DISTRIBUTION AND CLASSIFICATION STUDIES ON THE WILD YEASTS OR BUDDING FUNGI ON THE FRESH FRUITS IN HOKKAIDO

By

YUJI SASAKI and TADASHI YOSHIDA*

Introduction

In the past, many kinds of yeasts which are widely spread in nature have been isolated and investigated with reference mainly to their characterization and for practical uses. And yet, systematic studies on the distribution of these wild yeasts should be a fundamental and very important investigation to ascertain their ecological characters. Such studies contribute to the progress of industry.

From these stand points, at first, the present writers have isolated the wild yeasts from the fresh fruits of Hokkaido and studies have been made on the classification and distribution of them. So far as the writers are aware no such systematic investigations about the wild yeasts of fruits have been published.

Experimental Methods

I. Samples.

a. Kinds of samples.

Mainly, apples and grapes were collected for they have been managed on a comparatively large scale in Hokkaido, and in addition to them, cherries and strawberries were collected too. Cherries and strawberries were collected from the vicinity of Sapporo. The season of ripeness and for picking is about middle of July. Though many varieties of apples are growing in Hokkaido, the following 7 varieties are more common and were collected. Ripeness and picking season are also indicated.

* Institute of Applied Microbiology, Faculty of Agriculture, Hokkaido University, Sapporo, Japan.

[Journ. Facul. Agr. Hokkaido Univ., Sapporo, Vol. 51, Pt. 1, 1959]

Variety :	Ripeness and picking season :
Benisakigake	Middle to latter part of August.
Iwai	Early to middle September.
Asahi	Late September to middle of October.
Delicious	Middle of October to middle of November.
Kogyoku	Late October to middle of November.
Kokko	November.
Indo	Middle of November.

Three varieties of grapes (Delaware, Niagara and Campbell Early) was collected. The season of ripeness and for picking ranges from late October to the middle of November.

b. Places for sampling.

Samples were collected from 16 places as follows. The names of zones are also added about each place on the basis of the climate, terrain and agricultural cultivation. (cf. map)

Places:	Zones:
Sobetsu	Iburi
Nanai	Oshima
Yoichi, Oe	Shiribeshi
Mashike, Teine, Misumai, Hiragishi, Sapporo	Ishikari
Osamunai, Otoe, Bibai, Iwamizawa	Sorachi
Kamui, Furano	Kamikawa
Kitami	Kitami

c. Method of collection.

Fruit was wrapped in sterilized parchment paper package and picked. Two samples were collected for each variety of fruit.

II. Isolation of Yeasts.

a. Culture medium for isolation (Potato-glucose agar).

Composition of the culture medium for isolation of yeast is as follows:

Washed and peeled potato	300 g.
Malt-root	50 g.
Glucose	20 g.
Agar	15 g.
Water	1000 ml.

After the finely chopped potato and malt-root had been decocted with water, material was filtered. This filtrate is made up to 1000 ml. by adding water, adjusted the pH to about 6.8 by $NaHCO_3$ saturated solution, and after dissolution of the washed agar and glucose in this solution, it was poured into separate test-tubes then autoclaved at 25 lbs. momentarily and prepared as slants.

b. Isolation and pure culturing.

For isolation of yeasts from samples use was made of glucose agar plate which was acidified to pH 4 by lactic acid. Surface of fruits were rubbed on the agar plate and incubated at 27°C. Appeared yeast colonies were selected in respect to their growing character with the naked eye or under a low magnifying microscope (15×10), and were isolated and purified by means of repeating the plate technique.

III. Method for Classification of the Isolated Yeast Strains.

Isolated and purified yeast strains were classified by LODDER and KREGER-VAN RIJ's method. Studies were made about the properties of cultures as follows:

1. Characteristics of the vegetative reproduction on malt agar by slide culture method.
2. Shape and size of the cells cultured in malt extract and on malt agar.
3. Ascospore formation.
4. Ballistospore formation.
5. Shape of the ascospores or of the ballistospores.
6. Macromorphological characteristics of agar streak cultures.
7. Pellicle formation on liquid media.
8. Sugar assimilation.
9. Sugar fermentation.
10. Assimilation of nitrate.
11. Utilization of ethanol as sole source of carbon.
12. Production of carotenoid pigments.
13. Production of starch-like compounds.
14. Production of ester.
15. Reaction in litmus milk.

Results

I. Classification of the Isolated Yeast Strains.

Taxonomic studies were made on isolated 272 strains of wild yeast with referencing to LODDER and KREGER-VAN RIJ's method. As a result, they were classified into 7 genera, 14 species as follows. A number of classified strains are added.

Dematium pullulans DE BARY	100 strains
Candida robusta DIDDENS et LODDER	6 strains
Candida tropicalis (CAST.) BERKHOUT	4 strains
Candida tenuis DIDDENS et LODDER	3 strains
Kloeckera apiculata (REESS emend. KLÖCKER) JANKE	5 strains
Torulopsis candida (SAITO) LODDER	77 strains
Torulopsis aeris (SAITO) LODDER	6 strains
Debaryomyces kloeckeri GUILLIERMOND et PÉJU	10 strains
Debaryomyces nicotianae GIOVANNOZZI	1 strain
Sporobolomyces roseus KLUYVER et VAN NIEL	16 strains
Sporobolomyces pararoseus OLSON et HAMMER	1 strain
Rhodotorula glutinis (FRES.) HARRISON	32 strains
Rhodotorula mucilaginosa (JÖRGENSEN) HARRISON	6 strains
Rhodotorula flava (SAITO) LODDER	5 strains

The taxonomical characteristics and outlines of references of each of these species will be described in the next chapter.

II. Description.

Dematium pullulans **DE BARY**[(1)(3)(5)(10)] (Pl. I-1)

Syn. *Hormonema pullulans* (DEBARY) LAGERBERG et MELIN.
Pullularia pullulans (DEBARY) BERKHOUT.

Colonies on CZAPEK's solution-agar abundant, spreading, rugose, moist, luster, slimy, white to pinkish buff, often black spots.

Malt extract culture: after 3 days at 25°C. cells are oval to ellipsoidal, 3–8 × 4–15 μ. Thick walled and dark colored large cells are present and sometimes cells are separated 2 or 3 by septa. Thick and flocky creeping rings are formed. After one month, medium changes strongly viscid, flocky and pasty sediment in bottom and thick mats occasionally black colored on surface are formed.

Malt agar streak culture: After 3 days at 25°C. growth is considerably good, convex, rhizoidal, smooth, glistening, membraneous and viscid,

greyish buff to pinkish buff colored. Cells are oval to ellipsoidal, 3–8×4–15 μ. Large cells with septa are there. After one month, culture changes to dark reddish buff or black, rugose, folded and rhizoidal. Cells containing oil drops.

Slide culture: True mycelium is well developed, oval to long oval conidia, 2–6×4–8 μ, are formed abundantly on the mycelium, which is little branched.

Sugar fermentation: Absent.

Sugar assimilation : Glucose +, Galactose +, Sucrose +, Maltose +, Lactose —.

Assimilation of KNO_3: Assimilated.

Ethanol utilization: Weak.

Growth in litmus milk: Strongly peptonized, somewhat changes to blue.

Temperature range for growth: 5–32°C.

Optimum temperature: 15–20°C.

In this strain, at first, vegetative reproduction is by multilateral budding of individual cells and colony composed of oval to long oval cells is strongly viscid, smooth, glistening and greyish buff to brownish white on potato-glucose agar plate. After 3 or 4 days culture, mycelium appears abundantly and colony changes gradually dark reddish color, rugose, rhizoidal, and folds all over the surface and occasionally aerial hyphae become like needles. Liquid media changes to mucous and mycelium makes a thick mat on the surface and is dark colored. This strain can not grow at the temperature higher than 35°C. and the higher the temperature the more mycelium appears and color changes to black.

Conidia of this species are formed by means of budding on mycelium or budding of themselves. These characters are identical to the description of SASAKI (1950)[10], BAUER (1938)[1], J. GILMAN (1945)[3], MIYAJI (1945)[6], HENRICI (1930)[4], BREFELD (1895)[2] and STELLING-DEKKER (1931)[11] who pointed out this species as imperfect fungi reproducible by budding.

100 strains were observed.

Candida robusta DIDDENS et LODDER[5] (Pl. I-2)

Syn. *Saccharomyces hutensis*, nom. nud.

Malt extract culture: After 3 days at 25°C. cells are round to oval, 2.5–8 × 2.5–8 μ and occasionally elongated. Ring and sometimes dry pellicle and clayish sediment are formed. Vegetative repro-

duction is by multilateral budding and it forms occasionally a tree-like chain.

Malt agar streak culture: After one month, yellowish white, soft, smooth, glistening and sometimes partly powdered. Margin is ciliated and fimbricated.

Slide culture: Pseudomycelium is formed.

Sugar fermentation: Glucose +, Galactose +, Sucrose +, Maltose +, Lactose −, Raffinose + (1/3).

Sugar assimilation: Glucose +, Galactose +, Sucrose +, Maltose +, Lactose −.

Assimilation of KNO_3: Absent.

Ethanol utilization: Weak. After one month almost forms a pellicle.

Growth in litmus milk: No visible change.

