Yeasts From the Osaka Bay and the Yodo River

Tokio FUJITA* and Kei YAMAGATA*

Synopsis

The yeast flora of the Osaka Bay and the Yodo River has been studied and 42 species (8 genera) enumerated. The predominant genera were *Rhodotorula, Torulopsis* and *Candida;* and the most frequently isolated yeast was *Rhodotorula rubra* (Demme) Lodder. It is noteworthy that two ascosporogenous genera (*Pichia* and *Hansenula*) and one basidiomycetous genus (*Rhodosporidium*) were isolated.

Introduction

During the recent three years (1977–1979) our laboratory isolated number of 975 strains from sea water[1-2] of the Osaka Bay and river water[3] of the Yodo River. These yeasts can be classified into eight groups by the characteristics of vegetative reproduction, morphology of the vegetative cells grown in liquid and on solid media, formation of pseudomycelium and true mycelium, formation of chlamydospores, formation of vegetative endospores, formation of ballistospores, characteristics of the ascus and ascospores, and characteristics of the teriospores. This classification held also for their pattern of utilization of carbon compounds, utilization of nitrogen compounds, growth in vitamin-free medium, pigment formation and production of extracellular, and amyloid compounds. The isolates of the eight groups isolated from the Osaka Bay and the Yodo River were identified as genus *Hansenula, Pichia, Kloeckera, Candida* and *Torulopsis* according to the taxonomic methods of van der Walt[4], J. Lodder[5], MacMillan & Phaff[6] and Kreger van Rij[7].

Methods

Culutral and morphological tests

The cultural characteristics of the strains were studied on malt agar[4] (van der Walt, 1970) and on YM agar (Difco Bact). Cultures were examined after 3 days at 25°*C* and after 1 month at 15 or 25°*C*. The morphological characteristics of the cells were studied during exponential growth at 20°*C* in shaken defined liquid medium[8] (Barnett & Ingram, 1955) containing D-glucose as the sole carbon source. Further morphological studies were made on cultures in 2% glucose-yeast extract-peptone water (Difco Bacto) and malt extract broth[4] (van der Walt, 1970) incubated for 3 days at 25°*C*, and on potato dextrose agar (Difco Bacto) incubated for 6 days at 20°*C*.

Filamentous growth was examined on slide cultures (van der Walt, 1970) made with potato dextrose agar and with corn meal agar (both Difco Bacto).

* Lab. of Applied Microbiology, Dept. of Agricultural Chemistry, Kinki Univ., Higashiosaka, Osaka, 577 Japan.

The media used to test for ascospore formation were those described by van der Walt (1970)[4]. The strains were gorwn individually and collectively on YM agar and then streaked on to carrot plugs, potato plugs, malt extract agar (Difco Bacto), McClary's acetate agar, Grodkowa agar (modified), potato dextrose agar (Difco Bacto) and V8 vegetable juce agar. The plates were incubated at 25°C and the search for ascospores was carried out periodically for up to 6 weeks.

The method for detecting ballistospore formation was that described by do Carmo Sousa & Phaff (1962)[9]. Inoculated potato dextrose plates and corn meal plates (both Difco Bacto) were inverted over similar agar plates containing sterile glass slides and incubated at 20 or 5°C.

Phisiological tests

The aerobic substrate utilization tests were carried out by the method of Buhagiar & Barnett (1971)[10] on liquid defined medium[6] (Bernett & Ingram, 1955) but the rocked tubes of liquid media were incubated at 20°C for 28 days. The anaerobic fermentation test was as described by van der Walt (1970)[4] but 0.1 M D-glucose was used as the sole carbon source.

The strains were examined for amyloid material, pulcherrimin pigment, acid production, hydrolysis of urea, acid and arbutin splitting and the liquefaction of 12% (*w/v*) gelatin (Difco Bacto) by the method described by van der Walt (1970)[4].

Results

Strains of group I showed properties of the genus Hansenula; cells are spheroidal, ellipsoidal, or cylindrical.

Pseudohyphae and true hyphae may occur. One to four ascospores are produced. Ascospores are produced. Ascospores are hat-shaped, hemispheroidal or spherical. Sugars may or not be fermented; pellicles may or may not be formed; ester may be produced.

Nitrate is assimilated.

The yeasts belong to this group are mainly found in the fresh waters of the Yodo River basin.

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

Strain no: R-3-1, R-3-2, R-3-4, R-3-5, R-3-6 etc.

Cells from the edges of seven-day-old colonies may vary greatly in size. The more glistening colony form often produce spheroidal and broadly ellipsoidal cells occurring singly, in paris, or small groups. Cells at the edges of such colonies vary from (2–5) × (3–5)μ (Fig. 1). Edges of colonies are usually crenate, and hyphal tips are usually absent at seven days, but older colonies may have a band of hyphae. Pellicles vary from thin and smooth to absent for strains that produce glistening colonies, to gray or even white pellicles that are smooth or folded for strains that form smooth or rugose mat colonies. Mat colony forms usually produce pseudohyphae but no true hyphae under the cover glass (Fig. 2). The cells convert directly to asci containing one to four hat-shaped ascospores. They are produced abundantly on potato agar and carrot plugs. It fermented sucrose, but could not assimilated inulin and L-rhamnose.

