Studies on Yeasts in Slime Fluxes

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Introduction

Since 1957, the present author has performed a taxonomical and ecological reseach on yeasts from a number of trunk exudations (slime fluxes) in Japan and also in Papua & New Guinea. Subsequently, in 1971, he has begun an additional study on yeasts in slime fluxes found in a particular locality of Japan.

It is well known that the slime flux plays one of the important roles in the circulation of yeasts in nature, but it is not clear whether or not these fungi found therein are existent only by chance in such ways as surviving, reproducing and succeeding.

Thus, there are following items remaining unsolved:

(1) Relationships between species of yeasts in slime flux and species of plant from which the slime flux is obtained.

(2) Correlation between the distribution of yeast and the enviromental conditions including latitude, climate, temperature, etc.

(3) What is the major role of the yeasts in the cycle of the biological succession in slime flux, and what is the function of their individual species.
...In fact, yeast appear to execise in a individual slime flux as a stuff of the microflora in nature.

Since the yeasts are never stand alone in nature, behavior and reproduction of them should be considered not on the yeast alone but as one of the communities which are composed of various other organisms. The communities consist of host plant, mold, bacteria, protozoa, algae, insects and higher animals. Since the yeasts are a member of ecosystem, and may act as a primary decomposor in the cycle of the biological succession.

The yeast as a heterotrophic organism can grow well on carbohydrates, such as sugars, alcohols, organic acids and some other simple compounds synthesized by green plants, especially those found in tree sap such as soluble carbohydrates and amino acids. In this respect,slime fluxes are an excellent habitat for the yeasts.

Slime flux is an exudate inhabited by various microorganisms, and it flows from wound or creviceson trunks and stump of trees. Generally, the slime flux is divided into two categories: One is called **natural slime flux** which occurs naturally and the other is called **artificial slime flux** which occurs by artificial damage of trees. There is a slight difference between those two cases. In the former, yeasts continue to take nutrition from the exudate so far as the host plant is alive. In the latter, they can accept their foods only in spring.

Environmental factors are very important in determining the ecological character of an accumulation culture of yeasts growing in the slime flux. Population densities occurring in the accumulation may be contingent depending upon the availability and concentration of organic matters required for growth and reproduction of indigenous yeast flora.

Therefore, the present author investigated the successive flora of the yeasts from the initial to the final stages of slime fluxes.

Slime flux is the only one for the habitate of yeasts, through which one can observe their successive colonization by the naked eye.

Review of The Previous Investigations on Slime flux

Historically, a lot of ecological and taxonomical data have been presented on the slime fluxes. The slime flux has been utilized by yeast taxonomists as one of the first experimental materials.

The first report was published by Ludwig (1886) who isolated *Endomyces magnusii* Ludwig.

Following the above, researches of Hansen (1889), Brefeld (1891), Holtmann (1898), Rose (1910), Nadson and Konokotina (1911, 1912, 1926), Batschinokaia (1914), Saito (1916, 1932), Guilliermond (1919), Chaborski (1919), Kostka (1925), Saito and Ootani (1932), Cauch (1944), Sherwin (1948), Wickerham (1951), Kobayasi (=Kobayashi) (1953), Lund (1954), Shihata and Mrak (1952), Shihata and others (1955), Carson and others (1956), Phaff and Knapp (1956), Soneda (1957, 1964), Kodama and others (1962), Phaff and others (1964) and others have isolated a number of peculiar yeasts from slime fluxes.

Among them, Hansen (1889) examined exudates of *Quercus*, *Ulmus*, *Aesculus* and *Tilia*, and isolated *Saccharomycodes ludwigii*, unidentified *Saccharomyces*, *Pichia membranaefaciens*, *Candida mycoderma*, *Kloeckra apiculata* and some *Torulopsis* spp.. In the taxonomical and cytological reports of Brefeld (1891), that on *Ascoidea* and *Endomyces* were significant. Haltmann (1898) established two new fungal genera, *Conidiascus* and *Oscarbrefeldia*, and described *Ascoidea rubescens* from a slime flux in Java. Rose (1910) isolated *Saccharomyces ludwigii* (=*Saccharomycodes ludwigii*), *Saccharomyces apiculatus* (=*Kloeckera apiculata*), *Endomyces magnusii* and unidentified species of *Torulopsis* from a mucous secretion of *Quercus*. Nadson and Konokotina (1911, 1912, 1926) isolated *Guilliermondia fulvescens* (=*Nadsonia fulvescens*) with *Endomyces magnusii*, *Streptococcus* (*Leuconostoc*) *laherheimii* and *Debaryomyces globosus* from exudates of an oak in the neighborhood of St-Petersburg. Saito (1916, 1932) isolated following yeasts from exudates of various trees: *Endomycopsis monosporus*

(=? *Endomycopsis monospora*) from the exudate of cocconut palm, *Hanseniaspora lindneri* from exudation of tree in Russia, *Saccharomycodes ludwigii* from oak, *Schizosaccharomyces pombe* from cocconut-tree, *Zygopichia chevalieri* var. *fermentati* (=? *Pichia membranaefaciens*) from cocconut-tree, *Zygosaccharomyces chevalieri* (=*Pichia membranaefaciens*) from oak and cocconut-tree and *Zygosaccharomyces paradoxus* (=*Saccharomyces chevalieri*) from birch in Russia. Guilliermond (1919) isolated *Zygosaccharomyces pastori* (=*Pichia pastoris*) from the exudate of chestnut tree in France. Chaborski (1919) isolated some non-identified "Mycoderma" from sap of *Arenga* and *Betula*. Kostka (1925) isolated *Nadsonia richteri* (*Nadsonia elongata*) and several species of *Torulopsis* from exudates of *Uimus*, *Aesculus* and *Carpinus* found near Brunn (Brno). Saito and Ootani (1931) studied on slime fluxes, and they isolated *Endomyces magnusii*, *Schizosaccharomyces japonicus* and *Kloeckera apiculata* from *Quercus acutissima*. Cauch (1944) isolated *Nadsonia fulvescens*, although this strain was identified as *Nadsonia elongata* by other workers, from the sap of a recently cut *Betula nigra* in North Carolina. Sherwin (1948) isolated *Schizosaccharomyces octosporus* from sap of *Acer* and *Nadsonia fulvescens* from sap of *Betula* in same locality of Cauch. Wickerham (1951) isolated some species of *Hansenula* from saps and gums of trees. Kobayashi (Kobayasi) (1953) investigated on yeasts and molds in trunk exudates in Japan. He described twelve species including three new species as follows: *Dipodascus albidus* from *Fagus* (in June), *Cercidiphyllum* (in June) and *Betula* (in May); *Hanseniaspora valbyensis* from *Quercus* (in June); *Saccharomyces rosei* from *Quercus* (in June); *Saccharomyces bailii* from *Kalopanax* (in June); *Saccharomyces chevalieri* from *Alnus* (in June); *Debaryomyces kloeckri* (=*Debaryomyces hansenii*) from *Kalopanax* (in June); *Sporoboromyces salmonicolcr* from *Kalopanax* (in June); *Nectoria hakonensis* Kobayashi from *Picris* (in June); *Fusarium roseum* from various trees (in June); *Endomycopsis fibliger* (*Endomycopsis fibligera*) (imperfect form) from *Alnus* (in June); *Trichosporon merulioides* Kobayashi from *Kalopanax* (in June); *Trichosporon neofermentans* Kobayashi from broad-leaf tree (in June). Lund (1954) observed that yeasts in the majority of the sample from exudates of trees often amount to three million per gram. In his study, only asporogenous yeasts were isolated, viz. three *Torulopsis* and six *Candida* species. He concluded that the exudate may be an excellent habitate for yeasts. Shihata and Mrak (1952) described the presence of *Torulopsis* and *Rhodotorula* species in slime fluxes. Shihata and others (1955) isolated species of *Hansenula*, *Pichia*, *Candida* and *Trichosporon* from *Quercus*; example are *Pichia fluxuum*, *Hansenula angusta*, *Hansenula mrakii* and others. Carson and others (1956) also described that most frequent species in slime flux were *Pichia fluxuum* and *Pichia silvestris*. Phaff and Knapp (1956) isolated *Debaryomyces fluxorum* (=*Pichia fluxuum*) from slime fluxes of *Quercus* and *Abies*. Soneda (1957) isolated *Nadsonia elongata* from pinkish trunk exudation of *Betula japonica* in Japan and discussed taxonomically. After that, Soneda (1964) isolated fourteen species of yeasts from deciduous tree in Mt. Asahi of Japan as follows: *Candida krusei, Candida melinii, Candida rugosa, Cryptococcus albidus, Cryptococcus diffluens, Debaryomyce kloeckeri* (=*Debaryomyces hansenii*), *Dipodascus albidus, Hanseniaspora valbyensis, Kloeckera apiculata, Rhodotorula mucilaginosa* (=*Rhodotorula rubra*), *Saccharomyces chevalieri, Torulopsis famata, Torulopsis pinus, Trichosporon cutaneum*. Kodam and others (1962) isolated *Pichia saitoi* Kodama et al. from exudate of a stump of *Carpinus* in Japan. Phaff and others (1956) studied on the yeast flora of a slime flux from *Ulmus carpinifolia* in California. Population of *Pichia pastoris* attained $18\times10^3-79\times10^4$ viable units per gram dry weight, the highest counts being observed in March. *Trichosporon penicillatum* attained $4\times10^2-17\times10^4$ viable units per gram dry weight, the highest counts being in April and May. They believe that other yeasts occasionally present were probably contaminants brought in by insect and dust.

Methods and Materials

616 samples from Japan and 132 samples from Papua & New Guinea have been collected during the survey on distribution of yeasts and were used for the isolation.

Originally, a direct smearing method was applied to the isolation by using malt extract agar with 0. 001% chloramphenicol for inhibition of bacteria (Soneda 1962, Soneda and Uchida 1971).

Seven trees were chosen at randam as experimental materials for the survey on yeast succession at Nanasawa, at the foot of Mt. Tanzawa (Kanagawa, Japan), during the active flowing period for about sixty days from March to May, 1971. Samples were collected priodically under natural situation and used for the immediate isolation. And tried to find out some possible new relationships between the successive colonization established by yeasts and molds in the naturally developing slime flux on the stumps.

The following procedures were employed for the taxonomical study of yeasts.

Examination under the Microscopie :

The shapes and dimensions of the yeast cells were first examined by microscope for their identification. Culture on malt extract at 20°C for three days was found to be most suitable condition for this purpose.

Characteristics of the Culture :

Malt extract agar (an aqueous of 10% of powdered malt plus 2% of agar) was used. When the cultures were kept at 20°C for a month, characteristics of the streak culture were observed especially on the surface and border of the cultures.

Examination of Pseudomycelium Formation :

Dalmau plate culture on corn meal agar was adopted for the observations of shape and modility of the formation of pseudomycelium branching.

Sporulation :

The ability to produce ascospore is tested on Gorodokowa agar (1% of pepton, 1% of meat extract, 0. 25% of glucose, 0. 5% of NaCl, 2% of agar), carrots agar and oat meal agar. Microscopic examination was begun at five day-culture and continued for twenty days.

Carrot agar (modificated by Soneda):

Extract of 150% (weight per volume) of grated carrots were prepared, brought to a 4% aqueous agar, heated in boiling water bath for 60 minutes, and filtered through two or three layers of gauze. It was sterilized in grass tubes at 120°C for 15 minutes.

Oat meal agar:

Ten grams of commercial oat-meal were extracted with 100 ml of distilled water at around 70°C for 30 minutes and kept at room temperature for 1 hr., then filtered through two or three layers of gauze and made up to 200 ml by addition of distilled water to which 2 grams of agar was also added.

Malt extract:

An aqueous extract of 15% of powdered malt was sterilized by autoclaving.

Fermentation of sugars:

The fermentability of 2% each sugar solutions in a solution of 0.5% dry yeast extract was tested in Durham fermentation-tube. The sugars used were glucose, galactose, saccharose, maltose, lactose and raffinose. Trehalose, melibiose and inulin were tested in some cases.

The tubes were kept at 25°C and observed every day; the final check was made after fifteen days. A concentration of 4% was used exceptionally for raffinose.

Assimilation of carbon compounds:

Important taxonomic value has been attached to the results of assimilation tests.

The present author used 32 carbon compounds according to Wickerham's formula (1951). The tests were carried out the replica method on a solid medium (0.67% of yeast-nitrogen base with 2% of agar) (Plate 6).