The strains classified in this species do not form ascospore and true mycelium, but present a pseudomycelium. Cells are reproduced by way of multilateral budding. Glucose, galactose, sucrose and maltose are well fermented and assimilated, and raffinose is fermented its 1/3. KNO_3 is not assimilated. According to taxonomical systems of LODDER and KREGER-VAN RIJ these strains identified as *Candida robusta*.

On account of the correspondence with description of *Candida robusta* these strains were classified to *Candid robusta* DIDDENS et LODDER. It is said that *Candida robusta* is the imperfect form of *Saccharomyces cerevisiae*.

6 strains were studied.

Candida tropicalis (CAST.) BERKHOUT[5] (Pl. II-2)

Syn. *Oidium tropicale* CAST.
Endomyces tropicalis CAST.
Monilia candida BON. sensu HANSEN
Monilia bonordenii VUILL.
Endomyces paratropicalis CAST.
Endomyces bronchialis CAST.
Endomyces entericus CAST.
Endomyces faecalis CAST.
Endomyces niveus CAST.
Endomyces pulmonalis CAST.
Monilia butantanensis GOMES
Monilia onychophila POLL. et NANN.
Mycotorula interdigitalis RED.

Mycotorula dimorpha RED. et CIF.
Mycotorula trimorpha RED. et CIF.
Candida kefyr nom. nud.

Malt extract culture: After 3 days at 25°C. cells are round to oval, 3–6×5–10 μ. A clayish sediment and a ring are formed. After one month, no visible change from above mentioned characteristics.

Malt agar streak culture: After 3 days at 25°C. cells are short oval to oval, 3–5×5–10 μ, reproduced by multilateral budding. After one month, the color is yellowish white, appearance of culture is raised, surface smooth and little folded, with a ciliated margin.

Slide culture: Pseudomycelium is abundantly developed.

Sugar fermentation: Glucose +, Galactose +, Sucrose +, Maltose +, Lactose —, Raffinose weak or —.

Sugar assimilation : Glucose +, Galactose +, Sucrose +, Maltose +, Lactose —.

Assimilation of KNO_3: Absent.

Ethanol utilization: Weak.

Growth in litmus milk: No visible change.

These strains similar to the *Candida robusta* in their character, but these present a pseudomycelium abundantly and could not ferment raffinose. Also these strains were identical to *Candida tropicalis* and correspond to the description of LODDER et al[5].

4 strains were treated.

This species had been isolated from wine produced in Yamanashi and Nagano prefectures, Japan, by YOKOZUKA et al[13].

Candida tenuis DIDDENS et LODDER[5] (Pl. II-1)

Malt extract culture: After 3 days at 25°C. cells are small oval, 1–3×2.5–5 μ, clayish sediment and ring are formed. After one month, no visible change from above.

Malt agar streak culture: After 3 days at 25°C. cells are small oval, 1–3×2.5–5 μ, reproduced by multilateral budding. After one month, colony is yellowish white color, soft and smooth occasionally wrinkled surface, with fimbricate margin.

Slide culture: Pseudomycelium is well developed.

Sugar fermentation: Glucose +, Galactose +, Sucrose weak or —, Maltose weak or —, Lactose —, Raffinose —.

Sugar assimilation : Glucose +, Galactose +, Sucrose +, Maltose +, Lactose +.

Assimilation of KNO_3: Absent.
Ethanol utilization: Not utilized.
Growth in litmus milk: No visible change.

These strains grouped here formed neither ascospore nor true mycelium. Pseudomycelium is well developed and cells reproduced by multilateral budding. Glucose and galactose are fermented and assimilated. Sucrose, maltose and lactose are assimilated. KNO_3 is not assimilated. The characters of these strains identical to *Candida tenuis* described by DIDDENS and LODDER.

3 strains were studied.

Kloeckera apiculata (REESS **emend. KLÖCKER**) **JANKE**[5] (Pl. III-1)

Syn. *Kloeckera austriaca* (KLÖCKER) JANKE
Kloeckera germanica (KLÖCKER) JANKE
Kloeckera lindneri (KLÖCKER) JANKE
Kloeckera muelleri (KLÖCKER) JANKE
Kloeckera brevis LODDER

Malt extract culture: After 3 days at 25°C. cells are mainly lemon-shaped, 2–5×5–7.5 μ, occasionally large cells and elongated cells may occur. Flocky or clayish sediment and ring are formed.

Malt agar streak culture: After 3 days at 25°C. cells are almost lemon-shaped, 2–5×5–7μ, large cells and elongated cells may appear. After one month, colony appeared greyish white color, flat or raised, smooth, glistening and occasionally powdered partly with entire margin.

Slide culture: No pseudomycelium is formed.
Sugar fermentation: Glucose +, Galactose −, Sucrose −, Maltose −, Lactose −, Raffinose −.
Sugar assimilation : Glucose +, Galactose −, Sucrose −, Maltose −, Lactose −.
Assimilation of KNO_3: Absent.
Ethanol utilization: Not utilized.
Growth in litmus milk: No visible change.

These strains do not form the mycelium, ascospore nor pigment. Cells are lemon-shaped and reproduced by polar budding. Only glucose is fermented and assimilated. These characters are correspondent to the description of *Kloeckera apiculata* (REESS emend. KLÖCKER) JANKE.

5 strains were treated.

Torulopsis candida (SAITO) LODDER[5] (Pl. III-2)

Syn. *Torula candida* SAITO

Candida flareri (CIF. et RED.) LANGERON et GUERRA

Malt extract culture: After 3 days at 25°C. cells are round to oval, 2.5–6×3–8 μ, rings may be present, sediment is clayish and abundantly formed. After one month, ring is present.

Malt agar streak culture: After 3 days at 25°C. cells are round to oval, 2–6×3–8 μ, reproduced by multilateral budding. After one month, cultures appeared yellowish white to cream colored, pulvinate to capitate, smooth, glistening and sometimes stride radiated from center, partly powdered.

Slide culture: No mycelium is formed. Tree-like chains may occur formed by elongated cells.

Sugar fermentation: Absent.

Sugar assimilation: Glucose +, Galactose +, Sucrose +, Maltose +, Lactose +.

Assimilation of KNO_3: Absent.

Ethanol utilization: Some utilized. After one month, ring may be formed.

Growth in litmus milk: Usually litmus milk is reduced and peptonized.

In these strains ascospore, mycelium and pigment are not formed. Cells are round to oval and capsule is not formed. These strains have not any sugar fermentability but glucose, galactose, sucrose, maltose and lactose are assimilated. KNO_3 is assimilated and these strains must belong to the species *Troulopsis candida* (SAITO) LODDER.

It is noticiable that these strains usually showed strong peptonization of litmus milk.

77 strains were treated.

Some strains of this species had been isolated from the air of Tokyo, Japan, as *Torula candida* by SAITO[9].

Torulopsis aeria (SAITO) LODDER[5] (Pl. III-3)

Syn. *Torula aeria* SAITO

Malt extract culture: After 3 days at 25°C. cells are round, 3–8×4–10 μ. A clayish sediment is formed abundantly. After one month, ring may be formed.

Malt agar streak culture: After 3 days at 25°C. cells are round, 3–8×4–10 μ, reproduced by multilateral budding. After one month,

culture appears yellowish gray color, pulvinate or capitate, soft, smooth, glistening with entire margin, translucent part may occur.
Slide culture: Mycelium is not formed.
Sugar fermentation: Absent.
Sugar assimilation : Glucose +, Galactose +, Sucrose +, Maltose +, Lactose +.
Assimilation of KNO_3: Positive.
Ethanol utilization: No growth.
Growth in litmus milk: Strongly peptonized.

These strains do not form an ascospore, mycelium nor any pigment. Cells are round to oval and capsule is not formed. No sugar fermentability but glucose, galactose, sucrose, maltose and lactose are assimilated. KNO_3 is assimilated too. These strains were decided as *Torulopsis aeria* (SAITO) LODDER, because of correspondence of characters to the description of the species by LODDER et al.

6 strains were treated.

Some strains of this species were isolated from the air of Tokyo, Japan, as *Torula aeria* by SAITO[9].

Debaryomyces kloeckeri GUILLIERMOND et PÉJU[5] (Pl. IV-1)

Syn. *Debaryomyces matruchoti* GRIGORAKI et PÉJU
Debaryomyces hudeloi DA FONSECA
Debaryomyces hildegaardi OTA
Debaryomyces fabryi OTA
Debaryomyces gruetzii OTA
Debaryomyces sake SAITO et OTA

Malt extract culture: After 3 days at 25°C. cells are usually round, 2.5–7 × 2.5–8 μ, ring and clayish sediment are formed. After one month, dry dull pellicles may be formed.
Malt agar streak culture: After 3 days at 25°C. cells are usually round, 2.5–7 × 2.5–8 μ, reproduced by multilateral budding. After one month, colony appeared convex, yellowish to greyish white-colored, smooth glistening and powdered part or stripe in center may be present.
Slide culture: No mycelium is formed.
Sporulation: Usually one round and worty ascospore is formed in the ascus which is formed by conjugation between mother and daughter cells.
Sugar fermentation: Absent.
Sugar assimilation : Glucose +, Galactose +, Sucrose +, Maltose +,

Lactose —.
Assimilation of KNO_3: Not assimilated.
Ethanol utilization: Weak.
Growth in litmus milk: No visible change.

