol ; W : Weak ; R : Ring formation ; a) + or ⊥ ; b) ⊥ occationally − ; c) − or ⊥ ; d) − or + ; e) ⊥ very weak.

1) OHARA, Y. and H. NONOMURA : *J. Soc. Brew., Japan*, (醸協) 50, K71 (1955)

2) OHARA, Y. and H. NONOMURA : *J. Agr. Chem. Soc. Japan*, (農化) 30, 524 (1956)

3) LODDER, J. and N. J.W. KREGER van RIJ : *The Yeasts, a Taxonomic study*. Amsterdam (1952)

* 小原巖，野々村英夫：ブドウ酒醗酵中の酵母に就て (第2報補遺) 各分離菌株の性質

1. *Saccharomyces cerevisiae* Hansen Group II Lodder et van Rij

Strain S-1, S-3, S-4, S-5. (Fig. 1)

Growth in malt extract: After 3 days at 28°C, cells are oval, (3.3–6.5)×(5–8)μ, single or in pairs. A sediment is formed. After one month at 20°C. a thin ring is present. Growth on malt agar: After 3 days at 30°C. cells are oval, (2.5–6.5)×(4–8)μ, single or in pairs. After one month at 20°C. the streak culture is pale yellowish brown, smooth and glistening. Slide cultures on potato agar: Very primitive pseudomycelium. Sporulation: Aboundant on gypsum block. The ascospores are oval, (2–3.2)×(2–4)μ, 1–4 per ascus.

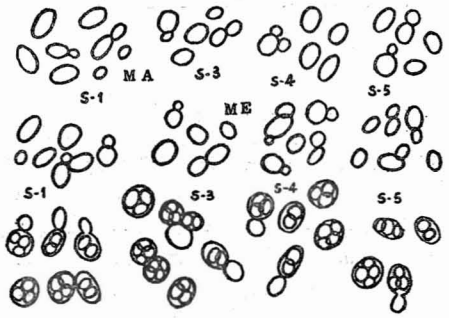

FIG. 1. *S. cerevisiae*
After 3 days in malt extract (ME) and on malt agar (MA); Spores on gypsum block after 1 week.

2. *Saccharomyces cerevisiae* Hansen form. a nov. f.

Strain S-8, S-9. (Fig. 2)

Growth in malt extract: After 3 days at 28°C. cells are small round to short oval, (2–4)×(2–5)μ, in pairs or single. A sediment is formed. After one month at 20°C. a sediment and a thin ring are present. Growth on malt agar: After 3 days at 30°C. cells are round to oval (2–5)×(2–6.5)μ, single or in pairs. After one month at 20°C. the streak culture is pale yellowish brown, smooth and glistening. Slide cultures on potato agar: Little developed pseudomycelium. Sporulation: Conjugation usually precedes ascus formation. The ascospores are round, (2.4–3.2)μ in diameter usually with an oil drop inside and 1–2 per ascus (rarely 3–4 ascospores found on carrot plugs or V-8 agar).

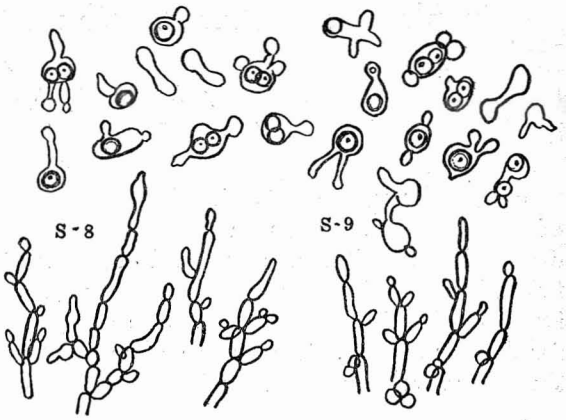

FIG. 2. *S. cerevisiae* form. a nov. f.
Spores on gypsum block after 1 week.
Slide cultures, Potato agar.

3. *Saccharomyces oviformis* Osterwalder

Strain S-12.

Growth in malt extract: After 3 days at 28°C. cells are oval, (3.5–5)×(5–6.5)μ, single, in pairs or in small chains. A thick ring is present after one month at 20°C. Growth on malt agar: After 3 days at 30°C. cells are oval, (2–6.5)×(3–8)μ, single or in pairs. After one month at 20°C. the streak culture is pale yellowish brown, hard and rough, somewhate raised in the middle. Slide culture: On potato agar no pseudomycelium is formed. Sporulation: Aboundant; often occur even in the slide cultures. The spores are oval, (1.5–2)×(2.4–3.2)μ, 1–4 per ascus.

