

Dynamic Aspect of Yeast-flora during Vinous Fermentation

Part 4. Characteristics of Identified Strains¹⁾ (II)

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各分離菌株の性質 (II)

This paper describes the taxonomic characteristics of the yeasts found in Japanese grape musts in succession to Part 2 of the study.¹⁾

In the preceding paper Part 3, about five hundred isolates of yeasts were classified into five groups, *e. g.*, *Saccharomyces* yeasts (S), Apiculate yeasts (A), *Torulopsis bacillaris* type yeasts (T), Kahm yeasts (K), and others (O). Sixty representatives of these groups have been purified by repeating a dilution plate method and studied according to the system of LODDER & KREGER-van RIJ²⁾. These groups included the following species :

	(Group)	(Genera and species)	(No. of isolates identified)
a)	S	: <i>Saccharomyces cerevisiae</i>	24
		<i>Saccharomyces rosei</i>	4
		<i>Saccharomyces oviformis</i>	1
b)	A	: <i>Kloeckera apiculata</i>	7
c)	K	: <i>Pichia fermentans</i>	1
		<i>Candida mycoderma</i>	5
		<i>Candida pelliculosa</i>	1
d)	T	: <i>Torulopsis bacillaris</i>	5
e)	O	: <i>Torulopsis glabrata</i>	2
		<i>Torulopsis colliculosa</i>	2

The other several isolates which could not be identified with any known species of yeasts are being compared with the related species at C. B. S., Delft, and their details will be discussed in other paper.

As is listed above, twenty four isolates belonged to *Sacch. cerevisiae* but none of them was identified with the variety *ellipsoideus* of the species. This reason may be due to the original flora on the grapes and, at the same time, to the use of this species as starter.

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Most of the other species isolated here have previously been obtained from musts. But one of the wild yeasts, namely *C. pelliculosa*, belongs to the species which appear not to have been found previously in grape musts.

Saccharomyces cerevisiae Hansen, Medd. Carlsberg Lab.,

2 : 29, 168, 220 (1883, 1886, 1888)

Fermentation : Glucose +, galactose +, sucrose +, maltose +, lactose —, raffinose +, melibiose —. Assimilation : Glucose +, galactose +, sucrose +, maltose +, lactose —. Splitting of arbutin : —. Assimilation of KNO_3 : —. Pseudomycelium : —. Pellicle : In malt extract after 30 days at 30°C. usually no pellicle but in some cultures islets and in a few cultures (S-18, S-32) very thin pellicle are formed. Sporulation : Oval ascospores, 2~4 per ascus. Streak culture on malt agar after 30 days at 25°C : Cream colored, soft shining, raised in the middle. Shape and size of the cells : In malt extract after 3 days at 30°C. cells of almost all cultures are oval, $3.5\sim6\times5\sim8\mu$, in a few cultures (*e. g.*, S-18, S-27, S-33) more roundish. On malt agar cells are oval, $3\sim7\times4\sim8\mu$, but in some cultures (*e. g.*, S-24, S-28, S-42) there are also much elongate cells besides oval ones. However their cells are generally not so elongate as the variety *ellipsoideus* of the species.

Twenty four isolates were studied : S-18~S-41. These isolates may belong to the Group II of this species.

Saccharomyces rosei (Guill.) Lodder et Kreger-van Ri,

The Yeasts, Amsterdam, 187 (1952)

Syn. *Torulaspora rosei* Guilliermond, Arch. Protistenk., 28 : 52 (1913)

Fermentation : Glucose, sucrose and raffinose only. Assimilation : Glucose and sucrose. Splitting of arbutin : —. Pseudomycelium : —. Assimilation of KNO_3 : —. Pellicle : After 30 days some islets or very thin pellicle are formed. Sporulation : Round or slightly oval spores, 1~4 per ascus. Streak culture : Greyish cream colored, shiny, nearly flat, covered with small warts. Shape and size of the cells : In malt extract, round or almost round $3\sim5\mu$; on malt agar, $2\sim6\times3\sim7\mu$.

Four isolates were studied : S-14~S-17. They were isolated at the early stage of fermentation. The species has been suggested for vinification of musts low both in sugar and acid content by CASTELLI ('48)²⁾

Saccharomyces oviformis Osterwalder, Zentr. Bakt.