The strains belonging to this group possess round cells and usually one round and worty ascospore is formed in the ascus which formed by conjugation between mother and daughter cells. Mycelium is not formed and cells are reproduced by multilateral budding. KNO_3 is not assimilated. After one month, pellicle is formed in the surface of liquid media. Sugar fermentability is weak or nearly non-existent. Glucose, galactose, sucrose and maltose is assimilated.

These strains are identical to the species *Debaryomyces kloeckeri* GUILLIERMOND et PÉJU, described by LODDER et al.[5]

10 strains were treated.

Debaryomyces nicotianae GIOVANNOZZI[5] (Pl. IV-2)

Syn. *Debaryomyces membranaefaciens* NAGANISHI
var. *hollandicus* LODDER
Debaryomyces nicotianae GIOVANNOZZI
var. *minor* GIOVANNOZZI
Debaryomyces marylandii GIOVANNOZZI

Malt extract culture: After 3 days at 25°C. cells are round, 2.5–5 × 2.5–6 μ, dry powdery creeping pellicles and abundant powdery sediment are formed.
Malt agar streak culture: After 3 days at 25°C. cells are round, 2.5–5 × 2.5–5 μ, reproduced by multilateral budding. After one month, colony appeared yellowish to greyish white color, glistening and convex, powdered part may be present.
Slide culture: No mycelium is formed.
Sporulation: Usually one round and worty ascospore is formed in the ascus which is formed by conjugation of mother and daughter cells.
Sugar fermentation: Absent.
Sugar assimilation: Glucose +, Galactose +, Sucrose +, Maltose +, Lactose —.
Assimilation of KNO_3: Not assimilated.
Ethanol utilization: Growth. Pellicle is formed.
Growth in litmus milk: No visible change.

Although the characters of these strains are similar almost entirely

to those strains grouped in *Debaryomyces kloeckeri*, these strains formed a dry creeping pellicle in early state of culture. And then these strains might be classified into the species *Debaryomyces nicotianae* GIOVANNOZZI, described by LODDER et al.

One strain was treated.

Some strains of this species had been isolated from soy sauce mash and "Koji" in Japan by OHARA and NONOMURA[7].

Sporobolomyces roseus KLUYVER et VAN NIEL[5] (Pl. V-1)

Syn. *Sporobolomyces tenuis* KLUYVER et VAN NIEL
Sporobolomyces photographus CIF. et RED.
Amphiernia rubra GRÜSS
Sporobolomyces salmoneus DERX
Sporobolomyces pollaccii VERONA et CIFERRI

Malt extract culture: After 3 days at 25°C. cells are oval, ellipsoidal to long oval, 3–8×8–10 μ, sometimes worty due to formation of sterigmata. Ring and occasionally thin pellicle are formed. Flocky or soft clayish sediment is present. After one month, thick, flocky ring or mat is formed on the surface.

Malt agar streak culture: After3 days at 25°C. cells are oval, ellipsoidal to long oval, 3–8×8–10 μ, reproduced by multilateral budding, culture appeared violet-red color, convex, smooth, glistening with entire margin. After one month, wrinkled surface may be present.

Slide culture: Occasionally sterigmata elongated and pseudomycelium may be formed but true mycelium is not present. Mirror image is formed.

Sporulation: At the top of the sterigmata which elongated from side of the vegetative cells kidney- to sickle-shaped ballistcspores develop in an oblique direction and discharged.

Sugar fermentation: Absent.

Sugar assimilation: Glucose +, Galactose +, Sucrose +, Maltose +, Lactose —.

Assimilation of KNO_3: Assimilated.

Ethanol utilization: Not utilized.

Growth in litmus milk: After one month, usually showed strong peptonization.

These strains formed a violet-red colony, and kidney- or sickle-shaped spores are developed in an oblique position to the well developed aerial sterigmata and discharged and present a "mirror image".

No true mycelium. KNO_3 is assimilated. No sugar fermentation but glucose, galactose, sucrose and maltose are assimilated. The characters of these strains are in accordance with the description of *Sporobolomyces roseus* KLUYVER et VAN NIEL.

16 strains were treated.

Some strains of this species have been isolated from the air of Tokyo, Japan, as *Sporobolomyces* sp. by YAMAZAKI[12].

Sporobolomyces pararoseus OLSON et HAMMER[5] (Pl. V-2)

Syn. *Torula shibatana* OKUNUKI
Sporobolomyces shibatanus (OKUNUKI) VERONA et CIFERRI

Malt extract culture: After 3 days at 25°C. cells are oval to ellipsoidal, 3–8×8–10 μ, ring and flocky to soft clayish sediment are formed. After one month, flocky ring or mat is formed on the surface.

Malt agar streak culture: After 3 days at 25°C. cells are oval to ellipsoidal, 3–8×8–10 μ, reproduced by multilateral budding. Colony appeared light orange-red to coral-red color, convex, smooth and glistening. After one month, wrinkled over the surface.

Slide culture: Mycelium or pseudomycelium is not formed. Mirror image is formed.

Sporulation: At the top of the sterigmata which elongated from side of the vegetative cells kidney-shaped spores are developed in an oblique position and discharged.

Sugar fermentation: Absent.

Sugar assimilation: Glucose +, Galactose +, Sucrose +, Maltose +, Lactose −.

Assimilation of KNO_3: Not assimilated.

Ethanol utilization: Ethanol utilized and ring is formed.

Growth in litmus milk: Islets and ring are formed, peptonized.

The characters of these strains similar to those strains classified into the species *Sporobolomyces roseus* but different in the lacking of KNO_3 assimilation. These characters identical to species *Sporobolomyces pararoseus* OLSON et HAMMER as described by LODDER et al[5].

One strain was treated.

Rhodotorula glutinis (FRES.) HARRISON[5] (Pl. VI-1)

Syn. *Rhodotorula bronchialis* (CIF. et RED.) LODDER
Torula suganii OKUNUKI
Torula infirmo-miniata OKUNUKI

Malt extract culture: After 3 days at 25°C. cells are oval to ellipsoidal, 3–8×5–10μ, ring and sometimes islets and clayish sediment are formed. After one month, thick mat is formed on the surface.

Malt agar streak culture: After 3 days at 25°C. cells are oval to ellipsoidal, 3–8×5–10μ, usually containing an oil drop, reproduced by multilateral budding. Colony appeared orange to orange-red color, convex, smooth and glistening with entire margin.

Slide culture: No mycelium is formed.

Sugar fermentation: Absent.

Sugar assimilation: Glucose +, Galactose +, Sucrose +, Maltose +, Lactose −.

Assimilation of KNO_3: Assimilated.

Ethanol utilization: Weak.

Growth in litmus milk: Peptonization may occur.

The strains belonging to this group produce an orange-red pigment but spores and mycelium are not formed. KNO_3 is assimilated. Cells are round to ellipsoidal. These characters correspond to the description of the species *Rhodotorula glutinis* (FRES.) HARRISON.

32 strains were treated.

Torula suganii which was included in the species *Rhodotorula glutinis* by LODDER et al. had been isolated from the air of Tokyo, Japan, by OKUNUKI[12].

Rhodotorula mucilaginosa (JÖRGENSEN) HARRISON[5] (Pl. VI–2)

Syn. *Torula mucilaginosa* JÖRGENSEN
Torula sanguinea SCHIMON
Cryptococcus ludwigi ANDERSON
Blastodendrion carbonei CIFERRI et REDAELLI
Eutorulopsis dubia CIFERRI et REDAELLI
Mycotorula pulmonalis CIFERRI et REDAELLI
Torulopsis sanniei CIFERRI et REDAELLI
Torulopsis biourgei CIFERRI et REDAELLI
Blastodendrion simplex CIFERRI et REDAELLI
Cryptococcus rubrorugosus CASTELLANI
Cryptococcus paraoseus CASTELLANI
Torula aclotiana nom. nud.
Rhodotorula aclotiana HARRISON
Torulopsis nitritophila CIFERRI et ASHFORD
Cryptococcus radiatus A. et R. SARTORY et MEYER

Torula decolans OKUNUKI
Torulopsis mannitica CASTELLI
Torulopsis aurantia ZACH
Mycotorula cisnerosi nom. und.

Malt extract culture: After 3 days at 25°C. cells are round to oval, 2.5–6×3–8 μ, ring and flocky to soft clayish sediment are formed. After one month, thick mat is present.

Malt agar streak culture: After 3 days at 25°C. cells are round to oval, 2.5–6×3–8 μ, reproduced by multilateral budding. After one month, culture appeared pink to red color, convex, smooth and glistening with entire margin.

Slide culture: Mycelium is not formed.

Sugar fermentation: Absent.

Sugar assimilation: Glucose +, Galactose +, Sucrose +, Maltose +, Lactose −.

Assimilation of KNO_3: Not assimilated.

Ethanol utilization: Weak or very weak.

These strains are different from *Rhodotorula glutinis* in lacking the ability of KNO_3 assimilation. The characters of these strains are identical to the description of the species *Rhodotorula mucilaginosa* (JÖRGENSEN) HARRISON.

6 strains were treated.

Some strains of this species had been isolated from the air of Tokyo, Japan, as *Torula decolans* by OKUNUKI[12].