Carbon compounds used are as follows:

glucose, galactose, L-sorbose, sucrose, maltose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, D-xylose, L-arabinose, D-ribose, L-rhamnose, ethanol, glycerol, erythritol, adonitol, dulcitol, D-mannitol, D-sorbitol, α-methyl-D-glucoside, salicin, glucono-δ-lacton, DL-lactic acid, succinic acid, citric acid, i-inositol.

Assimilation of nitrates used as nitrogen source:

The characteristic of nitrate assimilation was reproducible so that ability to utilize nitrate by yeasts could be useful for their classification. The auxanographic technique was adopted for nitrate assimilation using yeast-carbon basis method (1.17% of yeast-carbon base with 2% of agar).

Production of extracellular amyloid compounds:

Plate of glucose assimilation test was applied to the test of the formation of amyloid compounds. Extracellular polysaccharide gives a blue color with an iodin solution. After incubation for 15—20 days, the plate were flooded with dilute iodine solution.

Results

(1) Results of taxonomical studies on the yeasts isolated from slime fluxes with a discussion from a ecological viewpoint.

(A) In Japan....(1967)

In the analysis for the distribution of yeast species in various areas of Japan, *Nadsonia elongata*, *Dipodascus* sp., *Trichosporon pullulans*, *Candida sake*, and *Cryptococcus infirmo-miniatus*, *Pichia saitoi* were found to biquitously occur. Among them, *Nadsonia elongata*, *Dipodascus* sp., *Trichosporon pullulans* and *Pichia saitoi* were inherent yeasts in slime flux. In addition, *Saccharomyces chevalieri* and *Pichia pastris* appeared to be relatively common yeasts in slime flux, and this may be supported even by historical surveys. *Saccharomyces kluyveri*, *Candida parapsilosis*, *Kloeckera apiculata*, *Pichia membranaefaciens* and *Saccharomyces cerevisiae* seemed to distribute widely in the world in slime flux. *Metschnikowia pullicherrima* and *Metschnikowia reucauffii* whose habitats associate commonly with necter and insect, were found in slime flux in this survey. *Saccharomyces cerevisiae* seems to be related to host plant, which is *Quercus acutissima* as far as the present experiments are concerned. *Candida lambica* seems to be common in Japan, since Soneda (1960) has isolated eight strains of *Candida fimetaria* (=*Candida lambica*) from dung of animals in Japan.

However, with rare exceptions, there are found distinctively qualitative and quantative differences among the yeasts in all areas of Japan.

On the other hand, there are no clear relationships between species of yeasts and their hosts with a few exceptions. However, distribution and circulation of the yeasts varied depending on the species of the host plants, since season for the exudation from them are different.

Similarly, the yeast flora was affected indirectly by the distribution of host plants, and has some correlations to the environmental conditions such as latitude, climate, temperature etc..

Yeasts appear at first in a slime flux and then polluted fresh exudate, and they tend to form cellular masses spreading uniformly on the whole surface of the trunk of host plant, and always continuously obtain the nutritive substances nessesary for their reproduction in the slime flux. However, it can not be denied that the yeasts may have a way of utilizing those substances through the action of other organisms. It is also possible that the yeasts are affected by the metabolic products of these organisms which diffused into the slime flux. This may be supported by the fact that the natural balance with which the yeasts make a living in the slime flux in aid of molds which dicompose complex substances. Moreover, it should be considered that some successive actions are present between organisms, since the metabolic products of yeasts may beneficially influence the development of numerous fungi and insects in respect to the circulation of substances.

From these considerations, it seems to be reasonable that the climate and the flora and fauna of other organisms strongly influence the composition of the yeast flora in the slime flux.

◎**Nadsonia elongata** Konokotina; Bull. Jard. Imp. Botan. St. Pétersbourg, **13**, 32, 1913; Soneda, Jou. Jap. Bot. **32**, 11, 345, 1957

Host plants and localities:

No. 72, *Betula grossa*, (72 cm), white slime flux, Mt. Kunimi (Kumamoto) 1200 m (above sea level), 22th, April

No. 156, *Alnus pendula*, (25 cm), salmon pink, Mt. Uchimi (Kyoto) 100 m, 3rd, May

No. 158, *Fagus crenata*, (15 cm), white-pink, Mt. Uchimi (Kyoto) 100 m, 3rd, May

No. 246, *Betula tanschii*, (10 cm), gray, Kaida (Kiso) 1600 m, 7th, May

No. 255, *Bet. armoni*, (50 ɔm), white, Kaida (Kiso) 1600 m, 7th, May

No. 301, *Betula* sp., (30 cm), white, Mt. Fuji 750 m, 22th, May

No. 302, *Betula* sp., (26 cm), whitish red, Mt. Fuji 750m, 22 th, May

No. 306, *Betula* sp., (40 cm), foam, Mt. Fuji 750 m, 22th, May

No. 310, *Betula* sp., (20 cm), pink, Mt. Fji 750 m, 22th, May

No. 312, *Prunus* sp., (root), white, Mt. Fuji 750 m, 22th, May

No. 332, *prunus sargentii*, (30 cm), white, Yatani (Yayagata) 27th May

No. 333, *Aesculus turbinata*' (60 cm), white, Yatani (Yamagata) 27th, May

No. 335, *Bet. maximowicziana*, (40 cm), white, Yatani (Yamagata) 27th, May

No. 336, *Bet. maximowicziana*, (50 cm), white, Yatani (Yamagata) 27th, May

No. 337, *Bet. maximowicziana*, (29 cm), white and orange, Yatani (Yamagata), 27th, May

No. 343, *Bet. maximowicziana*, (40 cm), white, Yatani (Yamagata) 27th, May

No. 347, *Bet. maximowicziana*, (32 cm), white, Yatani (Yamagata), 27th, May

No. 396, *Cornus brachypoda*, (35 cm), black, orange-red, Inago (Yamagata), 29th May

Following lists shows, strain number, the host tree (diameter of trunk in cm), color of slime flux, locality and the collected date.

This species seems to be common in Japan. The genus *Nadsonia* was established by Sydow (1912), and the following three species were hitherto described; *N. fulvescens* (Nadonon et Konokotina) Sydow (1911) ; *N. elongata* Konokotina (1913) and *N. richteri* Kostaka (1927). However, *N. richteri* was identified as a synonym of *N. elongata* after taxonomic study by Lodder and Kreger-van Rij (1952) as the differential character, between the two species in using ethanol, described by Stelling, Dekker (1931), could not be maintained.

The genus *Nadsonia* is belonged to Nadsonieae accompanied with *Saccharomycodes* Hansen and *Hanseniaspora* Zikes by Stelling-Dekker in 1931.

Kudriavzev (1951) introduced Saccharomycodaceae for the same range of Nadsonieae treating *Saccharomycodes* as the initial taxon in this group.

Nòvak and Zolt (1961) indicated that *Nadsonia* belongs to the Lipomycetaceae of which Nòvak et Zsolt established as a new family including *Lipomyces* Lodder et Kreger-van Rij, *Pachysolen* Boidin et Azet and *Debaryolipomyces* Ramirez, for the reason that yeasts have same type of sporulation, called 'exozygotic ascus formation' by himself.

Afterward Saccharomycodoideae is proposed by Soneda (1967) for the almost similar reason of the introduction of the Saccharomycodaceae by Kudriavzev.

Saccharomycodoideae includes *Saccharomycodes* Hansen, *Nadsonia* Sydow, *Hanseniaspora* Zikes and *Wickerhamia* Soneda. The vegitative reproduction of those fungi occurrs by bud-fission at the both pole of the fungus cells.

◎**Dipodascus** sp.

No. 10, *Zelkova serrata*, (80 cm), orange-red, Mt. Aoi (Miyazaki) 19th, April
No. 38, *Camellia* sp., orange, Mt. Aoi (Miyazaki), 19th, April
No. 48, *Distylium sacemosum*, (20 cm), Yakobo (Miyazaki), 20th, April
No. 50, *Actinodaphane lanifolia*, (30 cm), white, Yakubo (Miyazaki), 20th, April
No. 51, *Actinodaphane lanifolia*, (15 cm), white-brown, Yakubo (Miyazaki), 20th April
No. 98, *Symplocos myrtacea*, (18 cm), orange-black, Mt. Kunimi (Kumamoto), 22th, April
No. 106, *Stewartia monoderpha*, (root), white, Mt. Kunimi (Kumamoto), 22th, April
No. 126, *Eurya acuminata* var. *montana*, (6 cm), orange-red, Kakinaro (Kochi), 27th, April
No. 247, *Cercidiphylum japonicum*, (65 cm), brown, Kaida (Kiso) 1600 m (above sea level), 7th, May
No. 279, *Prunus* sp., (10 cm), orange-gray, Otaki (Kiso), 10 th, May
No. 291, *Carpinus tschonoskii*, (40 cm), orange-red, Mt. Mitake (Kiso) 1000 m, 10th, May
No. 309, *Betula* sp., gray, Mt. Mitake (Kiso), 10 th, May
No. 339, *Betula maximowicziana*, (40 cm), white, Yatani (Yamagata) 27th, May
No. 341, *Betula maximowicziana*, (254 cm), white-orange, Yatani (Yamagata), 27th, May
No. 346, *Betula maximowicziana*, (50 cm), white, Yatani (Yamagata), 27th, May
No. 373, *Quercus* sp., (27 cm), pale yellow orange, Yatani (Yamagata), 27th, May
No. 377, *Q. crispula*, (30×60 cm), white, black purple, Yatani (Yamagata). 27th, May
No. 385, *Aesculus turbinata*, (40 cm), brown, Hibara (Fukushima), 27th, May
No. 407, *Betula tauschii*, (55 cm), white, Yabukawa (Morioka), 30th, May
No. 504, *Carpinus erosa*, (15 cm), orange-red, Tomakomai (Hokkaido), 5th, June
No. 506, *Carpinus erosa*, (23 cm), orange, Tomakomai (Hokkaido), 5th, June

No. 513, *Acer mono* var. *lupictum*, (15 cm), whitish orange, Tomakomai (Hokkaido), 5th, June

No. 519, *Acer mono* var. *lupictum*, (45 cm), white, Tomakomai (Hokkado), 5th, June

No. 536, *Bet. maximowicziana*, (23 cm), white, Tomakomai (Hokkaido), 5th, June

No. 572, *Tilia japonica*, (root), white, Yamabe (Hokkaido), 10th, June

No. 586, *Bet. maximowicziana*, (root), white, Yamabe (Hokkaido), 10th, June

No. 608, *Ulmus japonica*, (35 cm), white, Yamabe (Hokkaido), 10th, June

The all strains, listed above, show physiological differences from *Dipodascus albidus* Lagerh.

◎**Hanseniaspora valbyensis** Klöcker; Centr. Bakt. Parasitenk. Ab II XXV; Kobayashi Bull. Nat. Sci. Mus. No. 33, 1953

No. 588, *Bet. maximowicziana*, (35 cm), pale white-yellow-orange, Yamabe, 10th, June

No. 589, *Bet. maximowicziana*, (26 cm), pale white-yellow-orange, Yamabe, 10th, June

The two isolates from birch does not form more than two spores in an ascus.

Genus *Hanseniaspora* established by Klöcker (1912) for sporulating apiculate yeast. Three species were described in the genus, which classified by the criteria of the ascospore shape, number of spores in each ascus and assimilative activity of maltose.

◎**Hansenula saturnus** H. et P. Sydow var. **saturnus**;

Ann. Mycol. **17**, 33, 1919

syn. *Saccharomyces saturnus* Klöcker; Zentr. Bakteriol. Parasitenk., II, **35**, 369, 1912

Williopsis saturnus (Kl.) Zender; Bull. Soc. Botan. Geneve **17**, 258, 1925

No. 417, *Vitis kaempheri*, (15 cm), gelatinous, Yabukawa 1000 m (above sea level) (Morioka), 30th, May

No. 441, *Acer mono* var. *eupictum*, (35×40 cm), pale yellow-orange, Mt. Kurosawa 450 m (Morioka), 1st, June

No. 442, *Acer mono* var. *eupictum*, (40×45 cm), orange-red, Mt. Kurosawa, 1st, June

In 1925, Zender created *Williopsis* separating from *Hansenula* because it has saturn-shaped ascospores. In 1960, Kudriavzev also created *Zygowilliopsis* for the species which has saturn-shaped ascospores. Soneda (1967) accepted Zender's consideration and proposed to include *Zygowilliopsis* in *Williopsis*, However, Wickerham (1971) concluded that the both genera should be included in *Hansenula* after phylogenical examinations.