Parasitenk., Abt. II, 60 : 481 (1924)

Syn. *Saccharomyces cheresiensis* Pröstosserdow et Afrikan,
Das Weinland, 12 : 389 (1933)

Fermentation : Glucose +, galactose —, maltose +, lactose —, raffinose +.
Assimilation : Glucose +, galactose —, sucrose +, maltose +, lactose —. Splitting
of arbutin : —. Assimilation of KNO_3 : —. Pseudomycelium : —. Pellicle : After
30 days at 30°C. a pellicle is formed. Sporulation : Oval spores, 1~4 per ascus.
Streak culture : Yellowish brown, soft, raised in the middle, not shining. Shape
and size of the cells : In malt extract, oval to round, $4.5\sim6\times5.5\sim8\mu$: On malt
agar round or slightly oval, $5\sim7\times5\sim7\mu$.

One isolate was studied : S-13. It was obtained from a must after the fermenta-
tion finished. DOMERCQ ('57)⁴⁾ supposed the species may be dominant in the
latter part of must fermentation in Girond, France.

Kloeckera apiculata (Reess emend. Klöcker) Janke, Zentr.

Bakt. Parasitenk., Abt. II, 76 : 161 (1928)

Fermentation and assimilation : Glucose only. Splitting of arbutin : +. Assimil-
ation KNO_3 : —. Pseudomycelium : —. Pellicle : After 30 days a very thin pellicle
is present, but in the culture A-11 usually it is not present. Streak culture :
Greyish yellow, shining, flat. Shape and size of the cells : In malt extract
apiculate, lemon-shaped and oval, $1.5\sim5\times4\sim10\mu$; on malt agar, $1.5\sim5\times4\sim13\mu$.

Seven isolates were studied : A-6~A-11. This species is popular on grapes.
All isolates studied split arbutin. In our previous paper ('56)¹⁾, we pointed out that
Kl. apiculata, *Kl. magna* and *Kl. africana* could split arbutin weakly to moderately
and recently BARNETT ('56), and KREGER-van RIJ ('58)⁶⁾ also did, now the fact
should be accepted.

Pichia fermentans Lodder, Zentr. Bak. Parasitenk.,

Abt. II, 86 : 277 (1932)

Fermentation : Glucose only but strongly. Assimilation : Glucose only. Assi-
milatation of KNO_3 : —. Splitting of arbutin : —. Pellicle : After 3 days a
wrinkled, dry, creeping pellicle is formed. After 30 days a smooth, thin creeping
pellicle is present. Sporulation : The spores are hat-shaped, easily liberated from
the ascus. They are formed on FOWELL's acetate agar medium. Slide culture : Tree-
like primitive pseudomycelium, not abundant. Streak culture : Cream colored,
flat, dull, delicately wrinkled. Shape and size of the cells : In malt extract elongate

to cylindrical, $2\sim5\times4\sim10\mu$; on malt agar, $2\sim5.5\times3.5\sim13\mu$.

One isolate was studied : K-13.

Candida mycoderma (Reess) Loder et Kreger-van Rij,
The Yeasts, Amsterdam, 477 (1952)

Syn. *Mycoderma vini* Desmazieres *sensu* Lodder, Die anasko-
sporogenen Hefen, I Hälfte, Verhandl. Koninkl. Akad. Wetenschap.
Afd. Naturkunde, sect. II, 32 : 1 (1934)

Fermentation : Glucose, very weak, often no. Assimilation : Glucose only.
Splitting of arbutin : —. Assimilation of KNO_3 : —. Pellicle : After 3 days a thin,
smooth creeping pellicle. After 30 days the pellicle is present. Slide culture :
Tree-like pseudomycelium, not abundant and in some strains only primitive. The
differentiation between pseudomycelial cell and blastospore is little. Streak culture
: Greyish yellow, dull, flat, margin lobbed. Shape and size of the cells : In malt
extract oval to elongate, $2\sim5\times4\sim8\mu$; on malt agar, elongate, $2\sim4\times4\sim12\mu$.

Five isolates were studied : K-8~K-12. This species is a major member of
film forming yeast with *C. krusei*, *Pichia fermentans* and *P. membranaefaciens*
in musts.