4. *Candida robusta* Diddens et Lodder

Strain S-2. (Fig. 3)

Growth in malt extract: After 3 days at 28°C. cells are oval, (2.5–5)×(3–6.5)μ, in pairs or single. A sediment is formed. After one month at 20°C. a thin ring and brown sediment are present. Growth on malt agar: After 3 days at 30°C. cells are oval, (2.5–7)

$\times(4\text{–}8)\mu$, single or in pairs. After one month at 20°C. the streak culture is oak leaf brown, grey in the margin, smooth and glistening. There are rather little growth. Slide cultures on potato agar: Scanty and primitive pseudomycelium.

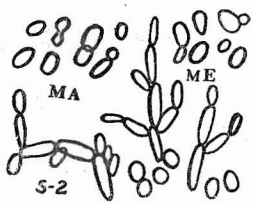

FIG. 3. *C. robusta*
After 3 days in malt extract (ME) and on malt agar (MA); Slide cultures, Potato agar.

5. *Candida guilliermondii* (Cast.) Langeron et Guerra

Strain S–6, S–7. (Fig. 4)

Growth in malt extract: After 3 days at 28°C, cells are oval, $(1.5\text{–}3)\times(2.5\text{–}5)\mu$, single, in pairs or in short chains. A ring and islets or thin pellicle are formed. After one month at 20°C, a thick ring is present. Growth on malt agar: After 3 days at30° C, cells are small oval, $(1.5\text{–}3)\times(2\text{–}4.8)\mu$, single or in pairs. The streak cultures after one month at 20°C, is milk white, glistening and fringed. Slide cultures on potato agar: The pseudomycelium is formed moderately, forming trees of equal cells.

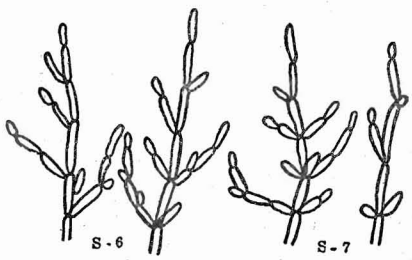

FIG. 4. *C. guilliermondii*
Slide cultures, Potato agar.

6. *Candida krusei* (Cast.) Berkhaut Group II Lodder et van Rji

Strain d–1, d–2, d–3, d–4. (Fig. 5)

Growth in malt extract: After 3 days at 28°C. cells are elongate to cylindrical, $(2\text{–}4.8)\times(3.2\text{–}9.6)\mu$, single, in pairs or in short chains. A dry creeping, wrinkled pellicle is formed. After one month at 20°C. a fragile pellicle is present. Growth on malt agar: After 3 days at 30°C. cells are oval to elongate, $(2\text{–}4)\times(3.5\text{–}9)\mu$, single, in pairs or in small chains. The streak culture after one month at 20°C. is yellowish grey, flat and delicately wrinkled all over the surface (d–1, d–2) or wrinkled at the middle (d–3, d–4). Slide cultures on potato agar: The pseudomycelium developes aboundantly. The oval or elongate blastospore usually develop in verticils.

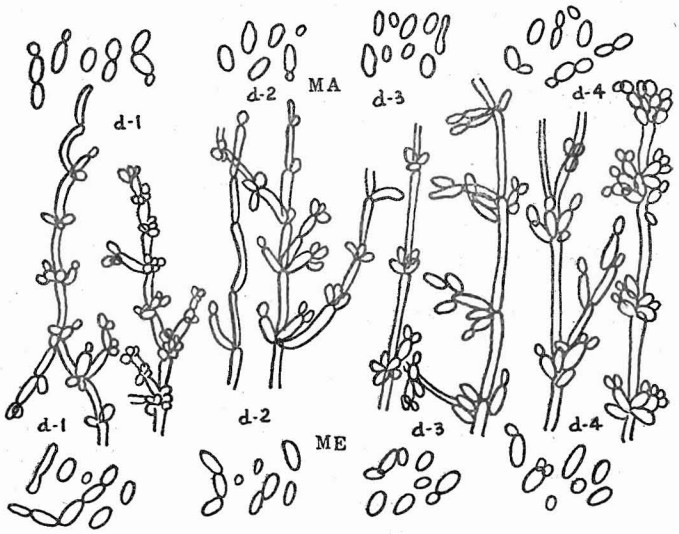

FIG. 5. *C. krusei*
After 3 days in malt extract (ME) and on malt agar (MA); Slide cultures, Potato agar.

