

## Dynamic Aspect of Yeast-flora during Vinous Fermentation

### Part 5. Characteristics of Identified Strains (III)\*

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小原巖, 野々村英夫, 湯目英郎: ブドウ酒発酵中の酵母について (第5報)  
各分離菌株の性質 (III)

In the preceding paper part 3, we isolated many strains of yeasts from grape musts, in which there were five strains difficult to identify with any hitherto known species. These five strains were isolated from the early stage of the fermentation and considered to be a members which had not the so large populations in the musts.

This paper describes the further details of their taxonomic characteristics.

One of them was recognized as a new species, *Candida vinaria* and two as new varieties, *Candida fimetaria* var. *diversa* and *Torulopsis* var. *obesa* respectively. Other two strains were regarded as *Candida parapsilosis* var. *intermedia* and *Candida guilliermondii* var. *membranaefaciens*.

These characteristics of the last two strains were slightly different from those of the standard descriptions of LODDER and KREGER-VAN RIJ<sup>2)</sup> but these differences are not enough to separate them from the species.

In studing the characteristic of these yeasts to be described the properties were determined by the system of LODDER and KREGER-VAN RIJ ('52).<sup>2)</sup>

*Candida vinaria* Oh., Nonom. et Yunome, J. Agr. Chem. Soc.  
Japan, 34 : 709 (1960) Fig. 1 ; 2

Growth in malt extract : Growth slow and poor. After 3 days at 25°C. cells are oval to elongate single or in pairs, measuring with large, oval cells, (2~3) × (3~3.5) μ; with smaller, elongate cells (1~1.5) × (2~6) μ. After one month at 17°C. a thin ring and a sediment are present, but no pellicle. Growth on malt agar : After

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\* A part of this paper has also been presented by the same authors in the note of J. Agr. Chem. Soc., Japan, 34, 709(1960). Part 4 : OHARA, NONOMURA & YUNOME ('57)

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3 days at 25°C. cells are elongate,  $(1.0\sim 1.4) \times (4.0\sim 10.8)\mu$ . single or in pairs. After one month at 17°C. the streak culture is smooth, glistening, diffused pale grey. Slide culture : Primitive pseudomycelium is rarely formed. Fermentation : Absent. Assimilation : Glucose+, galactose+, saccharose-, maltose-, lactose-. Assimilation of potassium nitrate : Absent. Ethanol as sole source of carbon : Moderate growth. Spritting of arbutin : Absent. Reaction in litmus milk : No change. Fat splitting : Absent or weakly positive.

One isolate was studied : 0-7.

The formation of a pseudomycelium of this strain is so rudimentary, that its position in the genus *Candida* seems dubious. However in the shape of the cells, not round but oval to longoval, it agree with *Mycotorula* WILL which is now synonym of *Candida*.

So we included this strain in the genus *Candida*.

Among all the *Candida* spp. this species is closely related to *C. rugosa*. But there is a clear distinction between the two in some characteristics : rare formation of pseudomycelium, smooth surface and shorter cells, poor growth in malt extract.

This strain, therefore, be considered as a new species for which we propose the name *Candida vinaria*.

### *Candida fimetaria* Soneda var. *diversa*

Oh., Nonom. et Yunome, J. Agr. Chem. Soc. Japan, 34 : 710  
(1960) Fig. 3 ; 4

Growth in malt extract : After 3 days at 25°C. cells are oval,  $(2.5\sim 4.3) \times (3.8\sim 7.0)\mu$ , single or in pairs. After one month at 17°C. a thin ring and sediment are present, pellicle is not formed. Growth on malt agar : After 3 days at 25°C. cells are oval to elongate, occasionally cylindrical,  $(1.8\sim 3.6) \times (3.2\sim 7.2)\mu$ . single or in pairs. After one month at 17°C. the streak culture becomes smooth, glistening, pale grey. Slide culture : Pseudomycelium is primitively developed on potato agar. There is little difference between the cells of the pseudomycelium and the blastospores. The development is according to the type "Mycocandida".

Fermentation and sugar assimilation : Glucose only. Assimilation of potassium nitrate : Absent. Ethanol as sole source of carbon : Weak growth. Growth in litmus milk : No change. Splitting of arbutin : Absent.

One isolate was studied : K-14.

Among all the *Candida* spp. this strain closely related to *C. krusei* and *C. mycoderma*. It resembles *C. krusei* in the fermentation of glucose ; strong positive,

however in the development of the pseudomycelium it resembles *C. mycoderma*.

Because of these properties, it has a position intermediate between *C. krusei* and *C. mycoderma*. Moreover absent of pellicle and cylindrical cells distinguish this strain from both species.