◎**Metschnikowia pullcherrima** Pitt et Miller;

Micologia **60**, 663, 1968

No. 3, *Zelkova serrata*, (40 cm), orange, Mt. Aoi (Miyazaki), 19th, April

No. 333, *Aesuculus turbinata*, (60 cm), white, Yatani, 27th May

◎**Metschnikowia reucaufii** Pitt et Miller;

Micologia **60**, 663, 1968

No. 492, *Actindia arguta*, (7 cm), colorless, Mt. Takamatsu (450 m above sea level) (Morioka), 1st, June

No. 480, *Castanea crenata*, (12 cm), cleam, Tozawa (Akita), 5th, June

No. 481, *Ulmus propingus*, (200 cm), Mt. Moiwa (Sapporo), 7th, June

The genus *Metschnikowia* is established by Kamienski (1899) for *Monospora* Metschnikoff which had already been applied by Hochstetter in 1841 to a genus of algae. In 1913, Genkel rejected the generic name of *Metschnikowia* which had been applied to a genus spongiarians and proposed a new name *Metschnikowiella*. In 1920, Keilin proposed the name *Monosporella* for *Monospora*. Soneda (1967) accepted *Monosporella* Keilin in his monograph. But spongiarians belong to the animal kingdom, therefore, the name *Metschnikowia* was available for fungi when Kamienski created.

Pitt and Miller (1968) described that *Candida pullcherrima* and *Candida reucaufii* could be induced to produce a long cylindrical peduncle, and differentiate into asci under suitable condition, such as low temperature, and one or two needle-shaped ascospores are formed in the asci, so that Pitt and Miller (1968) described *M. pulcherrima* and *M. reucaufii* respectively for perfect from of *Candida pulcherrima* and *Candida reucaufii*.

The isolates, from slime flux shown above, are in good agreement with the original descriptions of the each species by Pitt and Miller (1968).

◎**Pichia membranaefaciens** Hansen;

Zentr. Bakteriol. Parasitenk., Abt. II, **12**, 529, 1904

No. 58, *Quercus acutissima*, (12 cm), white, Kawaminami (Miyazaki), 20th, April

No. 60, *Quercus acutissima*, (20 cm), white, Kawaminami (Miyazaki), 20th, April

No. 305, *Betula* sp., (20 cm), white-pink, Mt. Fuji 750 m (above sea level), 22th, May

No. 335, *Bet. maximowicziana*, (40 cm), white, Yatani (Yamagata), 27th, May

◎**Pichia pastris** (Guilliermond) Phaff;

Ant. van Leeu. **22**, 113, 1956

syn. *Zygosaccharomyces pastori* Guilliermond; Compt. Rend. Soc. Biol. **82**, 466, 1919

No. 405, *Betula tauschii*, (70 cm), pale yellow-orange, Yabukawa (Morioka), 30th, May

No. 538, *Ulmus davidiana* var. *japonica*, (4 cm), white, Tomakomai (Hokkaido), 5th, June

◎**Pichia saitoi** Kodama, Kyono et Kodama;

J. Gen. Appl. Microbiol. **8**, 52, 1962

No. 30, *Distylium racemosum*, orang red, Mt. Aoi, 19th, April

No. 69, *Betula grossa*, (55 cm), Mt. Kunimi (Kumamoto), 22th, April

No. 110, *Sinoarundinaria aurea* var. *henonis*, (4 cm), orange, Kakinaro (Kochi), 27th, April

The genus *Pichia* is establishe by Hansen (1904) for "Kahmhefen". Science then difflerent diagnosis have been adopted on genus *Pichia* and there have been created a number of new species in it.

Boidin and Abadie (1954) proposed a new genus *Petasospora*, which unable to form a pellicle on liquid media, therefore, *Pichia pastris* had ought to be belonged in the new genus. However, Phaff (1956) interpreted a broad sense *Pichia* and he amended the definition of *Pichia* Hansen.

Pichia membranaefaciens, type species of *Pichia*, have been isolated from numerous different sources in the world.

Kodama and others (1952—1955) have written many reports on *Pichia* in Japan and dealt with *Pichia membranaefaciens* and *Pichia mandshurica* taxonomically. They proposed two new varieties named *Pichia membranaefaciens* var. *belogica* and *P. membranaefaciens* var. *mandshrica*, however Yokotsuka and Goto (1955), Ito (1964) and Kreger-van Rij (1970) did not accept those two varieties.

Pichia saitoi was described by Kodama, Kyono and Kodama (1962) who isolated it from slime flux of tree stump of *Carpinus* sp. in Japan. This peculiar yeast produces saturnshaped ascospores, while it resemble to *Pichia membranaefaciens* and *Pichia pastoris* in many physiological respects as follows.

Table 1. Assimilation capacity on carbon compounds of different species of *Pichia*.

	L-Rhamnose,	Glycerol,	Mannitol,	D-Sorbitol
P. membranaefaciens	—	—	—	—
P. saitoi	—	—	+	+
P. pastris	+	+	+	+

◎**Saccharomyces cerevisiae** Hansen;

Medd. Carlsberg Lab., 2, 29, 1883

No. 58, *Quercus acutissima*, (12 cm), white, Kawaminami (Miyazaki). 20th, April

No. 59, *Quercus acutissima*, (20 cm), white, Kawaminami (Miyazaki), 20th, April

No. 60, *Quercus acutissima*, (20 cm), white, Kawaminami (Miyazaki), 20th, April

No. 61, *Quercus acutissima*, (25 cm), white, Kawaminami (Miyazaki), 20th, April

No. 62, *Quercus acutissima*, (12 cm), white, Kawaminami (Miyazaki), 20th, April

◎**Saccharomyces chevalieri** Guilliermond;

Ann. Sci. Nat. Botan. et Biol. végét. XIX, 1, 1914;

Stelling-Dekker, Sporog. Hefen P155, 1931; Kobayashi, Bull. Nat. Sci. Mus. Tokyo, No. 33, 36, 1953

No. 13, *Zelkova serrata*, (1 cm), orange, black, Mt. Aoi (Miyazaki), 17th, April

No. 15, *Zelkova serrata*, (75 cm), pink, black, Mt. Aoi (Miyazaki), 17th. April

No. 60, *Quercus acutissima*, (20 cm), white, Kawaminami (Miyazaki), 20th, April
No. 145, *Sinearundinaria pubescens*, (10 cm), orange, white, Kamikamo (Kyoto), 2nd, May
No. 572, *Ulmus davidiana*, (30 cm), pink, Tomakomai (Hokkaido), 5th, June

◎**Saccharomyces florentianus** (Castelli) Lodder at Kreger-van Rij; The yeasts 213, 1952
syn. *Zygosaccharomyces florentianus* Castelli; Arch. Microbiol., **9**, 449, 1938
No. 12, *Zelkova serrata*, (1 cm), orange, Mt. Aoi, (Miyazaki) 19th, April
No. 343, *Betula maximowicziana*, (40 cm), white, Yatani (Yamagata), 27th, May
No. 344, *Betula maximowicziana*, (40 cm), white, Yatani (Yamagata), 27th, May
No. 343, *Prunus sargentii*, (80 cm), red, Yatani (Yamagata), 27th, May
No. 345, *Prunus sargentii*, (30 cm), white, Yatani (Yamagata), 27th, May

◎**Saccharomyces kluyveri** Phaff, Miller et Shifrine; Ant. van Leeu. **22**, 145, 1956
No. 11, *Zelkova serrata*, (40 cm), which orange, Mt. Aoi, (Miyazaki) 19th, April
No. 429, *Actnidia arguta*, (3 cm), white, orange-red, Mt. Takamatsu (Morioka), 1st, June
No. *Cornus controversa*, (25×30 cm), white, Mt. Kurosawa (Morioka), 1st, June
No. 446, *Pterocarya rhoifolia*, (15×20 cm), orange-red, Mt. Kurosawa (Morioka), 1st, June
No. 448, *Acer mono* var. *eupictum*, (35×40 cm), orange-red, Mt. Kurosawa (Morioka), 1st, June
No. 608, *Ulmus japonica*, (35 cm), white, Yamabe (Hokkaido), 10th, June

The generic name of *Saccharomyces* was introduced by Meyen (1838), afterward Reess (1870) defined the genus and described seven species. Lodder and Kreger-van Rij (1952) revised the diaginosis of the genus *Saccharomyces* based on the results of advanced genetics. Consequently *Zygosaccharomyces* Barker and *Toulaspora* Lindner were included *Saccharomyces* (Meyen) Reess.

On the contrary, Kudriavzev (1954, 1960) retained *Zygosaccharomyces*, and Novak and Zsolt (1961) reinstated *Torulaspora* and introduced another new genus *Zymodebaryomyces* for certain species which have been assigned to *Saccharomyces* by Lodder and Kreger-van Rij (1952). Capriotti (1958, abc) argued for the retention of *Torulaspora* because it is distinct taxon from either *Saccharomyces* or *Zygosaccharomyces.* Soneda (1957) also retained *Torulaspora* emphasizing manner of their sporulation and fermentation in his former report, however in the present paper. he does not accept generic name *Torulaspora*. Because, there is no real difference between *Torulaspora* and *Zygosaccharomyces* on account of cytological observations.

◎**Candida lambica** (Lindner et Genoud) van Uden et Buckely;
The Yeasts, 986, 1970
syn. *Mycoderma lambica* Lindner et Genoud; Woch. Brau. **30**, 363, 1913:
Candida fimetaria Soneda; Nagaoa, 6, 6, 1959

No. 107, *Sinoarundinaria aurea* var. *henonis*, (2 cm), red, Kakinaro (Kochi), 27th, April
No. 110, *Sinoarundinaria aurea* var. *henonis*, (4 cm), orange, Kakinaro (Kochi), 27th, April
No. 117, *Sinoarundinaria aurea* var. *henonis*, (3 cm), orange, Kakinaro (Kochi), 27th, April
No. 135, *Shiia sieboldi*, white-orange, Asakura (Kochi), 27th, April
No. 155, *Eurya acumi*, (7×3 cm), Iwakura (Kyoto), 2nd, May
No. 270, *Carpinus tschonoskii*, (15 cm), orange-black, Mt. Ontake (Kiso), 10th, May
No. 275, *Betula tauschii*, (10 cm), white, Shinkai (Kiso), 10th, May
No. 290, *Carpinus tschonoskii*, (30 cm), orange red, Mt. Mitake, 10th, May
No. 311, *Betula* sp., (21×27 cm), white-pink, Mt. Fuji (750 m above sea level), 22th, May
No. 315. *Betula* sp., (12 cm), pale yellow-pink, Mt. Fuji, 22th, May
No. 468, *Meliosma myriantha*, (25 cm), clear flux, Tozawa (Akita), 5th, June

◎**Condida maritima** (Siepmann) van Uden et Buckley;
The Yeasts, 1000, 1970
syn. *Trichosporon maritima* Siepmann; Veröff. f. Meeresforschung Bremerhaven, 8, 79, 1962

No. 27, *Taxus cupidata*, brown, Mt. Aoi, (Miyazaki) 19th, April
No. 66, unknown tree, black, Mt. Kunimi (Kumamoto), 22th, April
No. 78, *Betula grossa*, orange-red, Mt. Kunimi (Kumamoto), 22th, April
No. 247, *Cercidiphyllum japonicum*, (65 cm), brown, Kaida V. (Kiso), 7th, May
No. 371, *Bet. maximowicziana*, (75 cm), white-pink, Yatani (Yamagata), 27th, May
No. 385, *Carpinus tschonoshii*, (50 cm), white-yellow-red, Hibara (Fukushima), 10th, May

◎**Candida mesenterica** (Geiger) Diddens et Lodder;
Die anaskosporogenen Hefen, II Hälft, Amsterdam, 199, 1942

No. 78, *Betula grossa*, orange-red, Mt. Kunimi (Kumamoto), 22th, April
No. 218, *Alnus japonica*, (30 cm), orange, Shinkai (Kiso), 7th, May
No. 238, *Alnus Japonica*, (25 cm), orange, Shinkai (Kiso), 7th, May
No. 244, *Betula grossa*, (50 cm), brown, Kaida (Kiso), 7th, May
No. 248, *Perocarya rhoifolia*, (30 cm), orange, Kaida (Kiso), 7th, May
No. 283, *Carpinus tschonoskii*, (50 cm), white-yellow-red, Mt. Ontake, (Kiso), 10th, May

No. 285, *Hydrangea paniculata* var. *floribunda*, yellow-gray, Mt. Ontake, 10th, May