7. *Candida mycoderma* (Reess) Lodder et van Rij

Strain d–6, d–7. (Fig. 6)

Growth in malt extract: After 3 days at 28°C. cells are oval to elongate, $(1.5\text{–}3.2)\times(3.2\text{–}6.4)\mu$, single or in pairs. A very thin smooth pellicle is formed. After one

month at 20°C. a thin pellicle is present. Growth on malt agar: After 3 days at 30°C. cells are elongate to cylindrical, (2.5–3.5)×(4.5–15.5)μ, single, in pairs or in short chains. The streak culture after one month at 20°C. is yellowish grey, smooth, glistening to dull and flat. Slide cultures on potato agar: The pseudomycelium is primitive and consists of rather equal cells. There are little difference between preudomycelium cells and blastospores.

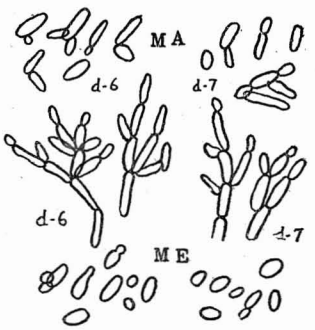

FIG. 6. *C. mycoderma*
After 3 days in malt extract (ME) and on malt agar (MA); Slide cultures, Potato agar.

8. ***Pichia membranaefaciens*** **Hansen**

Strain d–5. (Fig. 7)

Growth in malt extract: After 3 days at 28°C. cells are oval to elongate, (3.2–4.8)×(4.8–7.6)μ, single or in pairs. A thin smooth pellicle is formed. After one month at 20° a thick ring and a sediment are prese Growth on malt agar: After 3 days at 30° cells are elongate to cylindrical, (2.5–3) (6–18)μ, single, in pairs or in short chai The streak culture after one month at 20° is some brownish grey, wrinkled, flat raised. Slide cultures on potato agar: little developed pseudomycelium. Sporul tion: Ascospores are oval to round, 1.5–2.4 in diameter, 1–4 per ascus, in some asc spores are arranged on a linier position.

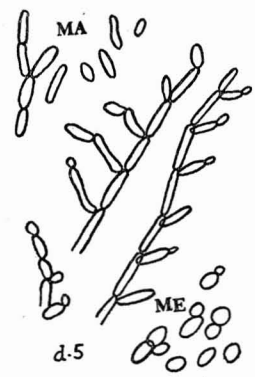

FIG. 7. *P. membranaefaciens*
After 3 days in malt extract (ME) and on malt agar (MA); Slide cultures, Potato agar.

9. ***Kloeckera apiculata*** **(Reess emen Klöcker) Janke**

Strain a–1, a–2, a–3. (Fig. 8)

Growth in malt extract: After 3 days 28°C. cells are small oval or lemon shape (1.5–4.5)×(3.2–8)μ, single, in pairs or in shc chains. A thin ring is formed. After o month at 20°C. a very thin ring is prese Growth on malt agar: After 3 days at 30° cells are lemonshaped or small oval, (1.5–4. ×(3–12)μ, single. The streak culture aft one month at 20°C. is yellowish brown, mi white in the margin, smooth, flat and shin Slide cultures on potato agar: The primiti pseudomycelium or no preudomycelium.

10. ***Kloeckera magna*** **(De'Rossi) Janl**

Strain a–4. (Fig. 8)

Growth in malt extract: After 3 days

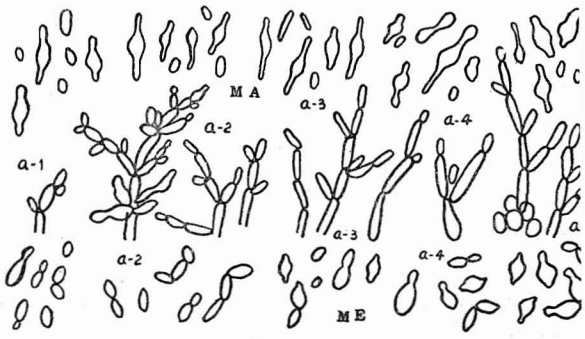

FIG. 8. *K. apiculata*, *K. magna*, and *K. afrikana*
After 3 days in malt extract (ME) and on malt agar (MA); Slide cultures, Potato agar.