Hansenula jadinii (A. et R. Sartory, Weill et Meyer) Wickerham 1932

Stain no: R-2-1

Cells at the edges of colonies are ellipsoidal and cylindroidal; occur singly, in pairs. They measure from (3–4) × (4–7)μ. Colonies are smooth, glistening, butyrous, and without hyphae. The mat form of this species is not known to exist. Primitive pseudohyphae are formed under slide culture. They consist of chain of cylindroidal cells. The pseudohyphae are highly branched, and produce few chain of cells sufficiently differentiated to be considered

as blastospores. Maximum length of hyphal cells is about 15μ. True hyphae are not produced. It fermented glucose and sucrose, but could not fermented maltose. It assimilated inulin, and growth in vitamin-free medium were positive.

Strains of group II showed properties of the genus Pichia;

Cells of various shape which reproduce by multilateral budding. True mycelium may occur to a very limited extent. The spores are spherical, hat-shaped, usually with an oil drop inside. The spores may have warts formed exclusively by the outer layer of the spore wall. One to four spores are formed in each ascus. Fermentation absent or present. Nitrate in not assimilated. The yeasts belong to this group could be only isolated from river water, especially its found in the collected water sample near by Hirakata area on the Yodo river basin.

Pichia ohmeri (Etchells et Bell) Kreger-van Rij 1950

Strain no : R-4-1, R-4-2, R-4-6 etc.

After 3 days at 25°*C* the cells are short-oval or cylindrical, (2–6) × (3–25)μ; single, in pairs or in chains. After one month at 15°*C* the streak culture is cream-colored to yellowish, dull, almost raised smooth to wrinkled over the whole surface. Pseudomycelium is well developed. It consits of chains of elongate cells. The spores are generally hat-shaped, although spherical spores without a ledge also occur; one to four are formed per ascus (Fig. 3 and Fig. 4). It fermented glucose, galactose and sucrose, and assimilated galactose, sucrose and maltose, but could not assimilated lactose and melezitose as a carbon source.

Strains of group III showed properties of the genus Kloeckera;

Cells are lemon-shaped (apiculate), ovoidal and sausage-shaped; reproduce by bipolar budding on a moderately broad base. A pseudomycelium is usually not formed, but some strains produce a pseudomycelium ranging from primitive to well developed. Ascospores are not formed. Fermentation of sugars occurs; nitrate is not assimilated; all species have an absolute requirement for inositol and phantothenic acid.

The yeasts belong to this group could be only isolated from river water, especially its found in the collected water sample near by Katura area on the Yodo River basin.

Kloeckera africana (Kloeckera) Janke 1912

Strain no.: R-4-3, S-4-4, R-4-5 etc.

After 3 days at 25°*C* the cells are apiculate, ovoid, or elongate, (2–5) × (5–10)μ, single, in pairs. Reproduction is by bipolar budding on a relatively broad base. A thin, incomplete ring may begin to form and there is some sediment. After 3 weeks a sediment and a thin ring are present. It assimilated sucrose and maltose as a carbon source.

Kloeckera apiculata (Reess emend. Kloecker) Jank 1870

Strain no: R-3-1, R-3-2, R-3-3 etc.

After 3 days in malt extract at 25°*C* the cells are lemon-shaped, ovoid, or elongate, single, in pairs, or occasionally in groups of three or four, (1.5–5.0) × (3–10)μ; reproduce by bipolar budding on a relatively broad base (Fig. 5). A sediment and thin has incomplete ring. The streak cultures are smooth, glossy, and umbonate in cross section. Pseudomycelium is absent. It could not assimilated sucrose and maltose.

Strains of group IV showed properties of the genus Cryptococcus;

Cells are spheroidal or ovoidal, occasionally elongate; reproduce by multilateral budding. Most strains are capsulated; extent of capsule formation depends on the medium. The capsule consist of starch-like compounds, which are release into the medium by many strains. All

species assimilate inositol as a carbon source for growth.

On solid media most species have a slimy appearance, psuedomycelium is either not formed or is rudimentary. In liquid media growth is slow and initially limited to the formation of a ring; later a sediment is produced. Ascospores, teriospores or ballistospores are not produced. Ability to ferment sugars is lacking.

The yeasts belong to this group are generally found in the both samples of sea and river water.

Cryptococcus albidus (Saito) Skinner var. *albidus*

Strain no: R-2-2, S-4-1, S-5-1, S-6-1, S-8-2, S-11-1 etc.

After 3 days at 25°*C* the cells are generally ovoidal to globose. These strains have rather small cells, (3–5) × (4–6)μ (Fig. 6). Cell morphology on malt agar is similar to that in malt extract. After one month the streak culture is cream-colored to slightly yellowish or tan, smooth, highly glossy, soft and slimy, and the border is entire. It assimilated sucrose, maltose and nitrate.

Cryptococcus flavus (Saito) Phaff et Fell *nov. comb.* 1922

Strain no: R-2-3, S-4-2, S-5-2, S-8-1, S-4-4, S-5-4 etc.

After 3 days in malt extract at 25°*C* the cells are oval, single and in pairs, (3–6) × (6–8)μ. A thin ring and a little sediment being to form. It assimilated sucrose, maltose and lactose, but could not nitrate. Starch formation was absent.