◎**Candiba parapsilosis** (Ashford) Langeron et Talice;
Ann. paras. hum. comp., **10**, 1: Soneda; Nagaoa, 6, 10, 1959
syn. *Monilia parapsilosis* Ashford; Am. J. Trop. Med., 8, 507, 1928
No. 301, *Betula* sp. (30 cm), white, Mt. Fuji (750 m above sea level), 22th, May
No. *Bet. maximowicziana*, (41 cm), white-orange, Yatani (Yamagata), 27th, May
No. 397, *Cornus controversa*, (20 cm), white, Inago (Morioka), 29th, May

◎**Candida sake** (Saito et Oda) van Uden et Buckely;
The Yeasts, 1034, 1970
syn. *Eutorulopsis sake* Saito et Oda; J. Brewery Sci., **12**, 159, 1934
No. 40, *Parthenocissus thunbergii*, (4 cm), Mt. Aoi, (Miyazaki) 19th, April
No. 68, *Betula grossa*, (55 cm), Mt. Kunimi (Kumamoto), 22th, April
No. 70, *Betula grossa*, (?), Mt. Kunimi (Kumamoto), 22th, April
No. 72, *Betnla grossa*, (72 cm), Mt. Kunimi (Kumamoto), 22th, April
No. 78, *Betula grossa*, (?), orange-red, Mt. Kunimi (Kumamoto), 22th, April
No. 116, *Sinoarundinaria aurea* var. *heninis*, (3 cm), red, Kakinaro (Kochi), 27th, April
No. 172, *Magnolia obovata*, (80 cm), Mt. Tate, 6th, May
No. 204, *Alnus japonica*, (30 cm), orange, Shinkai (Kiso), 7th, May
No. 212, *Alnus japonica*, (30 cm), orange, Shinkai (Kiso), 7th, May
No. 222, *Alnus japonica*, (35 cm), orange, Shinkai (Kiso), 7th, May
No. 224, *Alnus japonica*, (20 cm), orange, Shinkai (Kiso), 7th, May
No. 226, *Alnus japonica*, (26 cm), orange, Shinkai (Kiso), 7th, May
No. 232, *Alnus japonica*, (25 cm), yellow white, Shinkai (Kiso), 7th, May
No. 234, *Alnus japonica*, (35 cm), red, Shinkai (Kiso), 7th, May
No. 244, *Betula grossa*, (50 cm), brown, Kaida V. (Kiso), 7th,
No. 254, *Betula tauschii*, (10 cm), gray, Kaida V. (Kiso), 7th, May
No. 256, *Cercidiphyllum japonicum*, (15 cm), brown, Kaida V. (Kiso), 7th, May
No. 268, *Betula tauschii*, (25 cm), brown, Kaida V. (Kiso), 7th, May
No. 302, *Betula* sp., (26 cm), whitish-red, Mt. Fuji (750 m above sea level), 22th, May
No. 306, *Betula* sp., (40 cm), foam, Mt. Fuji (750 m), 22th, May
No. 307, *Betula* sp., (24×25 cm), pale yellow-orange, Mt. Fuji (750 m), 22th, May
No. 309, *Betula* sp., (?), gray, Mt. Fuji (750 m), 22th, May
No. 312, *Prunus* sp., (root), white, Mt. Fuji (750 m), 22th, May
No. 314, *Betula* sp., (27 cm), white, Mt. Fuji (750 m), 22th, May
No. 326, *Betula* sp., (?), pale yellow-brown, Mt. Fuji (750 m), 22th, May
No. 339, *Betula maximowicziana*, (41 cm), white, orange, Yatani (Yamagata), 27th, May
No. 391, *Betula ermani*, (28 cm), orange white, Yatani (Yamagata), 27th, May

No. 418, *Betula tauschii*, (48 cm), red, Yabukawa (Morioka), 30th, May
No. 424, *Betula tauschii*, (15 cm), white, orange-red, Yabukawa (Morioka), 30th, May
No. 478, *Aralia elata*, (?), gum, Tozawa (Akita), 5th, June
No. 554, *Ulmus davidiana* var. *japonica*, (8 cm), white, Nopporo (Hokkaido), 8th, June
No. 572, *Tilia japonica*, (root), Yamabe (Hokkaido), 10th, June
No. 580, *Betula maximowicziana*, (root), white, Yamabe (Hokkaido), 10th, June
No. 586, *Betula maximowicziana*, (root), white, Yamabe (Hokkaido), 10th, June
No. 588, *Betula maximowicziana*, (26 cm), pale-white, yellow-orange, Yamabe (Hokkaido), 10th, June
No. 616, *Acer* sp., (23 cm), orange-green, Yamabe (Hokkaido), 10th, June

In 1923, Berkhaut established the genus *Candida* for the yeast-like fungi hitherto included in the genus *Monilia*.

In the current monograph of van Uden and Buckely, eighty-one of species are accepted in the genus *Candida*.

The present author isolated five species in Japan.

◎**Cryptococcus albidus** (Saito) Skinner var. **albidus;**
Henrici's Mould, Yeasts and Actinomycetes, 2nd ed., 286 1947
No. 410, *Betula tauschii*, (30 cm), brown, Yabukawa (Morioka), 30th, May
syn. *Torula albida* Saito; Japan J. Bot., 1, 1, 1922
No. 5, *Zelkova serrata*, (40 cm), white, Mt. Aoi (Miyazaki), 19th, April
No. 102, *Stewartia monadelpha*, (root), white, Kunimi (Kumamoto), 22th, April
No. 129, *Calethra barbinervis*, (?), black, Asakura (Kochi), 27th, May
No. 353, *Betula maximowicziana*, (20 cm), gray, Yatani (Yamagata), 22th, May
No. 420, *Ostrya japonica*, (23 cm), colorless, Yabukawa (Morioka), 30th, May

◎**Cryptococcus infirmo-miniatus** (Okunuki) Phaff et Fell;
The Yeasts, 1113, 1970
syn. *Torula infirmo-miniata* Okunuki; Japan J. Bot., **5**, 285 1931
No. 25, *Zelkova serrata*, (?), pink, Mt. Aoi (Miyazaki), 19th, April
No. 84, *Styrax japonica*, (10 cm), orange, black spot, Mt. Kunimi (Kumamoto), 22th April
No. 102, *Stewartia monadelpha*, (?), white, orange, Mt. Kunimi (Kumamoto), 22th, April
No. 268, *Betula tauschii*, (25 cm), brown, Kaida V. (Kiso), 7th, May
No. 304, *Betula* sp., (23 cm), pale yellow-orange, Mt. Fuji (750 m), 22th, May
No. 309, *Betula* sp.. (23 cm), gray, orange, Mt. Fuji (750 m), 22th, May
No. 347, *Betula maximowicziana*, (32 cm), white, Yatani (Yamagata), 10th, June
No. 572, *Tilia japonica*, (root), white. Yamabe. (Hokkaido) 10th, June
No. 573, *Tilia japonica*, (38 cm), white, Yamabe, (Hokkaido) 10th, June

◎**Cryptococcus laurentii** (Kufferath) Skinner var. **laurentii;**
Henrici's Mould. Yeasts and Actinomycetes, 2 nd Ed., 286, 1947
syn. *Torula laurentii* Kufferath; Ann. Bull. Sci. Méd.
Nat. Bruxelles, *74*, 16. 1920

No. 4, *Zelkova serrata*, (25 cm), orange, Mt. Aoi, (Miyazaki) 19th, April
No. 16, *Zelkova serrata*, (root), orange, Mt. Aoi, 19th, April
No. 20, *Zelkova serrata*, (40 cm), black, Mt. Aoi, 19th, April
No. 32, *Ficus beecheyana*, (?), orange, Mt. Aoi, 19th, April
No. 50, *Actinodaphne lancifolia*, (30 cm), white, Yakubo (Miyazaki), 20th, April
No. 86, *Styrux japonica*, (15 cm), Mt. Kunimi, 22th, April
No. 128, *Celethra barbinervia*, black, Asakura (Kochi), 27th, April
No. 129, *Celethra barbinervia*, (6 cm), orange, Kakinaro, (Kochi) 27th, April
No. 130, *Styrax japonica*, (2 cm), orange, Kakinaro, 27th, April
No. 132, *Ficus erecta*, (3 cm), orange, Kakinaro, 27th, April
No. 206, *Acer* sp., (3 cm), black, Shinkai (Kiso), 7th, May
No. 208, *Pterocarya rhoifolia*, (5 cm), white, Shinkai (Kiso), 7th, May
No. 280, *Prunus* sp., (10 cm), gray-black, Otaki (Kiso), 10th, May
No. 305, *Betula* sp., (20 cm), white-pink, Mt. Fuji (750 m above sea level), 22th, May
No. 363, *Betula maximowicziana*, (47 cm), orange-red, Yatani (Yamagata), 27th, May
No. 378, *Alnus tinctoria* var. *obtusiloba*, (50 cm), pale-yellow, orange, Hibara (Fukushima), 27th, May
No. 383, *Aesculus turbinata*, (40 cm), brown, Yatani, (Yamagata) 27th, May
No. 387, *Quercus erispula*, (45 cm), black, Yatani, 27th, May
No. 420, *Ostrya japonica*, (23 cm), colorless, Yabukawa (Morioka), 30th, May
No. 422, *Hamamelis japonica*, (3 cm), orange-red, Yabukawa (Morioka), 30th. May
No. 491, *Alnus hirsuta* var. *siblica*, (?), gum, Mt. Moiwa (Sapporo), 7th, June
No. 493, *Acer mono* var. eupictum, (?), salmon-pink, Mt. Moiwa (Sapporo), 7th, June

◎**Cryptococcus macerans** (Frederiksen) Paff et Fell;
Mycologia **60,** 663. 1968
syn. *Rhodotorula macerans* Frederiksen; Fresia, **5,** 234, 1956

No. 139, *Ilex integra* var. *ellipsoidea*, (10 cm), black, Kagami (Kochi), 28 th, April
No. 497, *Betula maximowicziana*, (5 cm), orange, Tomakomai (Hokkaido), 5 th, June

In 1833, Kützing established genus *Cryptococcus* with latin diagnoses, and Vuillermin (1901) emended the definition of the genus and limited it to anascosporogenous parasitic yeasts. He gave the name, *Cryptococcus hominis* Vuillemin, to the parasitic and nonfermenting yeast isolated by Busse (1894). The yeast must be identical with a yeast previously named *Saccharomyces neoformans* Sanfelice (1895) according to the nomenclature code.

Hasegawa and others (1960) explained strictly Vuillermin's emendation; therefore they included in the genus only *Cryptococcus neoformans*, in which carotenoid pigment is not detected.

Phaff and Spencer (1969) proposed to include in *Cryptococcus* those species which are able to assimilate inositol as the sole source of carbon and to consist capsular polysaccharide. Both properties, inositol assimilation and capsule formation, correlate well except one or two species. Accordingly Phaff and Fell (1970) give an emendation, to add the above two properties, for the diaginosis of the genus *Cryptococcus* Kützing, and the yeasts that synthesize carotenoid pigment, such as *Cryptococcus infirmo-miniatus* and *Cryptococcus macerans* are included in the genus.

As it is difficult to classify the genus *Cryptococcus* on the viewpoint of morphological properties, it becomes to accept the the classification on the viewpoint of physiological properties such as assimilative activity of carbon compounds.