Rhodotorula flava (SAITO) LODDER[5] (Pl. VI–3)

Syn. *Torula flava* SAITO
Chromotorula flava (SAITO) HARRISON

Malt extract culture: After 3 days at 25°C. cells are round to oval, 3–5×3–6 μ, ring and clayish sediment are formed. After one month, no visible change.

Malt agar streak culture: After 3 days at 25°C. cells are round to oval, 3–5×3–6 μ, reproduced by multilateral budding. After one month, culture appeared dark orange color, translucent, pulvinate, smooth and glistening with entire margin.

Slide culture: No mycelium is formed.

Sugar fermentation: Absent.

Sugar assimilation: Glucose +, Galactose +, Sucrose +, Maltose +, Lactose +.

Assimilation of KNO_3: Not assimilated.
Ethanol utilization: Not utilized.
Growth in litmus milk: No visible change.

As former two species of *Rhodotorula*, these strains lacking the ability of spore and mycelium formation and of sugar fermentation. There is a little difference in the colony color as yellowish-orange, shiny and translucent. Considering the characters of these strains we might be classified as *Rhodotorula flava* (SAITO) LODDER.

5 strains were treated.

Some strains of this species had been isolated from the air of Tokyo, Japan, as *Torula flava* by SAITO.

KEY TO THE SPECIES OF WILD YEASTS OR BUDDING FUNGI ON THE FRESH FRUITS IN HOKKAIDO

—Colonies are white or creamy colored.
  —Warty spore, containing an oildrop, is formed in ascus after the conjugation of mother and daughter cells.
    Cells are round, multilateral budding.
    Nitrate is not assimilated. Mycelium is not formed.
      No sugar fermentation. (Genus *Debaryomyces* (KLÖCKER) LODDER et VAN RIJ)
        Glucose, galactose, sucrose and maltose are assimilated.
          —Dull, dry pellicle is formed on liquid media . . . . . *Debaryomyces nicotianae* GIOVANNOZZI
          —Pellicle is not formed . . . *Debaryomyces kloeckeri* GUILLIERMOND et PÉJU
  —No spore formation.
    —Cells are usually lemon-shaped, bipolar budding. (Genus *Kloeckera* JANKE)
      Glucose is fermented and assimilated . . . . . *Kloeckera apiculata* (REESS emend. KLÖCKER) JANKE
    —Cells are round, oval or ellipsoidal, multilateral budding.
      —No mycelium is formed. No "starch" formation. (Genus *Torulopsis* BERLESE)
        No sugar fermentation. Glucose, galactose, sucrose, maltose and lactose are assimilated.
          —Nitrate is assimilated . . . . . *Torulopsis aeria* (SAITO) LODDER
          —Nitrate is not assimilated . . . . . *Torulopsis candida* (SAITO) LODDER
      —Pseudomycelium is well developed. (Genus *Candida* BERKHOUT)
        —Glucose, galactose, sucrose and maltose are fermented and assimilated.
          —1/3 raffinose is fermented . . . . *Candida robusta* DIDDENS et LODDER
          —Raffinose is not fermented . . . . *Candida tropicalis* (CAST.) BERKHOUT
        —Glucose, galactose are fermented and assimilated.
          Sucrose, maltose and lactose are assimilated . . . . . *Candida tenuis* DIDDENS et LODDER
      —True mycelium is formed. (Genus *Dematium* PERSOON)

Glucose, galactose, sucrose and maltose are assimilated.
No sugar fermentation. Color of colonies changes creamy to black . . . . . *Dematium pullulans* DE BARY
—Colonies are reddish, orange or pinkish colored.
—Asymmetrical, kidney- or sickle-shaped ballistospore is formed. (Genus *Sporobolomyces* KLUYVER et VAN NIEL)
Glucose, galactose, sucrose and maltose are assimilated.
—Nitrate is assimilated . . . . . *Sporobolomyces roseus* KLUYVER et VAN NIEL
—Nitrate is not assimilated . . *Sporobolomyces pararoseus* OLSON et HAMMER
—No spore formation. (Genus *Rhodotorula* HARRISON)
No sugar fermentation.
—Glucose, galactose, sucrose and maltose are assimilated.
—Nitrate is assimilated . . . . . *Rhodotorula glutinis* (FRES.) HARRISON
—Nitrate is not assimilated . . . . . *Rhodotorula mucilaginosa* (JÖRGENSEN) HARRISON
—Glucose, galactose, sucrose, maltose and lactose are assimilated . . . . . *Rhodotorula flava* (SAITO) LODDER

IV. Distribution of the Isolated Strains.

The distribution of 272 yeast strains which were isolated from fresh fruits of Hokkaido was examined on each species and each sample which was collected seasonally.

Table 1 presents the local distribution of yeasts, table 2 their seasonal distribution and table 3 (1–14) the local and seasonal distribution of each species of yeasts.

It is known traditionally that "Saru-sake" (monkey wine) was made by natural fermentation of preserved fruits in a hollow of a tree by monkeys, and also natural fermentation is known to occur in undisturbed substances such as fruits, foods, milk and other materials containing the carbohydrates under natural conditions. After many observations, it is said that such fermentation may be concerned with *Saccharomyces ellipsoideus* in the chief role, and that tbis species is spread widely in nature especially on fruits. But the authors could not find any *Saccharomyces ellipsoideus*, even the genus *Saccharomyces*, from the fruits of Hokkaido.

Considering the local distribution of each species, *Dematium pullulans* showed abundant distribution in every part of Hokkaido, especially in the early ripening fruits. In general, the appearance of this species seems to decrease in the late ripening fruits and to be comparatively less in the Sorachi zone than in others.

Torulopsis and red yeasts are spread over a relatively wide range.

TABLE 1. Local distribution of yeasts.

Species \ Zones	Oshima	Iburi	Shiri-beshi	Ishikari					Sorachi				Kami-kawa		Kitami
Species \ Places	Nanai	Sobetsu	Yoichi	Oe	Mashike	Teine	Misumai	Hira-gishi	Sapporo	Osa-munai	Otoe	Bibai	Iwa-mizawa	Kamui	Furano	Kitami
Dematium pullulans	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Candida robusta	−	−	+	−	−	−	−	−	−	+	−	+	+	−	−	−
Candida tropicalis	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−
Candida tenuis	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Kloeckera apiculata	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Torulopsis candida	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+
Torulopsis aeria	+	−	+	−	−	−	−	+	−	−	−	−	−	−	+	−
Debaryomyces kloeckeri	−	+	+	−	−	−	−	−	−	−	+	−	+	−	−	−
Debaryomyces nicotianae	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Sporobolomyces roseus	+	−	+	+	−	+	−	+	−	+	+	+	+	−	−	+
Sporobolomyces pararoseus	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Rhodotorula glutinis	+	+	+	−	+	+	+	+	+	−	−	+	−	−	+	−
Rhodotorula mucilaginosa	+	+	−	−	−	+	−	−	−	+	+	−	−	−	−	−
Rhodotorula flava	+	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−

+ : Yeast was found. − : Yeast was not found.

TABLE 2. Seasonal distribution of yeasts.

Species \ Seasons	Early Summer (Cherries)	(Straw-berries)	Midsummer (Apples : Early-var.)	Autumn Apples : Late-var.)	(Grapes)
Dematium pullulans	+	+	+	+	+
Candida robusta	−	−	+	+	+
Candida tropicalis	−	−	+	−	−
Candida tenuis	−	−	−	+	+
Kloeckera apiculata	−	−	−	−	+
Torulopsis candida	+	+	+	+	+
Torulopsis aeria	−	+	+	+	−
Debaryomyces kloeckeri	−	−	+	+	−
Debaryomyces nicotianae	−	−	+	−	−
Sporobolomyces roseus	−	−	+	+	+
Sporobolomyces pararoseus	−	−	−	−	+
Rhodotorula glutinis	+	+	+	+	+
Rhodotorula mucilaginosa	−	−	+	+	+
Rhodotorula flava	−	−	−	+	−

+ : Yeast was found. − : Yeast was not found.