28°C. cells are lemonshaped or oval, (3.5–7) ×(3.2–8)μ, single or in pairs. A thin ring is present after one month at 20°C. Growth on malt agar: After 3 days at 30°C. cells are lemonshaped or small oval, (3–5)×(3.5–18)μ, single or in pairs. The streak culture after one month is yellowish brown, raised and glistening. Slide cultures on potato agar: No pseudomycelium is formed.

11. *Kloeckera africana* (Klöcker) Janke

Strain a–5. (Fig. 8)

Growth in malt extract: After 3 days at 28°C. cells are lemonshaped or oval, (3.2–7) ×(3.2–8)μ, single or in pairs. After one month at 20°C. a sediment and a thin ring are formed. Growth on malt agar: After 3 days at 30°C. cells are lemon-shaped or oval, (2–4.8)×(3.2–11.0)μ, usually single. After one month at 20°C. the streak culture is flat, glistening and some pointed, yellowish brown. Slide culture on potato agar: The primitive pseudomycelium is formed.

12. *Torulopsis glabrata* (Anderson) Lodder et de Vries

Strain S–10.

Growth in malt extract: After 3 days at 28°C. cells are small oval, (2.5–3.5)×(2.5–5)μ, single or in pairs. A ring is present after one month at 20°C. Growth on malt agar: After 3 days at 30°C. cells are small oval, (1.5–3)×(2.5–4.5)μ, in pairs or single. The streak culture after one month at 20°C. is greyish white smooth, flat and glistening. Slide culture on potato agar: No pseudomycelium is formed.

13. *Torulopsis colliculosa* (Hartmann) Saccardo

Strain S–11.

Growth in malt extract: After 3 days at 28°C. cells are round to short oval 3.5–5μ in diameter. A thin ring is present after one month at 20°C. Growth on malt agar: After 3 days at 30°C. cells are oval, (3–6)× (3–5.5)μ, single or in pairs. After one month at 20°C. the streak cultures is yellowish grey, smooth and glistening. Slide culture on potato agar: A very primitive pseudomycelium is formed.

14. *Torulopsis bacillaris* (Kroemer et Krumbh.) Lodder

Strain T–1, T–2, T–3, T–4, T–5. (Fig. 9)

Growth in malt extract: After 3 days at 28°C. cells are small oval, (1.5–4)×(3.5–5)μ, in short chains, in paires or single. A sediment is formed. After one month at 20°C. a dark thick sediment is present. Growth on malt agar: After 3 days at 30°C. cells are small bacillary to oval, (1.4–2.5)×(3–5)μ, single, in paires or in short chains. After one month at 20°C. the streak cultures is grey (soon change to darker), smooth, shiny, slow growth and perishable. Slide cultures: No formation of pseudomycelium.

FIG. 9. *T. bacillaris*
After 3 days in malt extract (ME) and on malt agar (MA).

15. *Torulopsis famata* (Harrison) Lodder et Van Rij

Strain R–1.

Growth in malt agar: After 3 days at 28°C. cells are round 3–8μ in diameter, single or in paires. A ring is formed. After one month at 20°C. a reddish ring is present. Growth on malt agar: After 3 days at 30°C. cells are round to short oval, (2–6)×(3–6)μ, single or in pairs. After one month at 20°C. the streak cultures is cream coloured, smooth, flat, glistening, striped at the margin. Slide culture on potato agar: No pseudomycelium is formed.

16. *Rhodotorula mucilaginosa* (Jörg.) Harrison

Strain R–2.

Growth in malt extract: After 3 days at 28°C. cells are oval, (3–3.2)×(4–6)μ, single or in pairs. A reddish ring is formed. After one month at 20°C. a thick orange-red ring and a sediment are present. Growth on malt agar: After 3 days at 30°C. cells are oval, (2–3)×(3–6)μ, single or in pairs. After one month at 20°C. the streak culture is light reddish orange, glistening and some mucous. Slide culture on potato agar: No pseudomycelium is formed.

17. *Rhodotorula glutinis* (Fres.) Harrison var. *rubscens* (Saito) Lodder

Strain R–3.

Growth in malt extract: After 3 days at 28°C. cells are oval, (2–5.5)×(3–6)μ, single or in pairs. A reddish ring is formed. After one month at 20°C. a orange red ring and a thin pellicle are present. Growth on malt agar: After 3 days at 30°C. cells are oval (2–3)×(3–6)μ, single or in pairs. After one month at 20°C. the streak culture is yellowish red, glistening and mucous. Slide culture on potato agar: No pseudomycelium is formed.