◎**Rhodotorula glutinus** (Fresenius) Harrison var. **glutinus**;

Trans. Roy. Soc. Can., V, **22**, 187, 1928

syn. *Cryptococcus glutinus*, Fresenius; Beitr. Mycol., **2**, 77, 1850–'63 (1852)

No. 20, *Zelkova serrata*, (25 cm), orange, Mt. Aoi (Miyazaki), 19th, April
No. 74, *Betula grossa*, Mt. Munimi (Kumamoto), 22th, April
No. 181, *Carpinus tschonoshii*, (10 cm), orange, Shinkai (Kiso), 9th, May
No. 198, *Pterocarya rhoifolia*, (22 cm), orange,, Shinkai, 9th, May
No· 200, *Pt. rhoifolia*, (20 cm), orange, Shinkai, 9th, May
No. 201, *Pt. rhoifolia*, (20 cm), orange, Shinkai, 9th, May
No. 213, *Alnus japonica*, (20 cm), orange, Shinkai, 9th, May
No. 214, *Alnus japonica*, (20 cm), gellow-white, Shinkai, 9th May
No. 219, *Alnus japonica*, (20 cm), white, Shinkai, 9th, May
No. 223, *Alnus japonica*, (20 cm), orange, Shinkai, 9th, May
No. 234, *Alnus japonica*, (35 cm), red, Shinkai, 9th, May
No. 243, *Pterocarya rhoifolia*, (11 cm), orange, Kaida (Kiso), 9th, May
No. 244, *Betula grossa*, (50 cm), brown, Kaida, 9th, May
No. 292, *Betula* sp., (20 cm), pink, Mt. Fuji (1500 m), 22th, May
No. 293, *Betula* sp., (25 cm), pink, Mt. Fuji (1500 m), 22th, May
No. 299, *Betula* sp., (15×25 cm), white, Mt. Fuji (750 m), 22th, May
No. 305, *Betula* sp., (20 cm), white-pink, Mt. Fuji (750 m), 22th, May
No. 307, *Betula* sp., (24×25 cm), pale yellow-orange, Mt. Fuji, 22th, May
No. 311, *Betula* sp., (21×27 cm), white-pink, Mt. Fuji (750 m), 22 th, May
No. 314, *Betula* sp., (27 cm), white, Mt. Fuji (750), 22th, May
No. 330, *Prunus sargentii*, (30 cm), white, Yatani (Yamagata), 22 th, May
No. 341, *Bet. maximowicziana*. (25.4 cm), white-orange, Yatani, 27th, May
No. 343, *Bet. maximowicziana*. (40 cm), white, Yatani, 27th, May
No. 359, *Prunus sargentii*, (31 cm), pale orange, Yatani, 27th, May

No. 366, *Bet. maximowicziana.* (28 cm), orange, Yatani, 27th, May
No. 373, *Quercus* sp., (27 cm), beigue, Hibara (Fukushima), 28th, May
No. 375, *Pterocarya rhoifolia,* (30 cm), beigue, Hibara, 28th, May
No. 385, *Magnoria* sp., (50 cm), white-yellow-red, Hibara, 28th, May
No. 391, *Betula ermani,* (28 cm), orange-white, Yatani, 28th, May
No. 397, *Cornus controversa,* (20 cm), white. Inago (Morioka), 29th, May
No. 405, *Betula tauschii,* (70 cm), pale yellow-orange, Yabukawa (Morioka), 30th May
No. 429, *Actinidia arguta,* (3 cm), white-orange, Mt. Kurosawa (Iwate), 1st. June
No. 443, *Betula tauschii,* (45 cm), orange-red, Mt. Kurosawa, 1st, June
No. 588, *Betula* sp., (35 cm), yellow-white, Yamabe (Hokkaido), 10th, June
No. 600, *Ostrya* sp., (10 cm), orange, Yamabe, 12th, June
No. 604, *Actinidia* sp., (10 cm), Yamaba, 12th, June
No. 607, *Magnolia ofovata* (7 cm), beigue, 12 th, June
No. 610, *Ulmus japonica* (65 cm), white, Yamabe, 12 th, June

◎**Rhodotorula rubra** (Demme) Lodder; Die anaskosporogenen Hefen, I Hälfte, Verhandel, Koninkel, Akad. Netenschap. Afd. Natuurkunde, sect. II, **32**, 1, 1934
syn. *Saccharomyces ruber* Demme; Ann. micrographie, 1889
Rhodotorula mucilaginosa (Jörg) Harrison; Trans. Roy. Soc. Can. V, **22**, 187, 1928
No. 388, *Quercus* sp., (30 cm), Beigue, Yatani (Yamagata), 27 th, May
No. 404, *Cornus controversa,* (20 cm), Beigue, Takizawa (Morioka), 30 th, May
No. 430, *Quercus* sp., (45×50 cm), white-orange, Mt. Takamatsu, 1 st, June

Genus *Rhodotorula* was created by Harrison (1928) for the asporogenous, red pigment-forming yeasts. Hasegawa and others (1960) divided two Sub-genera, *Rubrotorula* and *Flavotorula* for *Rhodotorula* in which carotenoid pigment is detected strictly. Phaff and Spencer (1969) proposed to exclude off those species which are able to assimilate inositol as the sole souce of carbon and to consist capsular polysaccharide from *Rhodotorula.* Phaff and Fell (1970) classified *Rhodotorula* and *Cryptococcus* by the criteria of Phaff and Spencer.

◎**Kloeckera apiculata** (Reess emend. Klöcker) Janke;
Centr. Bakt. II, 76, 161, 1928: Soneda; Nagaoa, 6, 15, 1969
No. 8, *Zelkova serrata,* (80 cm), white-orange, Mt. Aoi, (Miyazaki) 19th, April
No. 10, *Zelkova serrata,* (80 cm), orange, Mt. Aoi, 19th, April
No. 144, *Sinoarundinaria rubescens,* (8 cm), pink, Kamikamo (Kyoto), 2nd, May
No. 349, *Aeculus turbinata,* (25 cm), orange-red, Yatani (Yamagata), 27th, May
No. 516, *Acer mono* var. *eupictum,* (28 cm), white, Tomakomai (Hokkaido), 5th, June
No. 517, *Carpinus erosa,* (22 cm), gray, pale yellow-orange, Tomakomai, 5th, June

The genus *Kloeckera* is established by Janke (1928) for *Kloeckeria* Janke (1923). *Kloeckera apiculata* has been isolated from different sources by a number of researchers up to date. Soneda (1959) isolated four strains of the species from animal dung in Japan, and he described the morphological property of them that consists primitive branching cells on slide culture of potato agar.

◎**Torulopsis inconspicua** Lodder et Kreger-van Rij;
The yeasts 436, 1952: Soneda; Nagaoa, 6, 13, 1959
No. 26, *Taxus cuspidata*, brown, Mt. Aoi (Miyazaki), 17th, April
No. 27, *Taxus cuspidata*, brown, Mt. Aoi (Kumamoto), 17th, April
No. 78, *Betula grossa*, orange-red, Mt. Kunimi, 20th, April

The genus *Torulopsis* was introduced by Berlese (1895) and accepted by Lodder (1934), Skinner (1950) and Lodder and Kregervan Rij (1952).

◎**Trichosporon pullulans** (Lindner) Diddens et Lodder;
Die anaskosporogenen Hefen, II Hälfte, 410, 1942
syn. *Oidium pullulans* Lindner; Mikroskopische Betriebskontolle in den Gärungsgewerben mit einer Einfübrung in die Hefeneinkuetur. Infektionslehre und Hefenkunde (Ed. I) Berlin 1895.
No. 34, *Distylium racemosum*, Mt. Aoi (Miyazaki), 19th, April
No. 39, *Camellia japonica*, Mt. Aoi, 19th, April
No. 44, *Sakaki ochnacea*, orange-red, Mt. Aoi, 19th, April
No. 46, *Sakaki ochnacea*, orange-red, Mt. Aoi, 19th, April
No. 72, *Betula grossa*, (77 cm), white, Mt. Kunimi (Kumamoto), 22th, April
No. 75, *Betula grossa*, (50 cm), fresh pale red, Mt. Kunimi, 20th, April
No. 78, *Betula grossa*, orange-red, Mt. Kunimi, 20th, April
No. 81, *Betula grossa*, (45 cm), orange, Mt. Kunimi, 20th, April
No. 84, *Styrex japonica*, (10 cm), black spot, Mt. Kunimi, 20th, April
No. 92, *Cornus controversa*, (15 cm), orange, Mt. Kunimi, 20th, April
No. 97, *Stewartia monadelpha*, (root), white, Mt. Kunimi, 20th, April
No. 100, *Stewartia monadelpha*, (45 cm), orange, Mt. Kunimi, 20th, April
No. 102, *Stewartia monadelpha*, (18 cm), orange, Mt. Kuuimi, 20th, April
No. 103, *Stewartia monadelpha*, (root), white, Mt. Kunimi, 20th, April
No. 104, *Stewartia monadelpha*, (18 cm), orange, Mt. Kunimi, 20th, April
No. 106, *Stewartia monadelpha*, (root), orange, Mt. Kunimi, 20th, April
No. 126, *Eurya acuminata* var. *montana*, (6 cm), orange-red, Kakinaro (Kochi), 27th, April
No. 143, *Sinoarundinaria rubescens*, (10 cm), white Kamikamo (Kyoto), 2nd, May
No. 155, *Eurya acuminata* var. *montana*, (7×3 cm), Iwakura (Kyoto), 2nd, May
No. 156, *Alnus pendula*, (25 cm), salmon pink, Mt. Uchimi (Kyoto), 2nd, May
No. 158, *Fagus crenata*, (15 cm), white-pink, Mt. Uchimi (Kyoto), 2nd, May
No. 176, *Alnus japonica*, (28 cm), orange, Shinkai (Kiso), 9th, May

No. 178, *Bet, tauschii,* (20 cm), pink, Shinkai (Kiso), 9th, May
No. 180, *Carpinus tschonoskii,* (5 cm), red, orange, Shinkai (Kiso), 9th, May
No. 182, *Carpinus tschonoskii,* (6 cm), orange, pink, Shinkai (Kiso), 9th, May
No. 184, *Alnus japonica,* white pink, Shinkai (Kiso), 9th, May
No. 187, *Alnus japonica,* orange, Shinkai (Kiso), 9th, May
No. 188, *Betula tauschii,* (45 cm), orange, white, Shinkai (Kiso), 9th, May
No. 190, *Betula tauschii,* (45 cm), white, Shinkai (Kiso), 9th, May
No. 195, *Magnolia oborata,* (20 cm), black, Shinkai, 9th, May
No. 198, *Pterocarya rhoifolia,* (22 cm), orange, Shinkai, 9th, May
No. 217, *Quercus valiabilis,* (20 cm), gray, Shinkai, 9th, May
No. 219, *Alnus japonica,* (20 cm), white, Shinkai, 9th, May
No. 223, *Alnus japonica,* (20 cm), orange, Shinkai, 9th, May
No. 237, *Alnus japonica,* (18 cm), orange, Shinkai, 9th, May
No. 291, *Carpinus tschonoskii,* (40 cm), orange-red, Mt. Mitake (Kiso), 10th, May
No. 294, *Rhododendron meeternichit,* (4 cm), pink, Mt. Fuji (1900 m above sea level), 22th, May
No. 297, *Betula* sp., (20 cm), white, Mt. Fuji (750 m above sea level), 22th, May
No. 330, *Prunus sargentii,* (30 cm), white, Yatani (Yamagata), 27th, May
No. 331, *Prunus sargentii,* (12 cm), Yatani, 27th, May
No. 337, *Bet, maximowicziana,* (29 cm), white-orange, Yatani, 27th, May
No. 338, *Bet. maximowicziana,* (30 cm), white, Yatani, 27th, May
No. 339, *Bet. maximowicziana,* (41 cm), white-orange, Yatani, 27th, May
No. 340, *Prunus sargentii,* (80 cm), red, Yatani, 27th, May
No. 344, *Bet. maximowicziana,* (40 cm), white, Yatani, 27th, May
No. 348, *Bet. maximowicziana,* (28.5 cm), white-orange, Yatani, 27th, May
No. 349, *Aesculus turbinata,* (25 cm), orange-red, Yatani, 27th, May
No. 377, *Quercus crispula,* (30×60 cm), white, black purple, Yatani, 27th, May
No. 403, *Cornus controversa,* (20 cm), pale yellow-orange, Takisawa V. 220 m (above sea level) (Morioka), 29th, May
No. 410, *Bet. tauschii,* (30 cm), brown, Yabukawa, 30th, May
No. 412, *Ostrya japonica,* (20 cm), pale yellow-orange, Yabukawa, 30th, May
No. 414, *Cornus controversa,* (10 cm), orange, Yabukawa, 30th, May
No. 422, *Hamamelis japonica,* (3 cm), orange-red, Yabukawa, 30th, May
No. 428, *Actnidia arguta,* (7 cm), colorless, Mt. Takamatsu (Morioka) (450 m above sea level), 1st, June
No. 572, *Tilia japonica,* (root), white, Yamabe 500 m (above sea level) (Hokkaido), 10th, June

The genus *Trichosporon* is established by Behrend (1890). *Trichosporon pullulans* seems to be a mold which has true-mycelium and arthrospores on solid media as *Geotrichum, Endomyces* and *Dipodascus.* However, the surface of the culture of the species on solid media becomes white to yellowish white, glossy and to soft texture, and it consists budding cells, like the genus *Trichosporon* which is included in the category of the yeasts.