Cryptococcus laurentii (Kufferath) Skinner var. *laurentii*

Strain no: R-2-4

After 3 days at 25°*C* the cells are ovoidal to elongate, usually single or in pairs. In most strains the cells are approximately (2–6) × (3–7)μ. A thin or fairly well developed ring, a moderate sediment and sometimes film islets are present. In young cultures growth is hyaline. After one month the streak may be cream-colored, yellowish, pinkish, orange or tan-colored. Pseudomycelium is absent or rudimentary, consisting of a few chain of elongate, undifferentiated cells.

It assimilated sucrose, maltose and lactose, but could not assimilated nitrate.

Crypsococcus neoformans (Sanfelice) Vuillemin 1894

Strain no: S-8-4, S-6-3 etc.

After 3 days at 25°*C* cells are generally spherical or globose, occurring singly in pairs, or more rarely in small gorups. The cells are smaller, measuring (3–5) × (3–6)μ. After one month there is a well developed ring and heavy sediment. The streak culture after one month at room temperature is cream-colored to yellowish brown.

It assimilated sucrose, maltose and galactitol, but could not lactose and nitrate.

Cryptococcus uniguttulatus (Zach) Phaff et Fell *nov. comb.* 1934

Strain no: S-6-4-, S-6-5, S-6-6 etc.

After 3 days at 25°*C* the cells are globose to ovoidal, in pairs, singly, or in small clusters, (3–5) × (4–6)μ. After one month at 25°*C* the streak culture is whitish to cream-colored, smooth, semiglossy and soft.

It assimilated glucose, but could not lactose, galactitol and nitrate.

Strains of group V showed properties of the genus Rhodotorula;

Cells are spheroidal, ovoidal or elongate; reproduce by multilateral budding. Ascospores or ballistospores are not formed. Red and yellow carotenoid pgiments are synthesized in

young malt agar cultures. Not assimilated inositol as sole source of carbon. Starch-like compounds are not synthesized. Fermentative ability is lacking. Acid formation on chalk agar and gelatin liquefaction are generally negative.
The yeasts belong to this group found in the both samples of sea and river water.

Rhodotorula glutinis (Fres.) Harrison var. *glutinis*
Strain no: R-5-1, R-5-2, R-5-3, S-4-5, S-4-6, S-5-5, S-5-6, S-8-5, S-8-6, S-11-2, S-15-1 etc.

After 3 days in malt extract at 25°*C* the cells are ovoidal to globose, (2–5) × (4–10)μ; After one month a medium to heavy, pink to orange or salmon-colored ring, and a heavy sediment are present. Pseudomycelium generally absent. It assimilated raffinose, melezitose and nitrate, but could not assimilated melibiose.

Rhodotorula rubra (Demme) Lodder 1889
Sampling no: R--1, R-1-2, R-1-3, R-2-5, R-3-9, R-3-10, R-3-11, R-3-12, R-4-7, R-5-4, R-5-5, etc.

After 3 days in malt extract at 25°*C* cell of the different strains vary form short-ovoidal to elongate, single, in pairs, the width varies between 2 and 5μ (Fig. 7). The length of the short-celled strains varies from 3-7μ; those with intermediate cells from 5-10μ, and the long -celled strains range from 7-12μ. After one month there is a moderate to heavy pink or salmon -colored ring; usually no pellicle. Pseudomycelium is absent, consisting mainly of chains of elongate cells. It assimilated raffinose and melezitose, but could not assimilated nitrate.

Strains of group VI showed properties of the genus Rhodosporidium;

Vegetative cells are spheroidal, ovoidal or elongate, reproduce by multilateral budding and are uninucleate. A dikaryotic mycelial phase with septa and clamp connection develops from the conjugate cells. Thic-walled teriospores are produced on the mycelium. Pro mycelium is formed. Ballistospores have not been found. Sugars are not fermented. Growth on solid media is usually mucous and is orange to pink due to the formation of carotenoid pigments.
The yeasts belong to this group are only found in the collected samples on outside of the Osaka Bay.

Rhodosporidium sphaerocarpum Newell et Fell 1970
Strain no: S-6-7, S-11-3, S-114 etc.

After 3 days at 20°*C* the cells are spheroidal to ellipsoidal, (3–5) × (5–7)μ, single or in pairs (Fig. 8). Often daughter cells are budded from very short necks. An orange to pink-orange sediment, but no pellicle or ring is formed. After one month the sediment becomes very thick and a week ring has formed. Microscopically, large spheroidal cells, up to 6 × 6μ can be observed. Cell morphology is similar to that in liquid malt extract. Streak culture at 3 days is smooth, glossy, orange to pink-orange; texture is highly mucous; cross section is raised, soreading; border is entire. After one month at 20°*C* the culture becomes extremely mucous; if kept at 5°*C* for one month, the culture does not become as mucous as at 20°*C*, and develops a yellow pigmentation. Teliospores were shown smooth and spherical (Fig. 9 and Fig. 10).