TABLE 2. Local and seasonal distribution decided into each species of yeasts. (1—14)

Dematium pullulans DE BARY (1)

Seasons \ Places	Nanai	Sobetsu	Yoichi	Oe	Mashike	Teine	Misumai	Hiragishi	Sapporo	Osamunai	Otoe	Bibai	Iwa-mizawa	Kamui	Furano	Kitami
Cherries			+			+	+	+	+							
Strawberries			+			+	+		+							
Apples																
Early-var.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Late-var.	+	+	+			+	+	+		+	+	+				
Grapes	+	+	+			+		+				+		+		

Candida robusta DIDDENS et LODDER (2)

Seasons \ Places	Nanai	Sobetsu	Yoichi	Oe	Mashike	Teine	Misumai	Hiragishi	Sapporo	Osamunai	Otoe	Bibai	Iwa-mizawa	Kamui	Furano	Kitami
Cherries			−			−	−	−	−							
Strawberries			−			−	−		−							
Apples																
Early-var.	−	−	−	−	−	−	−	−	−	+	−	+	+	−	−	−
Late-var.	−	−	+			−	−	−		−	−	−				
Grapes	−	−	+			−		−				−		−		

Candida tropicalis (CAST.) BERKHOUT (3)

Seasons \ Places	Nanai	Sobetsu	Yoichi	Oe	Mashike	Teine	Misumai	Hiragishi	Sapporo	Osamunai	Otoe	Bibai	Iwa-mizawa	Kamui	Furano	Kitami
Cherries			−			−	−	−	−							
Strawberries			−			−	−		−							
Apples																
Early-var.	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−
Late-var.	−	−	−			−	−	−		−	−	−				
Grapes	−	−	−			−		−				−		−		

Candida tenuis DIDDENS et LODDER (4)

Seasons \ Places	Nanai	Sobetsu	Yoichi	Oe	Mashike	Teine	Misumai	Hiragishi	Sapporo	Osamunai	Otoe	Bibai	Iwa-mizawa	Kamui	Furano	Kitami
Cherries			−			−	−	−	−							
Strawberries			−			−	−		−							
Apples																
Early-var.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Late-var.	−	−	+			−	−	−		−	−	−				
Grapes	−	−	+			−		−				−		−		

Kloeckera apiculata (REESS emend. KLÖCKER) JANKE (5)

Seasons \ Places	Nanai	Sobetsu	Yoichi	Oe	Mashike	Tenie	Misumai	Hiragishi	Sapporo	Osamunai	Otoe	Bibai	Iwa-mizawa	Kamui	Furano	Kitami
Cherries			−			−	−	−	−							
Strawberries			−			−	−		−							
Apples																
Early-var.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Late-var.	−	−	−			−	−	−		−	−	−				
Grapes	−	+	−			−		−				+		−		

Torulopsis candida (SAITO) LODDER (6)

Seasons \ Places	Nanai	Sobetsu	Yoichi	Oe	Mashike	Teine	Misumai	Hiragishi	Sapporo	Osamunai	Otoe	Bibai	Iwa-mizawa	Kamui	Furano	Kitami
Cherries			−			−	−	−	+							
Strawberries			−			+	+		+							
Apples																
Early-var.	+	+	+	−	−	+	+	+	−	+	+	−	+	+	+	+
Late-var.	+	+	+			+	+	+		+	+	−				
Grapes	+	+	+			+		+				+		+		

Torulopsis aeria (SAITO) LODDER (7)

Seasons \ Places	Nanai	Sobetsu	Yoichi	Oe	Mashike	Teine	Misumai	Hiragishi	Sapporo	Osamunai	Otoe	Bibai	Iwa-mizawa	Kamui	Furano	Kitami
Cherries			−			−	−	−	−							
Strawberries			−			−	−		+							
Apples																
Early-var.	+	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−
Late-var.	+	−	+			−	−	−		−	−	−				
Grapes	−	−	−			−		−				−		−		

Debaryomyces kloeckeri GUILLIERMOND et PÉJU (8)

Seasons \ Places	Nanai	Sobetsu	Yoichi	Oe	Mashike	Teine	Misumai	Hiragishi	Sapporo	Osamunai	Otoe	Bibai	Iwa-mizawa	Kamui	Furano	Kitami
Cherries			−			−	−	−	−							
Strawberries			−			−	−		−							
Apples																
Early-var.	−	−	−	−	−				−	−	−	−	+	−	−	−
Late-var.	−	+	+			−	−	−		−	+	−				
Grapes	−	−	−			−		−				−		−		

Debaryomyces nicotianae GIOVANNOZZI (9)

Seasons \ Places	Nanai	Sobetsu	Yoichi	Oe	Mashike	Teine	Misumai	Hiragishi	Sapporo	Osamunai	Otoe	Bibai	Iwa-mizawa	Kamui	Furano	Kitami
Cherries			−			−	−	−	−							
Strawberries			−			−	−		−							
Apples																
Early-var.	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Late-var.	−	−	−			−	−	−		−	−	−				
Grapes	−	−	−			−		−				−		−		

Sporobolomyces roseus KLUYVER et VAN NIEL (10)

Seasons \ Places	Nanai	Sobetsu	Yoichi	Oe	Mashike	Teine	Misumai	Hiragishi	Sapporo	Osamunai	Otoe	Bibai	Iwa-mizawa	Kamui	Furano	Kitami
Cherries			−			−	−	−	−							
Strawberries			−			−	−		−							
Apples																
Early-var.	+	−	−	+	−	+	−	+	−	+	+	+	+	−	−	+
Late-var.	+	−	−			−	−	−		−	−	−				
Grapes	−	−	+			+		−				−		−		

Sporobolomyces pararoseus OLSON et HAMMER (11)

Seasons \ Places	Nanai	Sobetsu	Yoichi	Oe	Mashike	Teine	Misumai	Hiragishi	Sapporo	Osamunai	Otoe	Bibai	Iwa-mizawa	Kamui	Furano	Kitami
Cherries			−			−	−	−	−							
Strawberries			−			−	−		−							
Apples																
Early-var.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Late-var.	−	−	−			−	−	−		−	−	−				
Grapes	−	−	−			−		−				−		+		

Rhodotorula glutinis (FRES.) HARRISON (12)

Seasons \ Places	Nanai	Sobetsu	Yoichi	Oe	Mashike	Teine	Misumai	Hiragishi	Sapporo	Osamunai	Otoe	Bibai	Iwa-mizawa	Kamui	Furano	Kitami
Cherries			−			−	−	+	−							
Strawberries			+			+	+		+							
Apples																
Early-var.	+	−	+	−	+	+	+	+	+	−	−	−	+	−	+	−
Late-var.	+	−	+			−	−	−		−	+	−				
Grapes	−	+	−			−		−				+		−		

Rhodotorula mucilaginosa (JÖRGENSEN) HARRISON (13)

Seasons \ Places	Nanai	Sobetsu	Yoichi	Oe	Mashike	Teine	Misumai	Hiragishi	Sapporo	Osamunai	Otoe	Bibai	Iwa-mizawa	Kamui	Furano	Kitami
Cherries			−			−	−	−	−							
Strawberries			−			−	−		−							
Apples																
Early-var.	−	+	−	−	−	+	−	−	−	−	+	−	−	−	−	−
Late-var.	+	−	−			−	−	−		+	−	−				
Grapes	−	+	−			−		−				−		−		

Rhodotorula flava (SAITO) LODDER (14)

Seasons \ Places	Nanai	Sobetsu	Yoichi	Oe	Mashike	Teine	Misumai	Hiragishi	Sapporo	Osamunai	Otoe	Bibai	Iwa-mizawa	Kamui	Furano	Kitami
Cherries			−			−	−	−	−							
Strawberries			−			−	−		−							
Apples																
Early-var.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Late-var.	+	−	−			−	−	−		+	+	−				
Grapes	−	−	−			−		−				−		−		

+ : Yeast was found. − : Yeast was not found.

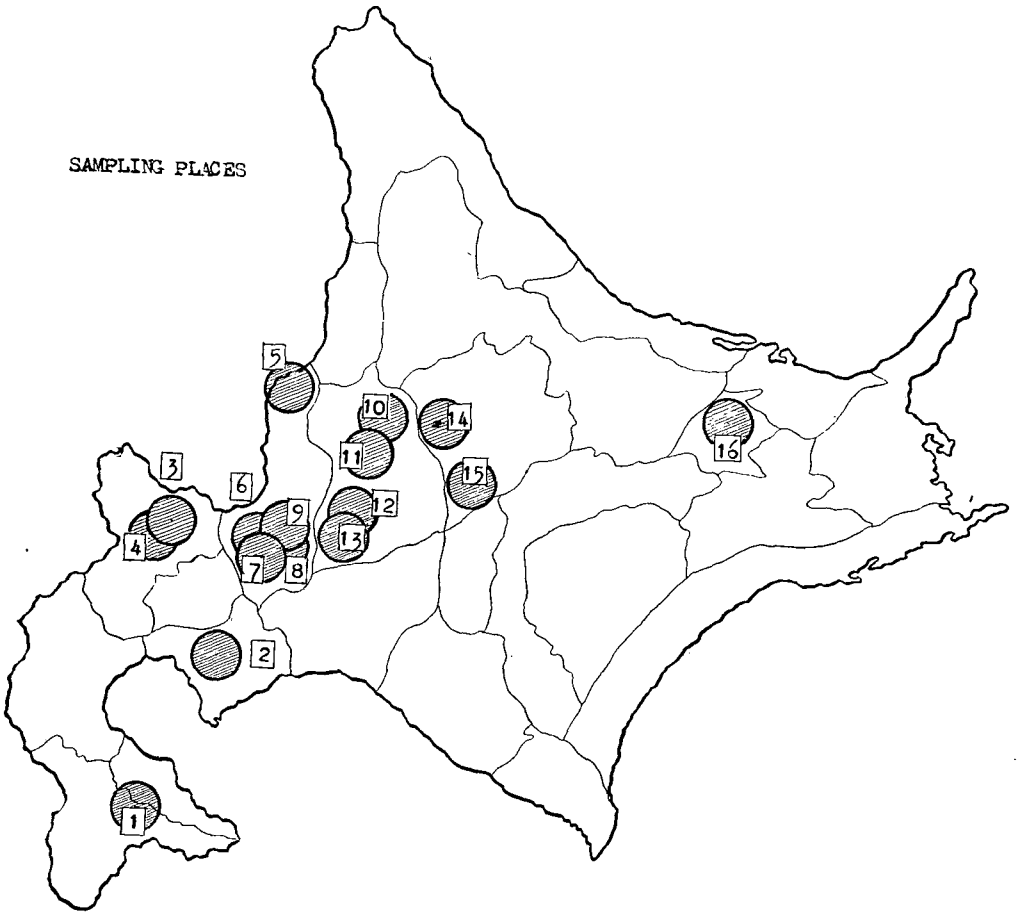
Torulopsis candida appeared in almost all areas commonly. *Sporobolomyces roseus* showed a wide distribution unconnected with the regions, and *Rhodotorula glutinis* distributed mainly in the southwestern part of Hokkaido.