◎**Prototheca zopfii** Krüger;

Hedwigia **33,** 241—266

No. 6, *Zelkova serrata,* (80 cm), white-orange, Mt. Aoi (Miyazaki), 19th, April

No. 481, *Ulmus propingua,* (200 cm), Mt. Moiwa (Sapporo), 7th, June

The genus *Prototheca* was established by Krüger (1894) and the characteristic morphological features of the genus are relatively clear-cut and include the presence of thick walled large cell containing endospores. Six species have been hitherto described.

Ciferri (1957) investigated genus *Prototheca* and described four species and one variety in the genus.

Tubaki and Soneda (1959) gave a study on morphology and physiology of *Prototheca* and recognized the following five species as valid taxons: *Prototheca zopfii* Krüger, *Prototheca moniliformis* Krüger, *Prototheca trispora* (Ash. Cif. et Dalm.) Cif., Montem. et O. Cif., *Prototheca ciferrii* Negroni et Blastin, *Prototheca wickerhamii* Tubaki et Soneda. The present isolates are identical *Prototheca zopfii* in their monograph.

(B) Slime flux yeasts collected in Papua & New Guinea...... **(1969—1970)**

The author isolated a number of yeasts from slime fluxes collected in Papua & New Guinea between 29 th, December 1969 and 4 th, February 1970.

In the tropical district, microorganisms develop very actively and decompose organic substances in a short period. During the very short period yeasts play as one of members of the cycle of biological succession in slime flux.

The result shows that there is not similarity between the floral analysis of Japan and those of Papua & New Guinea.

Seventeen species of yeasts were isolated from the natural slime fluxes of various tropical woody plants, such as mango tree, teak, tree fern and kasuarina.

The author ascertained that *Pichia membranaefaciens, Candida guilliermondii, Candida membranaefaciens, Rhodotorula glutines, Rhodotorula graminis* and *Rhodotorula rubra* are widely distributed in the present investigation. But no *Cryptococcus* nor *Trichosporon,* that produces starch-like compounds, could be observed, in the whole collection in the tropical district. Three new yeasts, *Pichia rabaulensis* Soneda et Uchida, *Candida buinensis* Soneda et Uchida and *Schizoblastsporion kobayasii* Soneda et Uchida were isolated severally there.

Those species mentioned above are indigenous to the areas of high temperature climate.

◎**Pichia rabaulensis** Soneda et Uchida;

Bull. Nat. Sci. Mus. Tokyo **14,** 448, 1971

Growth in malt extract: After 3 days at 25°C, cells short ovoid, long-ovoid to elongate, (3.2—5.5) × (3.4—8.8) μ, single, in pairs or in short chains, formed a little sediment and a thin creeping wrinkled pellicle. After one month at 20°C, present a heavy sediment and a grayish white, thin, creeping and delicately wrinkled pellicle.

Growth on malt agar: After one month at 20°C, the streak culture of the first strain (No. 28—1) colored yellowish white to cream, semi-glossy, soft, somewhat wrinkled, the second strain (No. 111—3) colored cream to brownish white, dull, somewhat tough and wrinkled over surface.

Sporulation: No conjugation immediately preceding ascus formation. Two to four hat-shaped ascospores are contained in an ascus. They are easily liberated from the ascus.

Dalmau plate culture on corn meal agar: Generally an abundant development of thin branched pseudomycelium and blastospores occurred. The small long-ovoid blastospores develop in to small chains.

Fermentation:

Glucose	+	Maltose	−
Galactose	−	Lactose	−
Sucrose	+	Raffinose	+

Assimilation of carbon compounds:

Glucose	+	D-Ribose	−
Galactose	+	L-Rhamnose	+
L-Sorbose	−	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erithritol	+ or −
Cellobiose	+ (weak) or L	Adonitol	+ (weak)
Trehalose	+ weak or −	Dulcitol	−
Lactose	−	D-Mannitol	+
Melibiose	−	D-Sorbitol	+
Raffinose	+	α-Methyl-d-Glucoside	+
Melezitose	+ (weak)	Salicin	+ (weak)
Inulin	−	DL-Lactic acid	+ (weak)
Soluble starch	−	Succinic acid	+ (weak)
D-Xylose	+	Citric acid	+ (weak)
L-Arabinose	+	Inositol	−
D-Arabinose	−		

Assimilation of potassium nitrate: Absent

Maximum temperature for growth: 45°C—43°C

Hab: No. 28—1, feces of African snail collected in Rabaul (31 th. Dec. 1969)

No. 111—3, natural slime flux of *Mangifera indica* collected in Rabaul (19 th, Jan. 1970)

This specimen closely resembles *Pichia ohmeri* (Etchells et Bell) Kreger van Rij (1970).

Table 2.

	Pichia rabaulensis	*Pichia ohmeri*
Galactose fermentation	+	+, weak or slow
Assimilation of xylose	+	—
Assimilation of rhamnose	+	—
Assimilation of melezitose	+ (weak)	—

◎**Pichia membranaefaciens** Hansen;

Medd. Calsberg Lab., **2,** 220, 1888

No. 118—1, *Kasuarina equisetifolia,* (18 cm), brownish, Goroka (New Guinea), 21 th, Jan.

◎**Candida buinensis** Soneda et Uchida;

Bull. Nat. Sci. Mus. Tokyo, **14,** 448, 1971

Growth in malt extract: After 3 days at 25°C, cells small, mostly short-ovoid, (2.0—3.6) × (2.5—4.0) μ, single or in pairs, formed a sediment. After one month at 20°C, present a moderate ring and a heavy sediment.

Growth on malt agar: The streak culture, after one month at 20°C, colored yellowish white to cream, semi-glossy, soft and delicately wrinkled in central part of streak.

Dalmau plate on potato agar: Pseudomycelium either very primitive consisting of branched chains of cylindrical cells, or displaying long pseudomycelial cells bearing spindle-shaped blastospores.

Fermentation: Absent

Assimilation of carbon compounds:

Glucose	+	D-Ribose	—
Galactose	+	L-Rhamnose	—
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	—
Maltose	+	Erythritol	—
Cellobiose	+	Adnitol	—
Trehalose	+	Dulcitol	—
Lactose	—	D-Mannitol	—
Melibiose	—	D-Sorbitol	—
Raffinose	—	α-Methyl-D-Gulcoside	+
Melezitose	+	Salicin	—
Inulin	—	DL-Lactic acid	—
Soluble starch	—	Succinic acid	—
D-Xylose	+	Citric acid	+

L-Arabinose	+	Inositol	−
D-Arabinose	+		

Assimilation of potassium nitrate: Absent
Maximum temperature of growth: 27—28°C
Hab: No. 65—A, slimy material of tree fern in Buin

This species somewhat similar to *Candida sake* differing mainly in fermentability of glucose and in assimilative activities of D-mannitol, D-sorbitol and salicin.

◎**Candida diddensii** (Phaff, Mrak et Williams) Fell et Meyer;
Mycopath. Mycol. Appl., **32,** 117, 1967
syn. *Trichosporon diddensii* Phaff, Mrak et Williams;
Mycologia, **44,** 431, 1952

No. 55—A, *Artocarpus* sp., (40 cm), tan, Rabaul, 3rd, Jan.
No. 62—2, tree fern, (15 cm), brown (gelatinous materials), Buin, 7th, Jan.
No. 68—1, unidentified tree, (35 cm), black, Buin, 8th, Jan.
No. 96—1, *Mangifera indica,* (120 cm), brown, Rabaul, 16th, Jan.
No. 97—3, *Mangifera indica,* (150 cm), black, Rabaul, 16th, Jan.
No. 133—1—3, *Mangifera indica,* (80 cm), black, sea side of Rabaul, 28th, Jan.

Among the seven strains, there are little differences to each others but no determinative discrepancy has been recognized between the original description of the species and those strains.

◎**Candida freyschussii** Buckley et van Uden;
Mycopath. Mycol. Appl., **36,** 256, 1968

No. 24—2, unidentified tree, (50 cm), greenish brown and black, 31th, Dec.

There are good agreements between the properties of the strain and the original description of *Candida freyschussii,* except assimilative activities of DL-lactic acid and citric acid.

◎**Candida guilliermondii** (Castellani) Langeron et Guerra var. **guilliermondii;**
Ann. parasitol. humaine et Comparèe, **16.** 136, 1938
syn. *Endomyces guilliermondii* Castellani; Brit., Med., J., **2,** 1208, 1912

No. 26—2, unidentified tree, (20 cm), grayish brown, Rabaul, 11th, Dec.
No. 137—1, *Mangifera indica,* (100 cm), colorless, Rabaul, 31th, Dec.
No. 138—2—1, *Mangifera indica,* (20 cm). white, Rabaul, 1st, Dec.

◎**Candida intermedium** (Ciferri et Ashford) Langeron et Guerra;
Ann. Parasitol. humaine et Comparèe, **16,** 36, 1938
syn. *Blastodendrion intermedium* Ciferri et Ashford;
Puerto Rico J. Pub. Health Trop. Med., **5,** 91, 1929

No. 8—2, *Musa paradisiaca,* gelatinous material, Rabaul, 28th, Dec.

◎**Candida melinii** Diddens et Lodder (imperfect stage of *Hansenula·wingii* Wickerham);

Die anaskosporogenen Hefen, II Hälfte, A'dam, 1942

No. 5—1, New Guinea Palm tree, (150 cm), Rabaul, 29th, Dec.

Ascospores of the strain could not be found on usual sporulating media, and the strain does not assimilate L-rhamnose for carbon source, in spite of van Uden and Buckley (1970) described the assimilative activity of L-rhamnose of the type strain.

◎**Candida membranaefaciens** (Lodder et Kreger-van Rij) Wickerham et Burton:

J. Bacteriol., **68,** 594, 1954

syn. *Candid melibiosi* Lodder et Kreger-van Rij var. *membranaefaciens* Lodder et Kreger-van Rij; The Yeasts 583, 1952

No. 65—1, unidentified tree, (25 cm), tan, Buin, 7th, Jan.

◎**Candida parapsilosis** (Ashford) Langeron et Talice;

Ann. parasitol. humaine et Comparèe, **10,** 1, 1932

syn. *Monilia parapsilosis* Ashf.; Am. J. Trop. Med., 8, 507, 1928

No. 19—1, *Mangifera indica,* (40 cm), redish brown, Rabaul, 31 th, Dec.

No. 52—1, *Tectona grandis,* (25 cm), brownish, Rabaul, 3rd, Jan.

◎**Candida sorbosa** Hedrick et Burk ex. van Uden et Buckley;

The Yeasts, 1052, 1970

No. 115—2—1, *Kasuarina equisetifolia,* (35 cm), black, Goroka, 21th, Jan.

◎**Candida tenuis** Diddens et Lodder;

Die anaskosporogenen Hefen, II Hälfte, 376, 1942

No. 140—1—2, *Mangifera indica,* (6 cm), white (within many insect labai), Rabaul, 1st, Feb.

This strain differs from the type strain in certain points of which described by van Uden and Burkley (1970).

	No. 140—1—2	type strain
Assimilation of D-ribose	—	+
Assimilation of salicin	—	+

◎**Rhodotorula glutinis** (Fres.) Harrison var. **glutinis;**

Trans. Roy. Soc. Canada, **V,** 22; 187, 1928

syn. *Cryptococcus glutinis* Fres.; Beitr. Myc., **2,** 77, 1852

No. 138—1—2, *Mangifera indica,* (20 cm), white, Rabaul, 1st, Feb.

◎**Rhodotorula graminis** di Mena;

J. Gen. Microbiol., **18,** 269, 1958

No. 27—1, unidentified tree, (35 cm), white moldy, Rabaul, 31 th, Dec.

No. 140—1—1, *Magnifera indica,* (6 cm), slime flux with many insect labai, 1st, Feb.

◎**Rhodotorula rubra** (Dumme) Lodder;

Die anaskosporogenen Hefen, I Hälfte, Verhandel Kon. Akad. Wetenschap., Afd. Natuurkunde, Sect II, **32,** 1, 1934

syn. *Saccharomyces ruber* Dumme; Ann. Micrographie, 1889

No. 26—2—1, *Mangifera indica,* (20 cm), grayish brown, Rabaul, 31 th, Dec.

No. 138—2—2, *Mangifera indica,* (20 cm), white, Rabaul, 31 th, Dec.