Strains of group VII showed properties of the genus Candida;

Cells were globose, ovoid, cylindrical or elongate, sometimes irregularly shaped, normally not ogival, apiculate or flask-shaped. Normally, vegetative cells reproduce by multipolar budding; cells with apparent bipolar budding do not normally bud on a broad base. The pseudomycelium is often differentiated into pseudohyphae and blastospores. Chlamydospores may be formed. Ture mycelium may be formed. Arthrospores absent. Ascospores, teriospores

or ballistospores are not formed. Visible pigmentation due to carotenoid pigments absent. The yeasts belong to this group found in the collected samples on river and outside of the bay.

Candida albicans (Robin) Berkhout 1853
Strain no: R-5-7, R-5-8, R-5-9, R-5-10 etc.

After 3 days at 25°*C* the cells are globose or short-ovoid, sometimes elongate, (3–5) × (6–9)μ (Fig. 11). A thin ring may be formed. After one month at 25°*C* the streak culture is cream-colored, glistening, or somewhat waxy, soft and smooth to slightly reticulated. A well differentiated pseudomycelium is abundantly formed. True mycelium also occurs. It assimilated glucose, sucrose, maltose, and soluble starch, but could not cellobiose, erythritol and nitrate.

Candida berthetii Buckley et van Uden 1968
Strain no: R-1-4, R-3-13, R-3-14, R-3-15, R-4-8 etc.

After 3 days at 25°*C* the cells are globose to ovoid, (3–6) × (4–10)μ; sausage-shaped cells also occur. A dry pellicle is present. It assimilated glucose, cellobiose, salicin and nitrate, but could not sucrose, maltose and lactose.

Candida bogoriensis Deinema 1961
Strain no: R-4-9, R-4-10 etc.

After 3 days at 25°*C*, the cells are long-ovoid to sausage-shaped, (2–5) × (7–12)μ. After one month at 25°*C* the streak culture is yellowish-brown, mucoid, soft and smooth. It assimilated glucose, maltose, cellobiose and trehalose, but could not sucrose, lactose and nitrate.

Candida boidinii Ramirez 1953
Strain no: R-1-5, R-3-16, R-4-11 etc.

After 3 days at 25°*C* the cells are long-ovoid to cylindrical, often slightly curved, (2–4) × (7–10)μ. A creeping, powdery pellicle is present. It fermented only glucose, and it could not assimilated sucrose, maltose, cellobiose, lactose and salicin.

Candida buffonii (Ramirez) van Uden et Buckley *nov. comb.* 1957
Strain no: R-1-6, R-5-11 etc.

After 3 days at 25°*C* the cells are elongate, (2–5) × (10–21)μ. A ring may be formed. After one month after 25°*C* the streak culture is greyish-brown, glistening, soft and smooth. On potato glucose agar the streak culture is mucoid. Psuedomycelium is abundantly formed. It assimilated glucose, maltose and nitrate, but could not sucrose, lactose, melibiose and L-rhamnose.

Candida conglobata (Redaelli) van Uden et Buckley *nov. comb.* 1925
Strain no: R-4-12, R-4-12, R-4-13, R-5-12 etc.

After 3 days at 25°*C* the cells are ovoid to cylindrical, (2–5) × (6–15)μ. A dry pellicle may be present. After one month at 25°*C* the streak culture is yellowish to cream-colored. It assimilated glucose, D-ribose and arabinose, but could not L-rhamnose, sucrose, maltose, lactose and nitrate.

Candida diddensii (Phaff, Mrak et Williams) Fell et Meyer 1952
Strain no: R-4-14, R-4-15 etc.

After 3 days the cells are globose, ovoid or long-ovoid, (2–4) × (4–10)μ. A ring and

islets may be present. It assimilated glucose, sucrose, maltose, trehalose, L-rhamnose, erythritol, ribitol and nitrate, but could not lactose, raffinose and inositol.

Candida foliarum Ruinen 1963
Strain no: R-1-7, R-1-8 etc.

After 3 days at 25°C the cells are slender, long-ovoid to cylindrical, (2–4) × (4–10)μ. The cells are encapsulated. It could not fermented glucose, and assimilated glucose, but could not assimilated sucrose, maltose and lactose.

Candida krusei (Sastellani) Berkhout 1910
Strain no: R-4-16, R-4-17 etc.

After 3 days at 25°C the cells are cylindrical and ovoid. A thin powdery pellicle that creeps up against the glass wall is readily formed in most strains. It fermented glucose, and assimilated glucose, but could not L-sorbose, sucrose, maltose, cellobiose, trehalose, lactose, D-xylose, ribitol and nitrate.

Candida lambica (Lindner et Genoud) van Uden et Buckley *nov. comb.* 1913
Strain no: R-1-9, R-3-17, R-3-18, R-5-13 etc.

After 3 days at 25°C the cells are ovoid, (3–6) × (5–10)μ. A pellicle is formed. After one month at 25°C the streak culture is cream-colored, soft, smooth or finely wrinkled. Pseudomycelium is abundantly formed. It fermented glucose, and assimilated glucose, but could not L-sorbose, sucrose, maltose, cellobiose, trehalose, lactose and ribitol.

Candida lusitaniae van Uden et do Carmo-Souda 1959
Strain no: R-5-14, R-5-15 etc.

After 3 days at 25°C the cells are subglobose to ovoid, (1.5) × (3–9)μ. After one month at 25°C the streak culture is cream-colored, glistening, soft and smooth. It fermented cellobiose, and assimilated glucose, galactose, sucrose, maltose, cellobiose, L-rhamnose and ribitol, but could not lactose, raffinose, erythritol and nitrate.