As *C. fimetaria* strain NI-7623 reported by SONEDA ('59)<sup>8)</sup> has resemblance to this strain, we examined these two strains and it appeared that there were differences between them. In the former strain, pseudomycelium is abundantly formed and there is much difference between blastospores and pseudomycelial cells. The development is according to the type "Candida" and "Mycotorula". The cells are oval and the size of the cells are all alike. In the malt extract, it forms very thin pellicle. On the contrary, in the latter strain "Mycocandida" type pseudomycelium is primitively formed. The cells are oval to elongate and diverse. No pellicle is formed. So we consider this strain as a new variety for which we propose the name *Candida fimetaria* var. *diversa*.

*Torulopsis bacillaris* (Kr. et Krumbh.) Lodder var.  
*obesa* Oh., Nonom. et Yunome, J. Agr. Chem. Soc. Japan, 34:  
710 (1960) Fig. 5

Growth in malt extract : After 3 days at 25°C. cells are oval (1.8~4.0) × (2.7~4.5) μ, single or in short chains. After one month at 17°C. a thin ring and a sediment are present. Growth on malt agar : After 3 days at 25°C. cells are oval (2.2~2.7) × (3.5~4.5) μ, single or in pairs. After one month at 17°C. the streak culture is smooth, glistening pale grey. Slide culture : No pseudomycelium is formed. Fermentation : Glucose+, galactose-, saccharose+, maltose-, lactose-, raffinose+1/3. Sugar assimilation : Glucose+, galactose-, saccharose+, maltose-, lactose- Assimilation of potassium nitrate : Absent. Ethanol as sole source of carbon : Slight, growth. Growth in litmus milk : No change. Splitting of arbutin : Absent.

One isolate was studied : 0-5.

This resembles both *T. stellata* and *T. bacillaris* in physiological properties but differs from them in morphological properties. In comparison with *T. stellata* its cells are smaller and there is no round cells. On the malt agar, it does not make the appearance which characterizes *T. bacillaris*; transparently white, in the middle darker, thin smooth and shining and there is no bacillary cells peculiar to *T. bacillaris*. Therefore this strain represents a new variety for which we propose the name *Torulopsis bacillaris* var. *obesa*.

*Candida parapsilosis* (Ashf.) Lang. et Talice var.  
*intermedia* v. Rij et Verona, Atti acad. naz.  
 Lincei, 7 : 249 (1949)

Growth in malt extract : After 3 days at 25° C. cells are round, oval and elongate (1.8~4.5) × (2.9~4.8) μ. After one month at 17° C. a ring, a islet and a sediment are formed. Growth on malt agar : After 3 days at 25° C. cells are oval (1.9~3.6) × (2.6~5.0) μ. After one month at 17° C. the streak culture is smooth, glistening, cream colored. Slide culture : The pseudomycelium is not very well developed. The development of pseudomycelium and blastospore occurs according to the types "Candida" and "Mycotorula".

Fermentation : Glucose+, galactose weakly+, sucrose-, maltose-, lactose-, Sugar assimilation : Glucose+, galactose+, sucrose+, maltose+, lactose-. Assimilation of potassium nitrate : Absent. Ethanol as sole source of carbon : A very weak growth. Growth in litmus milk : The litmus changes to blue slightly. Splitting of arbutin : Positive. Growth in litmus milk : The color slightly changes to blue.

One isolate was studied : C-6.

This strains forms only islet and the development of pseudomycelium is poor. In these properties it differs form those of the standard description of LODDER and KREGER-VAN RIJ.<sup>2)</sup>

*Candida guilliermondii* (Cast.) Lang. et Guerra var.  
*membranaefaciens* Lodder et v. Rij, The Yeasts,  
 Amsterdam, 518 (1952)

Growth in malt extract : After 3 days at 25° C. cells are elongate to pseudomycelial, (1.2~3.8) × (2.2~18.0) μ. After one month at 17° C. a rough, thick pellicle and a loose sediment are present. Sometimes the pellicle falls to the bottom. Growth on malt agar : After 3 days at 25° C. cells elongate to cylindrical, (1.3~3.1) × (2.4~8.5) μ. After one month at 17° C. the streak culture becomes hairy, surrounded by pseudomycelial cell, pale grey. Slide culture : The thin pseudomycelia are abundantly developed and true mycelia with few septa are also formed. The development of pseudomycelium is according to the type "Mycocandida".

Fermentation : Glucose+, galactose weakly+, sucrose+, maltose-or weakly+, raffinose+3/1. Sugar assimilation : Glucose+, galactose+, sucrose+, maltose+,

lactose—. Assimilation of potassium nitrate : Absent. Ethanol as sole source of carbon : Growth, a very thin pellicle is formed. Growth in litmus milk : The milk is peptonized. Splitting of arbutin : Positive.

One isolates was studied : 0-8.

The cells of this strain in malt extract are all pseudomycelial and the development of pseudomycelium differs from the standard description of LODDER and KREGER-VAN RIJ.<sup>2)</sup> But other characters are fairly the same.

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