◎**Schizoblastosporion kobayasii** Soneda et Uchida;

Bull. Nat. Sci. Mus. Tokyo **14,** 456, 1971

Growth in malt extract: After 3 days at 25°C, the cells ovoidal to elongate ovoidal or lemon-shaped, (4.8—10.0) × (7.5—18.0) μ, single or in pairs, or in short chains. Budding in both pole on a broad base. A septum formed across the constriction, separating bud and mother cell. The two cells separated by fission, sometimes nonpolar buds occur. A thin creeping pellicle and a sediment are formed. After one month at 20°C, thin pellicle and moderate sediment are present.

Growth in malt agar: After one month at 20°C, the streak culture colored yellowish white to cream, soft, semi-glossy, the center of streak somewhat rised.

Dalmau plate culture on corn meal agar: Primitive pseudomycelium consisted of short chains of cells.

Fermentation: Completely absent

Assimilation of carbon compounds:

Glucose	+	D-Ribose	−
Galactose	+	L-Rhamnose	−
L-Sorbose	−	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	−	Erythritol	−
Cellobiose	−	Adnitol	−
Trehalose	−	Dulcitol	−
Lactose	−	D-Mannitol	−
Melibiose	−	D-Sorbitol	−
Raffinose	−	α-Methyl-D-Glucoside	−
Melizitose	−	Salicin	−
Inulin	−	DL-Lactic acid	+ weak
Soluble starch	−	Succinic acid	−
D-Xylose	−	Citric acid	−

L-Arabinose	−	Inositol	−
D-Arabinose	−		

Assimilation of potassium nitrate: Absent

Maximum temperature of growth: 42—45°C

Hab: No. 115—2—2, *Kasuarina equisetifolia*, (35 cm), tanish, Goroka, 16 th, Jan.

Morphological properties: Cells reproduced by bipolar budding on broad base, ascospores not formed.

Fermentation: Absent

Because of the properties mentioned above, this strain is identified with the genus *Schizoblastosporion*, which is established by Ciferri (1930), for the strain isolated from soil in North America by Starkey and Henrici.

In bipolar budding, *Schizoblastosporion* resembles *Kloeckera*. However there is a difference between those genera in fermentability, absent in the former and present in the latter. The present author could find no ascosporogenous apiculate yeast which is unable to ferment.

Table 3. Specific differences in the characteristics of *Schizoblastosporion*

	S. starkey-henricii	*S. kobayasii*
Cell size	(2.5—6.8) × (4.5—20) μ	(4.8—10.0) × (7.5—18.0) μ
Pellicle	Negative	Positive
Fermentation	Absent	Absent
Assimilation of galactose	−	+
Assimilation of sucrose	−	+
Assimilation of cellobiose	−	+
Assimilation of succinic acid	+	−
Growth at 37°C	−	+

◎**Torulopsis inconspicua** Lodder et Kreger-van Rij;

The Yeasts, 436, 1952

No. 82—2, unidentified tree, (20 cm), tan, Namatanai (New Ireland I.), 11 th, Jan.

No. 90—1, *Compesderma* sp., (120 cm), brown, Rabaul, 14 th, Jan.

(2) **Successivc yeast flora in slime fluxes** (Table 6)

Successive yeast flora in slime fluxes on tree stumps has been considered to be related to decomposition of organic matters from trees. The present study on the yeast flora in the slime fluxes was carried out on the stumps of three species of trees which had been prepared beforehand.

The yeasts grew vigorously on the surface of the tree stumps acompanied with the molds which have similar physiological activities, therefore the slime fluxes changed their color variously.

Generally, inherent and transient yeasts grow together in a stump, while occasionally only the inherent yeast predominate in natural slime fluxes.

The soil yeasts such as, *Cryptococcus albidus* var. *albidus* and *Rhodotorula glutinis*, are found from the slime fluxes. Those yeasts can survive long period even under severe conditions. Therefore, most of them probably have been transported from a slime flux to others by insects, wind, splash, etc.

On the other hand, *Nadsonia elongata, Trichosporon pullulans* and *Cryptococcus macerans*, had been recorded frequently in slime fluxes in the author's survey in Japan. They have just appeared through the biological succession in his course of isolating procedure. Of course, it seems that the yeasts came to a new exudate from other trees in the neighborhood on which a slime flux occurred. The process of colony-formation of any yeasts in slime flux on tree trunks must be very similar in biochemical function, because the most species of yeasts are able to develop a colony on every ordinary exudates.

The interaction between yeasts and molds, living together in a slime flux, is not only the problem of competition in the tree exudate, although it must be one of very important factors.

Mucor hiemalis and *Aureobasidium pullulans* forms colonies with some yeasts. After them, *Fusarium* sp. (Nectoria-type) and *Cladosporium herbarum*, which can grow in common stumps of trees, appear as the next colonists decomposing wooden tissue by their extracellular enzymes.

There are some differences in the periods of developing of the yeasts through the biological succession. Slime fluxes of the first and the second trees of *Acer mono* var. *eupictum* were not typical being colorless and liquid-type, because the environmental condition for the maple tree was unfavourable. Never-the-less, yeasts and molds were found in both fluxes.

Pestalozia sp. and *Cladosporium herbarum* were collected like yeasts as a primary decomposor in a slime flux.

The writer observed comparatively well developed floras in the trees, numbered from Fourth to Seventh. *Aureobasidium pullulans, Cryptococcus laurentii* var. *laurentii*, *Mucor hiemalis* and *Cryptococcus macerans* seem to be dominant species in each trees during each stages. Those dominant species dominate the respective flora of the slime fluxes even it contains many other resident organisms. And the growth of dominant species may control the "niche" of flora according to seasonal change of temperature.

Judging from the facts described above, the present author dares to conclude that there is a yeast community, even in each stages of the slime fluxes.

The information of the distribution of yeasts in nature may vary with cultural methods isolating from original substrates that harbors yeasts. The present result indicates a drift of the yeast in slime fluxes by their distribution and succession.

(3) Additional data of the Yeasts in Slime Fluxes.

In this work, twenty-five samples were examined in the spring of 1958. The trees of their habitat were *Betula* (7), *Fagus* (4), *Ulmus* (2), *Quercus* (2), *Prunus* (1), *Acer* (1), *Castanea* (1) and *Pterocarya* (1). They are collected in mountainous districts of Tokyo, Saitama, Nagano, Yamagata and Miyagi in Japan by Soneda and Dr. Tubaki. Table fourth shows eighteen species of yeasts and eight species of molds isolated from the above samples. Among them, seven species; viz. *Endomycopsis fibuligera*, *Saccharomyces chevalieri*, *Pichia membranaefaciens*, *Debaryomyces kloeckeri*, *Rhodotorula glutinis*, *Trichosporon pullulans* and *Candida krusei* are common in slime fluxes, four species; viz. *Pichia farinosa*, *Candida humicola*, *Candida solanii* and *Torulopsis candida* are found widely from other plant sources. And this result shows that the survey was treated good chance can be obtained various yeasts on field survey with passage of year.

Table 4. Fungi Isolated from Slime Fluxes

Endomycopsis fibuligera (Lindner) Dekker

1 strain; *Bet. platyphylla* var. *japonica*, Okutama (Tokyo)

Saccharomyces chevalieri Guill.

2 strains; *Ul. laciniata*, Gaga SPA; *Bet. ermani* var. *cmmunis*, Mt. Ootaki (Nagano)

Hansenula anomala (Hansen) H. et P. Sydow var. *schneggi* (Weber) Dekker

6 strains; *Q. crispula*, Gaga SPA (Miyagi); *Fagus crenata* Gaga SPA: *Betula* sp., Mt. Asahi; *Ul. lacinata*, Mt. Asahi (Yamagata); *Ul. lacinata*, Kamikochi (Nagano): *Betula* sp., Kamikochi

Hansenula minuta Wickerham

1 strain; *Pr. pubescens*, Mt. Asahi (Yamagata)

Pichia membranaefaciens Hansen

2 strains; *Betula* sp., Mt. Asahi (2)

Pichia farinosa (Lindner) Hansen

1 strain; *Castanea crenata*, Okutama (Tokyo)

Debaryomyces kloeckeri Guill. et Peju

1 strains; *Q. crispula*, Okutama

Torulopsis dattila (Kluver) Lodder

7 strains; *Pt. rhoifolia*, Kamikochi; *Betula* sp., Kamikochi; *Ul. laciniata*, Kamikochi; *Bet. platyphylla*, Okutama; *Bet. ermani* var. *communis*, Mt. Yake (Nagano); *Betula* sp., Mt. Asahi (2)

Torulopsis candida (Saito) Lodder

2 strains; *Fagus crenata*, Gaga SPA; *Cast. crenata*, Okutama

Torulopsis versatilis (Et. et B.) Lodder et Kreger-van Rij

1 strain; *Cast. crenata*, Okutama

Torulopsis holmii (Jörg.) Lodder

1 strain; *Pt. rhoifolia*, Kamikochi

Candida krusei (Cast.) Berkhaut

3 strains; *Fagus crenata*, Gaga SPA (2); *Betula* sp., Mt. Asahi

Candida scottii Diddens et Lodder

1 strain; *Fagus crenata*, Gaga SPA

Candida humicola (Dasz.) Diddens et Lodder

1 strain; *Acer mono* var. *eupictum*, Okuchichibu

Candida solanii Lodder et Kreger-van Rij

1 strain; *Fagus crenata*, Gaga SPA

Rhodotorula glutinis (Fres.) Harrison

1 strain; *Fagus crenata*, Gaga SPA

Cryptococcus flavus (Saito) Phaff et Fell

1 strain; *Fagus crenata*, Mt. Asahi

Trichosporon pullulans (Lindner) Diddens et Lodder

3 strains; *Fagus crenata*, *Q. crispula*, *Acer mono* var. *eupictum*, Mt. Asahi

— * —

Aspergillus versicolor (Vuill.) Tiraboschi

1 strain; *Betula* sp., Mt. Asahi

Aspergillus flavus Link

1 strain; *Fagus crenata*, Gaga SPA

Aspergillus niger v. Tieghem

1 strain; *Ul. laciniata*, Kamikochi

Penicillium funiculosum Thom

1 strain; Fagus crenata, Mt. Asahi

Fusarium spp. (=Nectria ?)

3 strains; *Pt. rhoifolia*, Kamikochi (2); *Betula* sp., Mt. Asahi

Cladosporium herbarum Link

1 strain; *Fagus crenata*, Gaga SPA

Cephalosporium acremonium Corda

2 strains; *Q. crispula*, Okutama; *Pt. rhoifolia*, Kamikochi

Aureobasidium pullulans (De Bary) Arnaud

3 strains; *Q. crispula*, Gaga SPA (2); *Q. crispula*, Okutama

Mortierella pusilla Oud.

2 strains; *Cast. crenata*, Okutama; *Acer mono* var. *eupictum*, Okuchichibu

Table 5 shows also the result of the field work in Yamabe, Hokkaido. Thisteen species of yeasts and three unidentified organisms were isolated from thirty samples of slime fluxes of *Tilia japonica*.

Among them, *Entoamoeba* sp. was isolated after Soneda's procedure and *Chlorella* sp. were isolated from the slime fluxs by ordinary planting method.

Soneda (1962) observed symbiotic phenomena of *Entoamoeba* sp. with different yeasts, and he discussed about biological society among the two members or three members.

Chlorella sp., sometimes it is heterotrophic organism as far as we know, isolated easily as same as *Prototheca zopfii* from slime fluxes. This isolate sometimes producer and sometimes consumer in slime flux as far as tested, so that *Chlorella* sp. may be expected to play an important role on the circulation of substances in nature.

Table 5. Yeast Isolated from Slime Fluxes Yamabe, 1966

Hansenula anomala (Hansen) H. et P. Sydow var. *anomala*; 3 strains
Pichia farinosa (Lindner) Hansen; 2 strains
Pichia membranaefaciens Hansen; 3 strains
Candida catenulata Diddens et Lodder; 1 strain
Candida parapsilosis (Ashford) Langeron et Talice; 2 strains
Cryptococcus albidus (Saito) Skinner var. *diffluens* (Zach) Phaff et Fell; 2 strains
Cryptococcus laurentii (Kuff.) Skinner var. *laurentii*; 4 strains
Kloeckera corticis (Klöcker) Janke; 1 strain
Rhodotorula glutinis (Fres.) Harrison var. *glutinis*; 5 strains
Rhodotorula rubra (Dumme) Lodder; 3 strains
Torulopsis candida (Saito) Lodder; 2 strains
Torulopsis pinus Lodder et Kreger-van Rij; 1 strain
Geotrichum candidum Link ex Persoon emend. Carmichael; 2 strains
Fusarium sp.; 3 strains
Entamoeba sp.; 2 strains
Chlorella sp.; 3 strains

Summary

(1) During recent fourteen years the author obtained 413 strains of yeasts from Japan, Papua and New Guinea. After laboratorial examinations, fifty-eight species belonging to fifteen genera has been identified among the 413 strains.