Candida melinii Diddens et Lodder 1942
Strain no: R-1-10, R-5-16 etc.

After 3 days at 25°C the cells are ovoid, (2–4) × (3–6)μ, single or in pairs. After one month at 25°C the streak culture is greyish to cream-colored, semidull, soft, smooth. It assimilated glucose, sucrose, maltose, cellobiose, melezitose, salicin and nitrate, but could not lactose and raffinose.

Candida parapsilosis (Ashford) Langeron et Talic 1928
Strain no: R-1-11, R-5-17, S-6-8, S-4-10, S-8-8 etc.

After 3 days at 25°C the cells are short-ovoid to long-ovoid, measuring (3–4) × (3–9)μ. The streak culture after one month at 25°C is cream-colored to yellowish. It could not fermentation of sucrose, and assimilated glucose, sucrose and maltose, but could not cellobiose, lactose, soluble starch, erythritol and nitrate.

Candida rugosa (Anderson) Diddens et Lodder 1917
Strain no: R-1-12, R-3-19, R-3-20, R-4-18, R-4-19 etc.

After 3 days at 25°C the cells are ovoid to elongate, sometimes sausage-shaped or curved, (3–4) × (7–10)μ. Islets or pellicle are usually present. After one month at 25°C the streak culture is variable in appearance. It could not fermentation of glucose, and assimilated glucose,

galactose, D-xylose and arabinose, but could not sucrose, maltose, cellobiose, trehalose, lactose, L-arabinose and nitrate.

Candida salmanticensis (Santa Maria) van Uden et Buckley *nov. comb.* 1963
Strain no: R-2-6

After 3 days at 25°*C* the cells are ovoid, (3–5) × (5–9)μ. After one month at 25°*C* the streak culture is whitish cream-colored, semidull, soft and smooth. The pseudomycelium consists of sparsely branched formation of wavy pseudohyphae bearing chains and clusters of ovoid blastospores.

It fermented glucose, and assimilated glucose, sucrose, maltose, lactose, melibiose and raffinose, but could not nitrate.

Candida sorbosa Hedric et Burke ex van Uden et Burkley
Strain no: R-2-7

After 3 days at 25°*C* the cells are long-ovoid to cylindrical, (2–5) × (4–12)μ. A thin, creeping pellicle is present. After one month at 25°*C* the streak culture is cream-colored, semidull, soft, almost smooth. The pseudomycelium is constituted by a network of thin, often curved pseudohyphae bearing few ovoid blastospores. It fermented glucose, and assimilated glucose and L-sorbose, but could not sucrose, maltose, cellobiose, trehalose, lactose and nitrate.

Candida tropicalis (Castellani) Berkhout 1910
Strain no: R-4-20, R-4-21, S-4-11, S-6-10 etc.

After 3 days at 25°*C* the cells are short-ovoid to ovoid, sometimes nearly globose, (4–8) × (5–10)μ. A sediment and a ring are formed. After one month at 25°*C* the streak culture is whitish to cream-colored, dull to semidull, soft and smooth or partly striped, reticulate of wrinkled: old strains may become tough and hairy. Pseudomycelium is abundantly formed and consists of long-stretched, branched pseudohyphae bearing blastoconidia and verticils of blastospores in branched or simple chains. It fermented sucrose, and assimilated glucose, galactose, sucrose, maltose and cellobiose, but could not lactose, L-rhamnose, erythritol, inositol and nitrate.

Strains of group VIII showed properties of the genus Torulopsis;

Vegetative cells reproduce mutipolar budding. Pseudomycelium absent or rudimentary. Ascospores or teriospores are not formed. And ballistospores, endospores or arthorospores are not formed.

Inositol is not used as carbon source.

The yeasts belong to this group found in the collected samples on river and outside of the bay.

Torulopsis anatomiae Zwillenberg 1966
Strain no: R-4-20

After 3 days at 25°*C* the cells are spheroidal to short-ovoidal, (2–5) × (3 8)μ, single, in pairs or in short chains. Elongate cells may occur. A ring may be present. After one month at 25°*C* the streak culture is whitish cream-colored, glossy, soft and smooth. It could not fermentation of lactose, and assimilated glucose, cellobiose and trehalose, but could not galactose, sucrose, maltose, lactose, erythritol and nitrate.

Torulopsis candida (Saito) Lodder 1922

Strain no: R-1-13, R-1-4, R-2-8, R-3-21, R-3-22, R-4-20, R-4-21, R-5-19, S-4-11, S-4-12, S-5-9, S-8-9 etc.

After 3 days at 25°C cells are almost spherical to ovoid. The size of the cells varies with the strain from (2–4) × (3–4) to (4–6) × (5–8)μ. After one month at 25°C the streak culture is greyish-white to cream-colored, semidull to dull, soft, smooth, or partly striped, warty or slightly wrinkled. It could not fermentation of lactose, and assimilated glucose, sucrose, maltose, cellobiose and D-xylose, but could not nitrate.

Torulopsis ernobii Lodder et Kreger-van Rij 1952
Strain no: R-1-15, R-3-23, R-4-22, R-4-23, R-4-24 etc.