Other species, except the above mentioned, scattered in central and southern parts widely.

Strong glucose fermentative yeast, *Kloeckera apiculata*, was isolated abundantly from grapes alone of Iburi and Sorachi zones.

The appearance of sugar fermenting yeast, *Candida*, and non sugar fermentative yest, *Debaryomyces*, was limited to the Sorachi and Shiribeshi zones after midsummer.

From the point of view of the seasonal distribution it might be said that the three species, *Dematium pullulans*, *Torulopsis candida* and



SAMPLING PLACES IN HOKKAIDO

No.	Places	Zones	No.	Places	Zones
1	Nanai	Oshima	9	Sapporo	Ishikari
2	Sobetsu	Iburi	10	Osamunai	Sorachi
3	Yoichi	Shiribeshi	11	Otoe	"
4	Oe	"	12	Bibai	"
5	Mashike	Ishikari	13	Iwamizawa	"
6	Teine	"	14	Kamui	Kamikawa
7	Misumai	"	15	Furano	"
8	Hiragishi	"	16	Kitami	Kitami

Rhodotorula glutinis, appear through all seasons, because they can be found on every sample which was collected seasonally such as cherries, strawberries, early- and late-variety apples and grapes.

In early summer, the appearance of wild yeast is restricted to these three species and no other species could be found.

Sugar fermentable yeasts of *Candida*, ascospore forming *Debaryomyces*, ballistospore forming *Sporobolomyces* and other red yeasts showed a tendency to appear in later season, to wit, midsummer to autumn. And, especially, it is noticeable that strong glucose fermentative yeast, *Kloeckera apiculata*, was isolated only from grapes in late autumn.

It may be worth noticing that the appearance of these yeasts is restricted by regions, seasons and samples, and it should be considered that this species is specific to grapes, because it was not detected from other samples whose maturities are about the same time as grapes.

Concerning the average- and accumulated-temperature of agricultural season, Iburi, Shiribeshi and Sorachi zones are rather warm. There is more rain and sunlight in summer than in other districts. Accordingly these areas are suitable for fruit growing. Cultivation has been continued many years, and many fermentable yeasts could be found. As for other places, even in Ishikari zone considered as a fruit growing area in Hokkaido, no fermentable yeasts could be found. The relationships between these fermentable wild yeasts, and agricultural environment could not be clarified.

Furthermore, it should be mentioned, however, that the sugar fermentable yeasts of *Candida robusta* and *Candida tropicalis* which are similar to *Saccharomyces cerevisiae* were isolated, but the present writers could not found any yeast belonging to the genus *Saccharomyces* especially *Sacch. ellipsoideus* which is considered to play the main role in natural fermentation and to be widely distributed on fruits.

Summary

1. The wild yeasts were isolated from fresh fruits in Hokkaido and studied with respect to their classification and distribution.

2. Samples of cherries, strawberries, apples and grapes were collected from 16 main fruit growing localities in 7 zones in entire state so far as possible. Two hundred seventy two strains of yeasts were isolated from these samples: 9 strains from cherries, 27 strains from strawberries, 111 strains from early variety apples, 80 strains from late variety apples and 45 strains from grapes.

3. As the results of taxonomical studies, pure cultured yeast strains were classified into 7 genera, 14 species as follows:

Dematium pullulans DE BARY	100 strains
Candida robusta DIDDENS et LODDER	6 strains
Candida tropicalis (CAST.) BERKHOUT	4 strains
Candida tenuis DIDDENS et LODDER	3 strains
Kloeckera apiculata (REESS emend. KLÖCKER) JANKE	5 strains
Torulopsis candida (SAITO) LODDER	77 strains
Torulopsis aeria (SAITO) LODDER	6 strains
Debaryomyces kloeckeri GUILLIERMOND et PÉJU	10 strains
Debaryomyces nicotianae GIOVANNOZZI	1 strain
Sporobolomyces roseus KLUYVER et VAN NIEL	16 strains
Sporobolomyces pararoseus OLSON et HAMMER	1 strain
Rhodotorula glutinis (FRES.) HARRISON	32 strains
Rhodotorula mucilaginosa (JÖRGENSEN) HARRISON	6 strains
Rhodotorula flava (SAITO) LODDER	5 strains

4. As the results of examination regarding the distribution of the isolated yeast strains the following points became clear:

a. *Dematium pullulans* is distributed in all parts of Hokkaido in greatest frequency whilst *Torulopsis candida, Sporobolomyces roseus* and *Rhodotorula glutinis* are widely spread too. These strains seem to be commonly present in Hokkaido without any regard to the regions and seasons.

b. Sugar fermentative *Candida robusta, Candida tropicalis* and *Candida tenuis* were isolated in the later part of the agricultural season from Shiribeshi and Sorachi zones; strong glucose fermentative *Kloeckera apiculata* was abundantly isolated in Iburi and Sorachi zones from grapes alone.

Then it might be said that there is a tendency for a fermentative yeasts following the maturity of fruits. The fact that some strains are distributed in limited areas, perhaps, may suggest the existence of some interesting connections between the distribution of wild yeast and the climate, characters of soils, seasons and the kinds of fruit.

References

1. R. BAUER: Zentralbl. für Bakt. Parasit. u. Infektionskrankheit. II abt. Bd. 98, p. 133 (1938).
2. O. BREFELD: Untersuchungen aus dem Gesammtgebiete der Mykologie. 10 Heft. (1895).
3. J. GILMAN: A Manual of Soil Fungi. p. 286 (1945).
4. A. T. HENRICI: The Mold, Yeast and Actinomycetes. (1930).
5. J. LODDER and N. J. W. KREGER-VAN RIJ: The yeasts. (1952).
6. K. MIYAJI: Oyobaikingaku. (1945).
7. I. OHARA and H. NONOMURA: J. Agr. Chem. Japan. Vol. 28, p. 837 (1954).
8. K. OKUNUKI: Japan Journal of Bot. Vol. 5, p. 285 (1931).
9. K. SAITO: Ibid. Vol. 1, p. 1 (1922).
10. Y. SASAKI: J. Facul. Agr., Hokkaido Univ. Sapporo, XLIX, 2 (1950).
11. N. M. STELLING-DEKKER: Die Sporogenen Hefen. (1931).
12. I. YAMAZAKI and S. MORISHITA: J. Agr. Chem. Japan. Vol. 17, p. 547 (1943).
13. I. YOKOTSUKA and S. GOTO: J. Agr. Chem. Japan. Vol. 29, p. 39, p. 174 (1955).

EXPLANATION OF PLATES

Plate I

1 ····· *Dematium pullulans* De Bary

2 ····· *Candida robusta* Diddens et Lodder

a : After 3 days in malt extract.

b : After 3 days on malt agar.

c : Slide culture on malt agar.