Debaryomyces: D. kloeckeri
Endomycopsis: E. fibuligera
Hanseniaspora: H. valbyensis
Hansenula: H. anomala var. *anomala. H. anomala* var. *schneggii.*
H. minuta. H. saturnus
Metschnikowia: M. pulcherrima. M. reukaufii
Nadsonia: N. elongata
Pichia: P. farinosa. P. membranaefaciens. P. pastoris P. rabaulensis. P. saitoi
Saccharomyces: S. cerevisiae. S. chevalieri. S. florentianus. S. kluyveri

Candida: *C. catenulata.* *C. buinensis.* *C. diddensii.* *C. freyschussii*
C. guilliermondii var. *guilliermondii.* *C. humicola.* *C. intermedia*
C. krusei. *C. lambica.* *C. maritima.* *C. melinii.* *C. membranaefaciens*
C. mesenterica. *C. parapsilosis.* *C. sake.* *C. scottii.* *C. solanii*
C. sorbosa. *C. tenuis*

Cryptococcus: *C. albidus* var. *albidus.* *C. albidus* var. *diffluens.* *C. flavus*
C. infirmo-miniatus. *C. laurentii* var. *laurentii.* *C. macerans*

Kloeckera: *K. apiculata.* *K. corticis*

Rhodotorula: *R. glutinis.* *R. graminis.* *R. rubra*

Schizoblastosporion: *S. kobayasii*

Torulopsis: *T. candida.* *T. dattila.* *T. holmii.* *T. inconspicua.* *T. versatilis*

Trichosporon: *T. cutaneum.* *T. pullulans*

Geotrichum: *G. candidum*

Prototheca: *P. zopfii.*

(*Dipodascus* sp.)

(2) There are five genera and seventeen species of yeasts found in Papua & New Guinea and fourteen genera and fourty-seven species in Japan.

(3) *Dipodascus* sp., *Nadsonia elongata*, *Trichosporon pullulans*, *Candida sake* and *Cryptococcus infirmo-miniatus* were found to be ubiquitous yeast of slime fluxes in Japan.

(4) Distribution and circulation of the yeasts vary with kinds of trees, because the exudation seasons of the species of trees are different with each other.

(5) Common exudate is able to be colonized by majority of the yeast species, and inherent and transient yeasts are mixed in slime fluxes.

(6) The dominant species dominate the respective flora of the slime fluxes containing many other resident organisms and the growth of dominant species may control the niche of flora by seasoned change of temperature.

(7) *Entoamoeba* sp. which have symbiotic activities with different yeasts is found in slime fluxes.

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Table 6

Occurrence of yeasts and mold on different conditions in slime fluxes.

Tree No. 1: *Acer mono* var. *eupictum*

Species \ Date	13th, March	24th, March	29th, March	7th, April
Pestalotia sp.	△		△	
Torulopsis inconspicua		△		

Tree No. 2: *Acer mono* var. *eupitum*

Species \ Date	13th, March	24th, March	29th, March
Aureobasidium pullulans		○	
Cryptococcus macerans		○	
Cladosporium herbarum		△	

Tree No. 3: *Cornus controversa*

Species \ Date	13th, March	24th, March	29th, March	7th, April	15th, April	23rd, April	1st, May
Aureobasidium pullulans			○		⊙	⊙	⊙
Crypt. macerans			○	⊙			
Torul. inconspicua				⊙			
Mucor heimalis				⊙		◎	
Rhodotorula glutinis					⊙	◎	◎
Cryptococcus sp.					⊙		
Clad. herbarum					○	◎	◎

△ 1000 > colonies per 1 gr.
○ 1001—5000
● 5001—6000
◎ 6001—20000
⊙ 20001 <

Tree No. 4: *Cornus controversa*

Species \ Date	13th, March	24th, March	29th, March	7th, April	15th, April	23rd, April	1st, May
Trichosporon pullulans		●					
Aureobasidium pullulans		●	●	●	—	○	
Clad. herbarum		△					
M. heimalis		△	—	●	●	⊙	⊙
Nadsonia elongata			◎	●			
Crypt. laurentii v. *laurentii*				○	⊙	—	○
Crypt. albidus v. *albidus*					⊙	○	○
Rhod. glutinis					○		
Rhod. rubra						○	

Tree No. 5: *Cornus controversa*

Species \ Date	13th, March	24th, March	29th, March	7th, April	15th, April	23rd, April	1st, May
Crpt. laurentii v. *laurentii*	●	⊙	⊙	⊙	⊙	○	
M. heimalis	○	△	○	○	○		
Bacteria	⊙						
Crypt. albidus v. *albidus*		⊙	—	—	—	—	●
Nad. elongata	●	—	—	●			
Rhod. glutinis					○		
Cepharosporium sp.					○	◎	
Crypt. macerans							△
Rhodotorula sp.							△
Cryptococcus sp.					⊙		

Tree No. 6: *Cornus controversa*

Species \ Date	13th, March	24th, March	29th, March	7th, April	15th, April	23rd, April	1st, May
Aureobasidium pullulans	●						
Crypt. laurentii v. *laureniit*	●	⊙	⊙	⊙	⊙	●	●
Trichosporon pullulans	●						
Rhod. glutinis	○						
Clad. herbarum	△						
Fusarium sp. (Nectoria-type)		△				○	●
Prototheca zopfii		○					
Cryptococcus macerans			⊙				
Rhodotorula sp.				⊙			
Pichia membranaefaciens					○		

Tree No. 7 *Carpinus tschonoskii*

Species \ Date	13th, March	24th, March	29th, March	7th, April	15th, April	23rd, April	1st, May
Crypt. macerans			○	○	△	○	
Fusarium sp. (Nectoria-type)			○				
Crypt. laurentii v. *laurentii*				⊙	○	◎	
Clad. herbarum						△	

Plate 1

Different stages of tree trunk No. 4

(*Cornus controversa*)

(13th, March; 24th, March; 7th, April; 15th, April; 10th, July)

Plate 2

Different stages of tree trunk No. 3

(*Cornus controversa*)

(24th, March; 29th, March; 15th, April; 1st, May; 10th, July)

Plate 3

Different stages of tree trunk No. 7

(*Carpinus tschonoskii*)

(24th, March; 29th, March; 23rd, April)

Plate 4

Different stages of tree trunk No. 1

(*Acer mono* var. *eupictum*)

(13th, March; 24th, March)

Plate 5

Ascus of *Dipodascus* sp.

X 600

Plate 6

Replica method of carbon assimilation

(A) Sterilization of needles

(B) Picking up of yeasts

(C) Inoculation

Plate 1

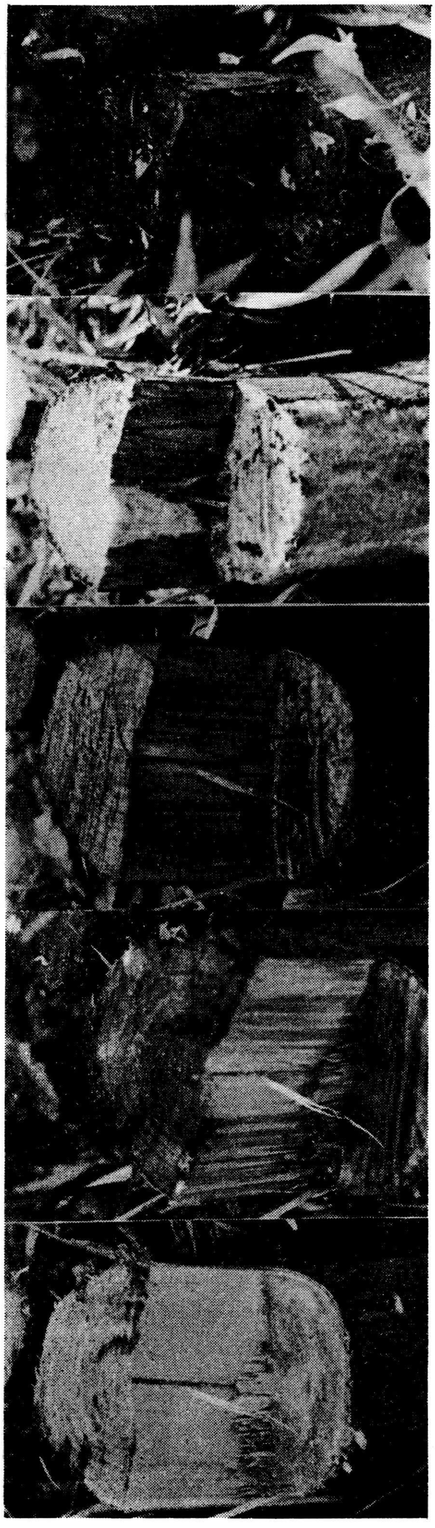

Plate 2

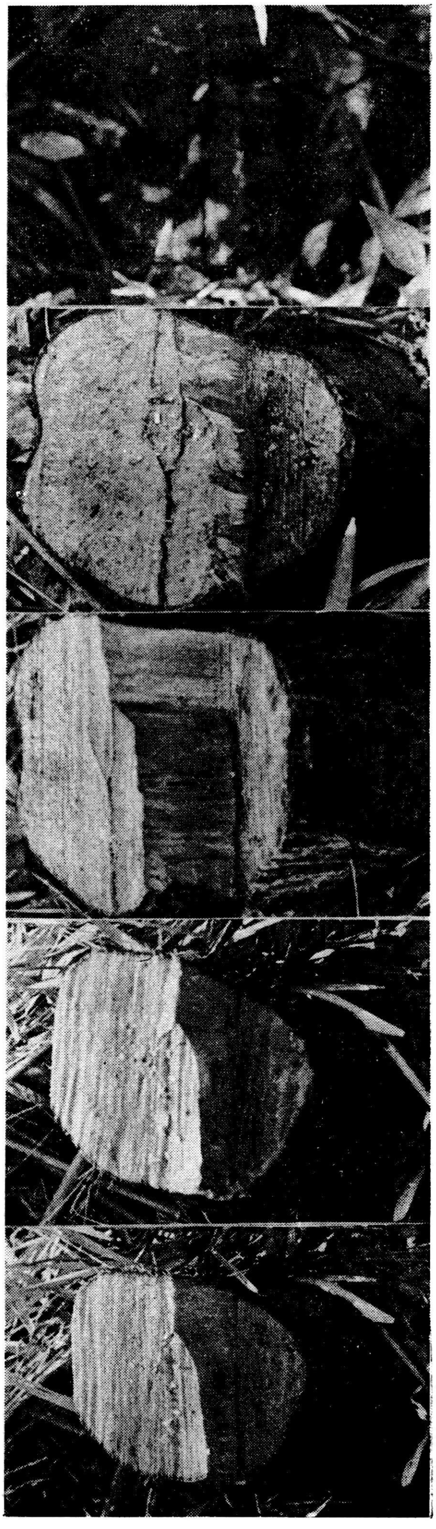

Plate 3

Plate 5

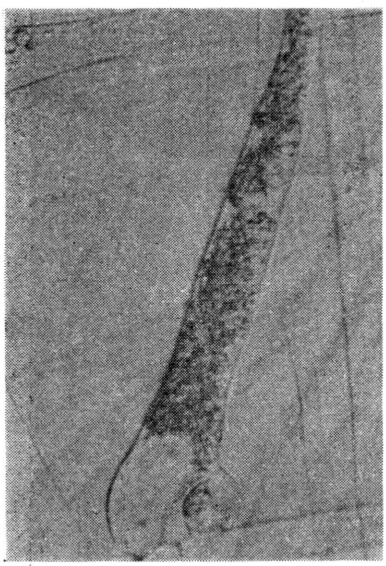

Plate 4

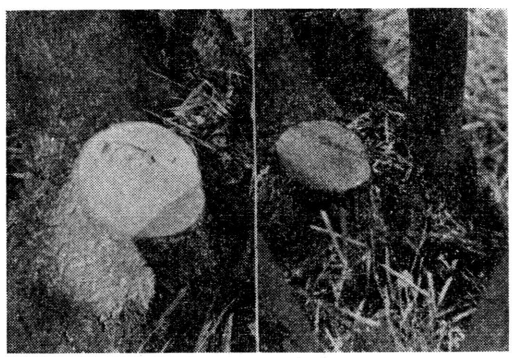

Plate 6

A B C