After 3 days at 25°C the cells are globose or nearly so, (3–4) × (3–6)μ. A ring may be present. After one month at 25°C the streak culture is brownish to greyish-white, almost dull, soft, smooth or slightly wrinkled.
It assimilated glucose, sucrose, maltose, cellobiose and nitrate, but could not lactose.

Torulopsis etchellsii Lodder et Kreger-van Rij 9152
Strain no: R-1-16, R-5-20 etc.

After 3 days at 25°C the cells are globose to short-ovoid, (2–4) × (3–6)μ (Fig. 12). A ring is present. After one month at 25°C the streak culture is greyish cream-colored, sometimes with a brownish tinge, glossy, soft, smooth or striped at the margin.

Torulopsis haemulonii van Uden et Kolipinski 1962
Strain no: R-1-7

After 3 days at 25°C the cells are ovoid to globose, rarely slender, (3–6) × (3–7)μ. A ring is present. After one month at 25°C the streak culture is cream-colored, glistening, soft and smooth. It could not fermented lactose, and assimilated glucose, sucrose, maltose and D-xylose, but could not cellobiose and nitrate.

Torulopsis halonitratophila Onishi ex van Uden et Vidal-Leiria
Strain no: R-3-24, R-3-25, R-5-21 etc.

After 3 days at 25°C the cells are globose to ovoid, (2–4) × (3–4)μ. A ring may be present. After one month at 25°C the streak culture is cream-colored, glossy, soft and smooth.
It assimilated glucose and nitrate, but could not sucrose, maltose, trehalose, cellobiose and lactose. These strains are not growth in sodium chloride-free media.

Torulopsis inconspicua Lodder et Kreger-van Rin 1952
Strain no: R-1-18, R-1-19, R-3-26, R-3-27, R-4-25, R-5-21 etc.

After 3 days at 25°C the cells are ovoid, (3–6) × (5–7)μ. After one month at 25°C the streak culture is greyish-white, semidull, soft, smooth or striped. It assimilated glucose, but could not galactose, sucrose, maltose, trehalose, lactose and nitrate.

Torulopsis maris van Uden et Zobell 1962
Strain no: R-3-28, R-5-23 etc.

After 3 days at 25°C the cells are globose, (3–5)μ. After one month at 25°C the streak culture is cream-colored, glossy, soft and smooth. No pseudomycelium is formed.
It assimilated glucose and galactose, but could not sucrose, maltose, cellobiose, trehalose, lactose and nitrate.

Torulopsis nitratophila Shifrine et Pharf 1956

Strain no: R-5-24

After 3 days at 25°C the cells are ovoid, (2–3) × (3–5)μ. Only a sediment is formed. After one month at 25°C the streak culture is greyish-white, glossy, soft, almost smooth. No pseudomycelium is formed. It fermented glucose, and assimilated glucose, trehalose and nitrate, but could not sucrose, maltose, cellobiose and lactose.

Torulopsis norvegica Reiersol 1958
Strain no: R-1-20, R-5-24 etc.

After 3 days at 25°C the cells are ovoid to globose, (2–5) × (3–5)μ. Only a sediment is formed. After one month at 25°C the streak culture is cream-colored, glossy, soft, smooth or finely punctilated. It assimilated glucose, cellobiose and nitrate, but could not sucrose, maltose and lactose.

Torulopsis torresii van Uden et Zobell 1962
Strain no: R-3-29, R-3-30, R-4-26, R-5-25, R-5-26, R-5-27 etc.

After 3 days at 25°C the cells are ovoid, (3–4) × (4–5)μ. After one month at 25°C the streak culture is cream-colored, glossy, soft and smooth. No pseudomycelium is formed. It assimilated glucose, galactose and trehalose, but could not sucrose, maltose, lactose and nitrate.

Discussion

The yeasts of number of 975 isolates from sea water on the Osaka Bay and river water on the Yodo River basin can be classified into the eight groups by the taxonomic methods of van der Walt[4] and J. Lodder[5].

At first, I group in the isolates belong to the eight groups were identified as genus Hansenula. The isolates include to II group identified to genus Pichia, III group to genus Kloeckera, IV group to genus Cryptococcus, V group to genus Rhodotorula, VI group to genus Rhodosporidium, VII group to genus Candida and VIII group to genus Torulopsis respectively. The predominant genera in them consist of Rhodotorula, Trulopsis and Candida. The isolates belong to genus Rhodotorula spread tendency in the both samples of the sea water and river water, the colonies most frequently isolated was Rh. rubra. The orginal strain was identified as Rh. rubra isolated from drunk milk and cheese on 1889–1897 by Demme R.[11-13]. His original description of this species was S. rubra.

Lodder J.[4] (1934) placed S. rubra in synonymy of Rh. mucilaginosa from several physiological properties. That is, glucose, galactose (weakly), sucrose, maltose and ethanol were assimilated, but not lactose or nitrate. But, Hasegawa et al[15-17] (1960, 1963) placed Rh. mucilaginosa in synonymt Rh. rubra and added to the description that almost all strains require thiamine stimulatively. In this paper, these isolates belong to Rh. rubra most frequently isolated from the collected samples near by shores of the Bay and Yodo River estuary.