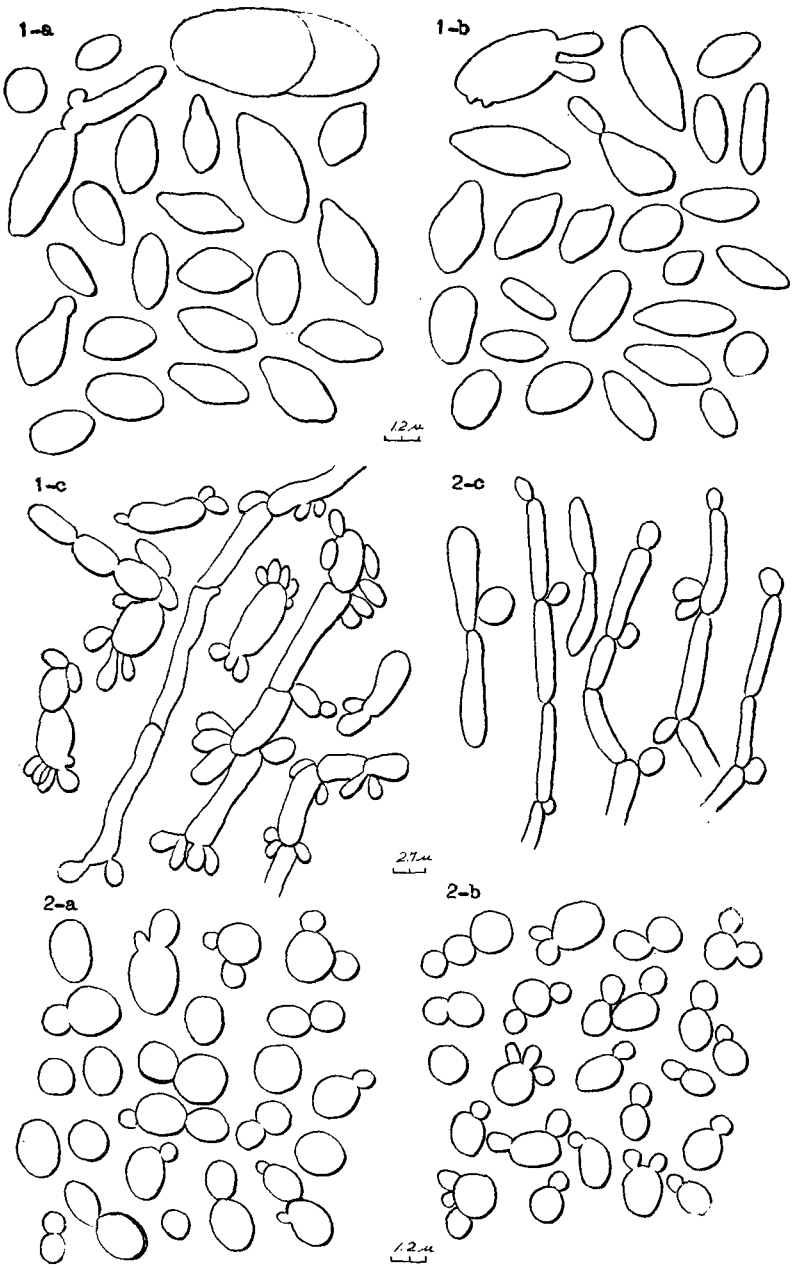

1-a
1-b
12μ
1-c
2-c
27μ
2-a
2-b
12μ

Plate II

1 ..... *Candida tenuis* DIDDENS et LODDER

2 ..... *Candida tropicalis* (CAST.) BERKHOUT

a : After 3 days in malt extract.

b : After 3 days on malt agar.

c : Slide culture on malt agar.

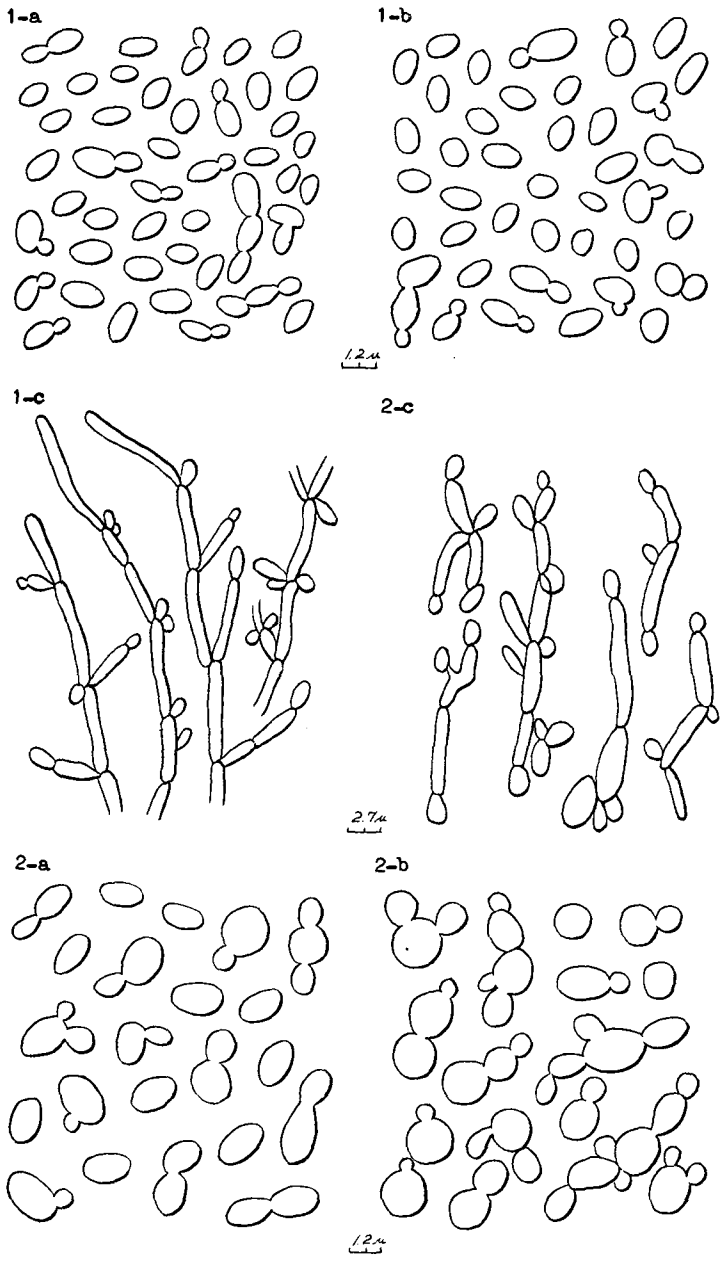

1-a
1-b
12μ
1-c
2-c
2.7μ
2-a
2-b
12μ

Plate III

1 ..... *Kloeckera apiculata* (REESS emend. KLÖKER) JANKE

2 ..... *Torulopsis candida* (SAITO) LODDER

3 ..... *Torulopsis aeria* (SAITO) LODDER

a : After 3 days in malt extract.

b : After 3 days on malt agar.

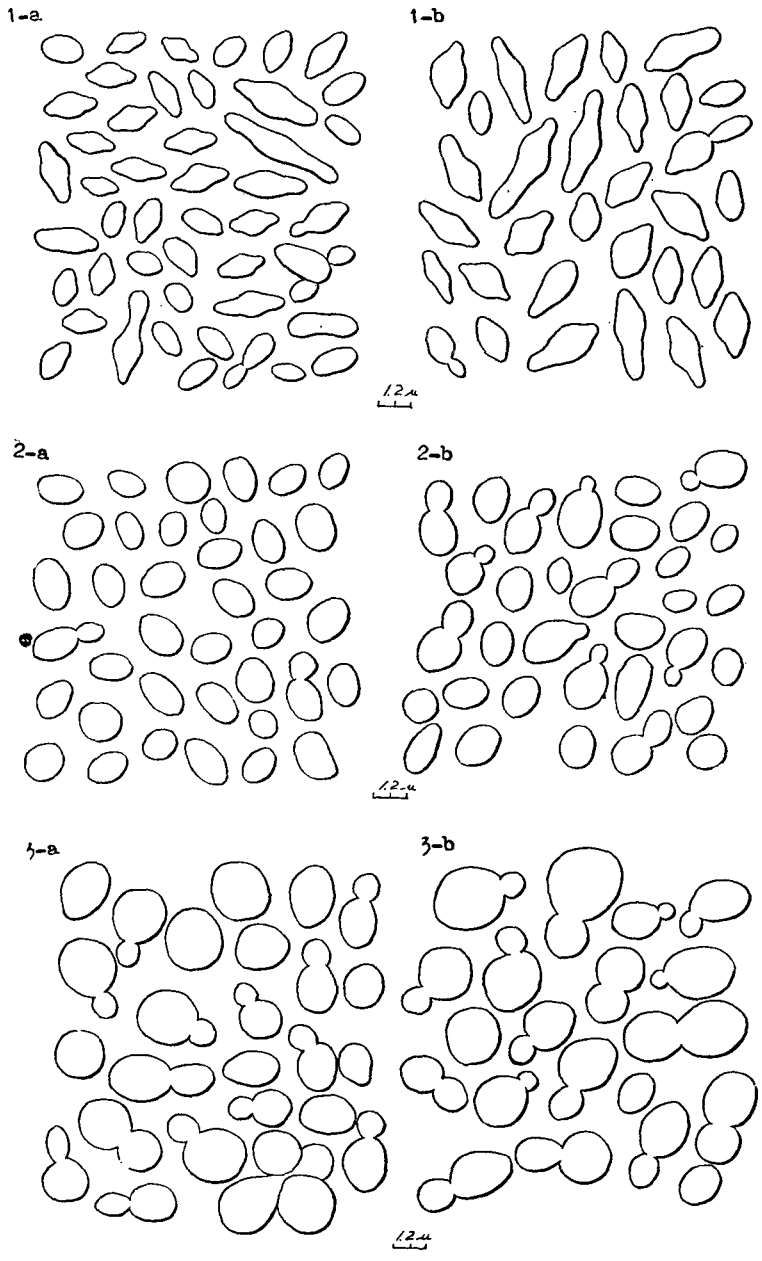

1-a
1-b
12μ
2-a
2-b
12μ
3-a
3-b
12μ

Plate IV

1 …… *Debaryomyces kloeckeri* GUILLIERMOND et PÉJU

2 …… *Debaryomyces nicotianae* GIOVANNOZZI

a : After 3 days in malt extract.

b : After 3 days on malt agar.

c : Ascospore on GORODKOWA's agar.