Candida pelliculosa Redaelli, Mem. prem. dal R. Ist.
Lombardo di Sci. e Lett., Pavia (1925)

Fermentation : Glucose +, galactose +, maltose +, lactose —, raffinose +,
sucrose +. Assimilation : Glucose +, galactose +, sucrose +, maltose +, lactose
—. KNO_3 as a sole source of nitrogen is assimilated and arbutin is splitted.
Pellicle : After 3 days, creeping pellicle ; after 30 days, a wrinkled creeping pellicle
is formed. Slide culture : Well developed pseudomycelia with many round to oval
blastospores. Streak culture : White to yellowish, soft, dry powdery to delicately
wrinkled. Shape and size of the cells : In malt extract oval, $2\sim6\times3\sim7\mu$; on
malt agar, $3\sim4\times3\sim5\mu$.

One isolate was studied : K-15. Whereas the culture was obtained from an
unfermented must, this species did not appear to be recorded previously from this
source.

Torulopsis bacillaris (Kroemer et Krumbholz) Lodder, Zentr.

Bakt. Parasitenk., Abt. II, 86 : 227 (1932)

Syn. *Saccharomyces bacillaris* Kroemer et Krumbholz, Arch. Mikrobiol., 2 : 252 (1931)

Brettanomyces italicus Verona et Florenzano, Atti. accad. naz. Lincei, Classe Sci., mat. e nat., 3 : 383 (1947)

Fermentation : Glucose, sucrose and raffinose. Assimilation : Glucose and sucrose. Splitting of arbutin : —. Assimilation of KNO_3 : —. Pseudomycelium : —. Pellicle : —. Starch like compound : —. Streak culture : Transparently white, darker in the middle, thin, smooth, shining. Shape and size of the cells : In malt extract, oval to elongate, $2\sim4 \times 3.5\sim5\mu$; on malt agar, bacillary to oval, $1\sim2.5 \times 2.5\sim5\mu$.

Five isolates were studied : T-6~T-10. This yeast has been isolated from various musts in large numbers and may be indigenous to grapes. On the preservation of the culture it should be noted that the species is very perishable.

Torulopsis glabrata (Anderson) Lodder et de Vries,

Mycopathologia, 1 : 98 (1938-'39)

Fermentation and assimilation : Glucose only. Splitting of arbutin : —. Assimilation of KNO_3 : —. Pseudomycelium : —. Pellicle : —. Starch like compound : —. Streak culture : Greyish brown (O-2) or cream colored (O-1), shining, margin smooth. Shape and size of the cells : In malt extract, round $1.5\sim5\mu$, (O-1), oval $4\sim5 \times 5\sim6.5\mu$ (O-2) ; on malt agar, round to oval, $3\sim4 \times 4\sim5\mu$, $3\sim4 \times 3\sim7\mu$ respectively.

Two isolates were studied : O-1, O-2. The species was considered to be exclusively connected with human or animal source until it was isolated from a sample of concentrated orange juice by RECCA & MRAK ('52)⁵⁾. Since we have isolated the species from grape musts repeatedly¹⁾, it may be considered as a not so peculiar one on grapes in Japan.

Torulopsis colliculosa (Hartmann) Saccardo, Syll.

Fung., 18 : 495 (1906)

Syn. *Cryptococcus colliculosa* (Hartmann) Skinner, Henrici's Mold,
Yeasts and Actimonycetes, New York (1947)

Fermentation : Glucose +, galactose -, sucrose +, maltose +, lactose -, raffinose +. Assimilation : Glucose +, galactose -, sucrose +, maltose +, lactose -. Assimilation of KNO_3 -. Starch like compound : -. Splitting of arbutin : Negative but in one culture (O-3) weak positive deviating from the standard description²⁾ of the species. Pellicle : After 30 days only ring is present. Pseudomycelium : -. Streak culture : Cream colored, raised in the middle, smooth, glistening. Shape and size of the cells : In malt extract, small round or slightly oval, $2.5\sim 5\mu$; on malt agar, $2\sim 4 \times 2\sim 5\mu$.

Two isolates were studied : O-3, O-4. The species was also isolated from grapes or early stages of fermenting musts repeatedly by ours¹⁾.

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