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要　約

水質汚染指標種を検索する目的で1977年から1979年にかけて比較的汚染の進んだ大阪湾とその最も主要な流入河川である淀川（木津川，桂川および宇治川を含む）から975株の酵母を分離同定した．その結果，*Hansenula* 属2種，*Pichia* 属1種，*Kloeckera* 属2種，*Cryptococcus* 属5種，*Rhodotorula* 属3種，*Rhodosporidium* 属1種，*Candida* 属17種，*Torulopsis* 属11種を同定した．分離試料中，湾口部から分離した酵母は *Cry. albidus var albidus, Cry. flavus, Cry. neoformans, Cry. uniguttlatus, Rh. glutinis var glutinis, Rh. rubra, Rhodosp. sphaerocarpum, C. parapsilosis, C. tropicalis, T. candida* の5属10種であり，湾奥及び沿岸周辺の試料中には *Rh. rubra* が多く認められた．

これに比較して淀川水系の試料中には *H. anomala var anomala, H. jadinii, P. ohmeri, Kl. africana, Kl. apiculata, Cry. albidus var albidus, Cry. flavus, Cry. laurentii var laurentii, Rh. glutinis var glutinis, Rh. lactosa, Rh. rubra, C. albicans, C. berthetii, C. buffonii, C. boidinii, C. conglobata, C. diddensii, C. foliarum, C. krusei, C. lambica, C. lusitaniae, C. melinii, C. parapsilosis, C. rugosa, C. salmanticensis, C. sorbosa, C. tropicalis, T. anatomiae, T. candida, T. ernobii, T. etchellsiii, T. haemulonii, T. halonitratophila, T. inconspicua, T. maris, T. nitratophila, T. norvegica, T. torresii* の7属28種を同定した．

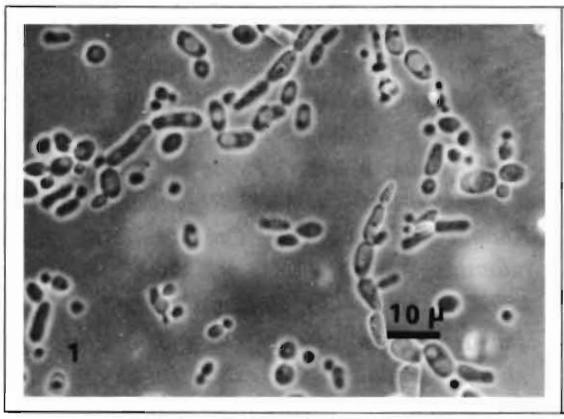

Fig. 1 H. anomala var anomala
After 7 days in malt extract.

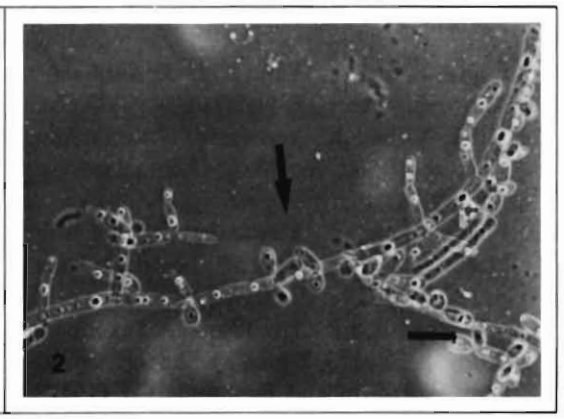

Fig. 2 H. anomala var anomala
Well developed pseudomycelium formation by slide culture.

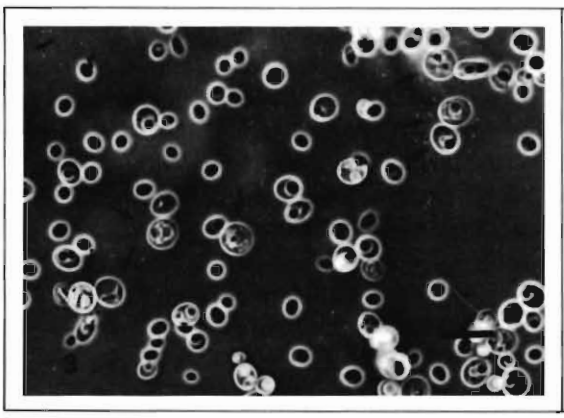

Fig. 3 P. ohmeri
Ascospore formation after 1 week on potato agar.

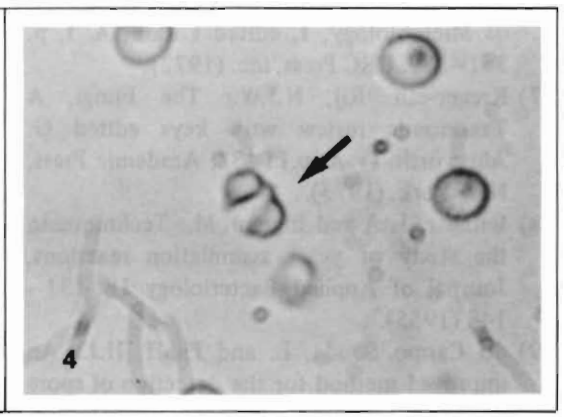

Fig. 4 P. ohmeri
Ascospore with hat-shaped on Gorodkowa agar.