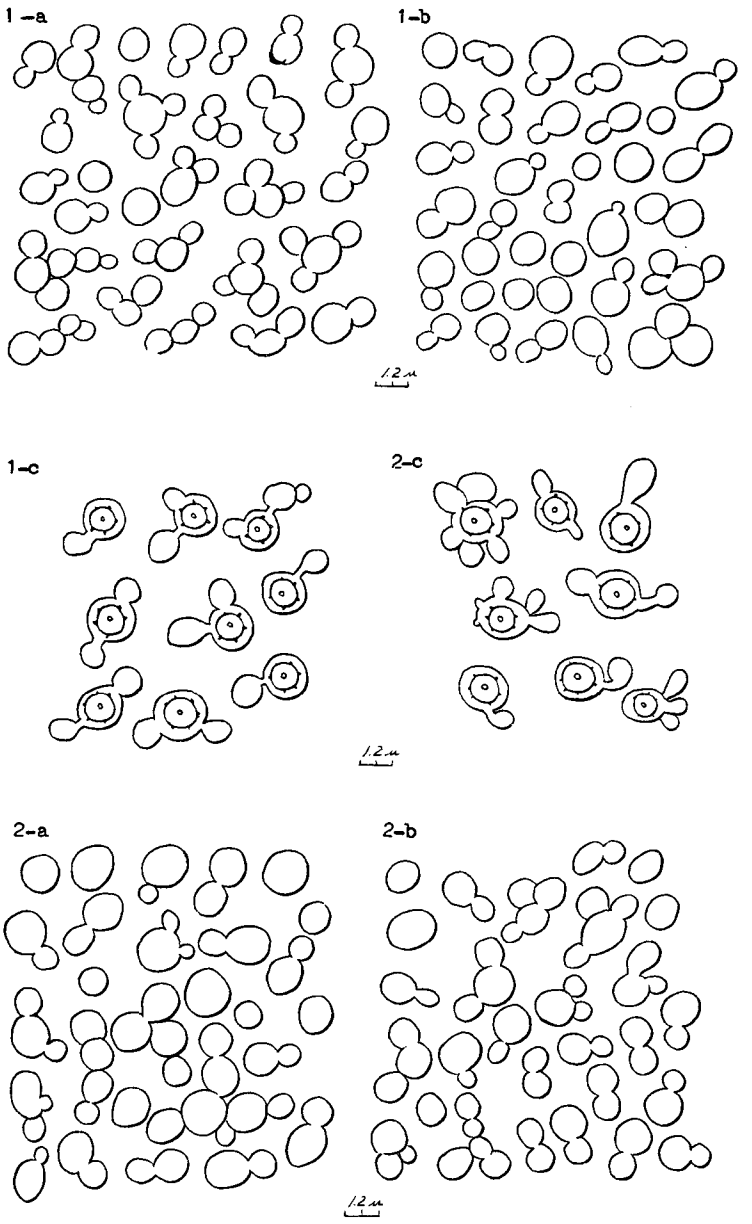

1-a
1-b
12μ
1-c
2-c
12μ
2-a
2-b
12μ

Plate V

1 ····· *Sporobolomyces roseus* KLUYVER et VAN NIEL

2 ····· *Sporobolomyces pararoseus* OLSON et HAMMER

a : After 3 days in malt extract.

b : After 3 days on malt agar.

c : Ballistospores on malt agar.

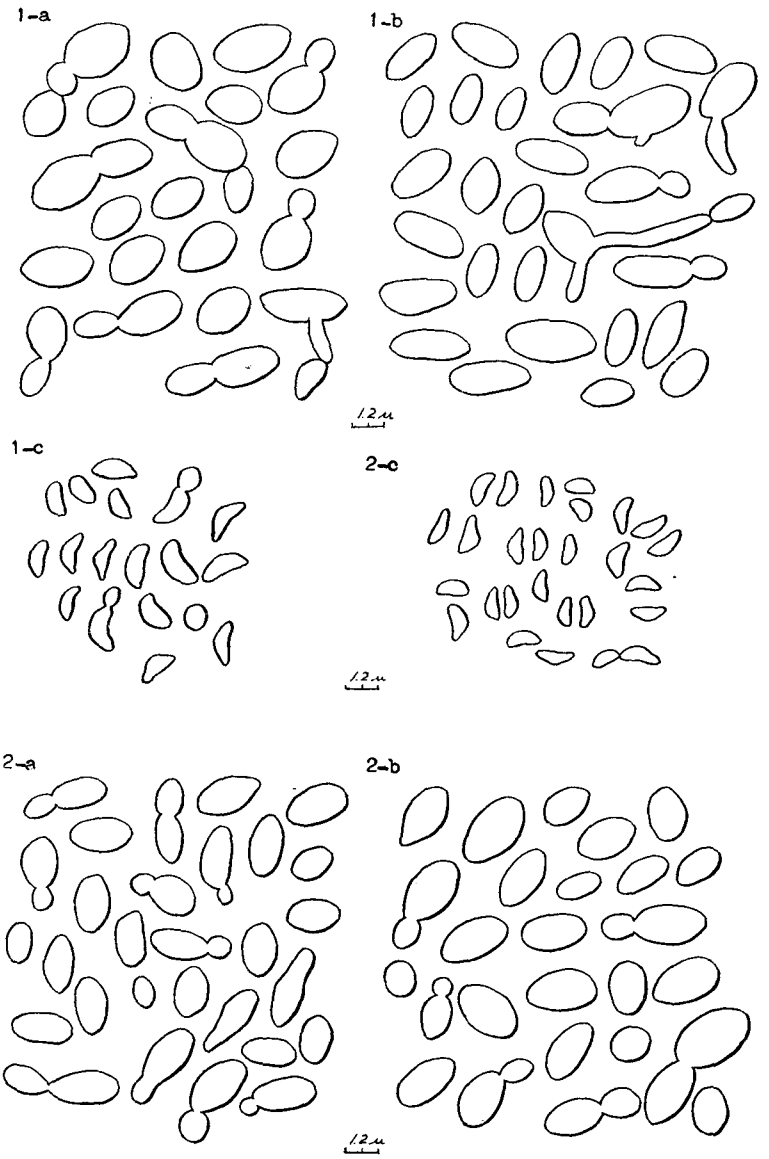

1-a
1-b
12μ
1-c
2-c
12μ
2-a
2-b
12μ

Plate VI

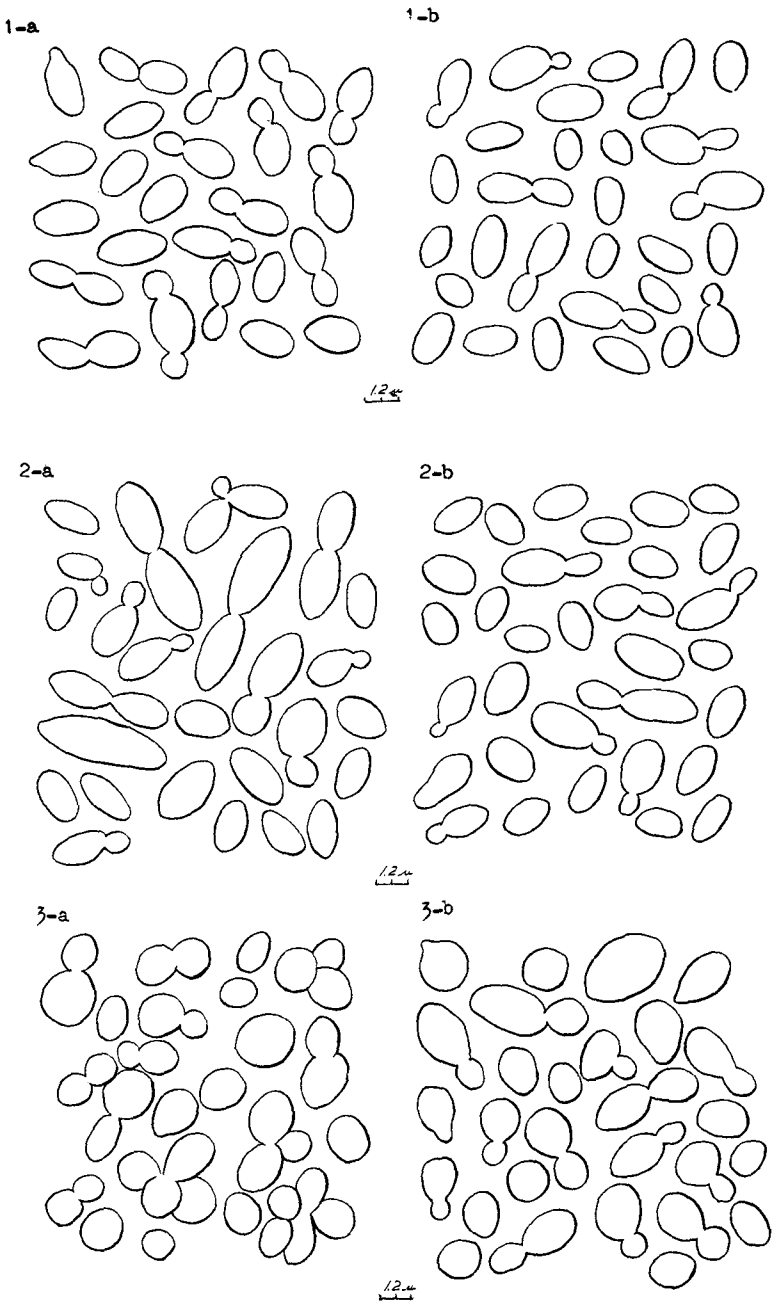
1 ..... *Rhodotorula glutinis* (FRES.) HARRISON

2 ..... *Rhodotorula mucilaginosa* (JÖRGENSEN) HARRISON

3 ..... *Rhodotorula flava* (SAITO) LODDER

a : After 3 days in malt extract.

b : After 3 days on malt agar.


1-a
1-b
12μ
2-a
2-b
12μ
3-a
3-b
12μ