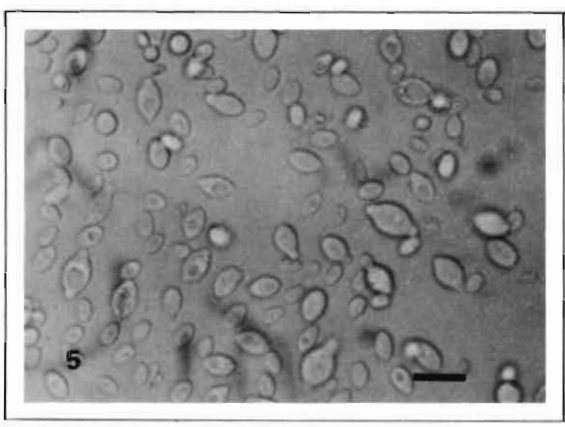

Fig. 5 Kl. apiculata
After 3 days in malt extract.

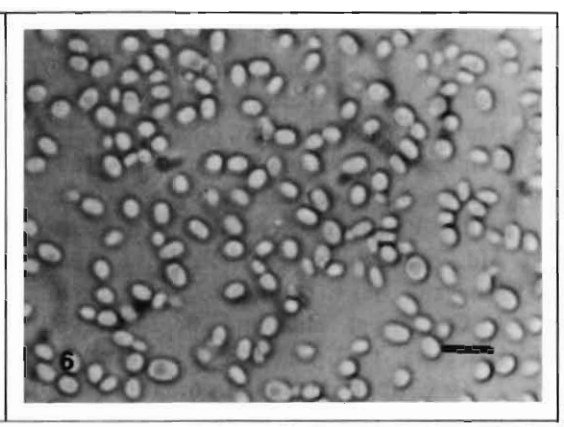

Fig. 6 Cry. albidus
After 3 days in malt extract.

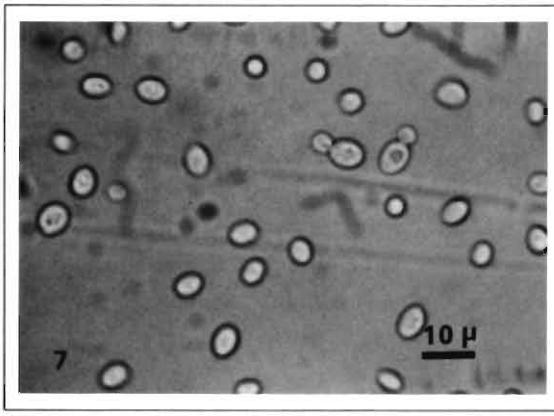

Fig. 7 Rh. rubra
After 3 days in malt extract.

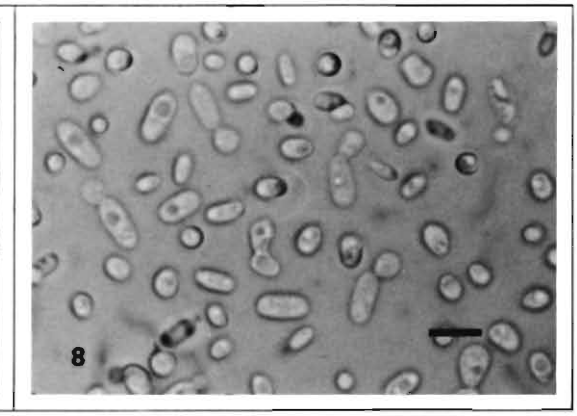

Fig. 8 Rhodosp. sphaerocarpum
After 3 days in malt extract.

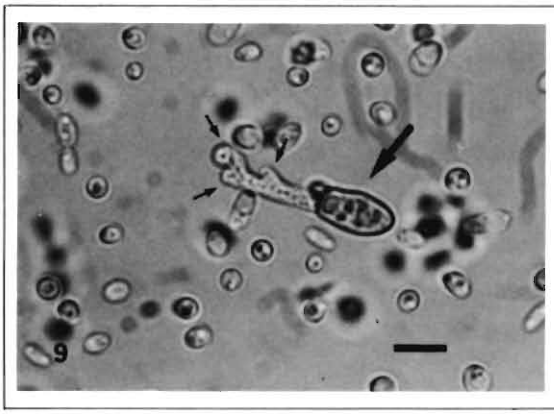

Fig. 9 Rhodosp. sphaerocarpum
Teliospore with promycelium, sporidia and clamp conection.

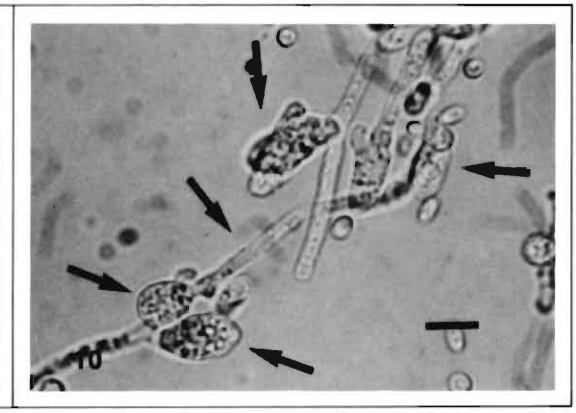

Fig. 10 Rhodosp. sphaerocarpum
Teilospore with well developed promycelium.

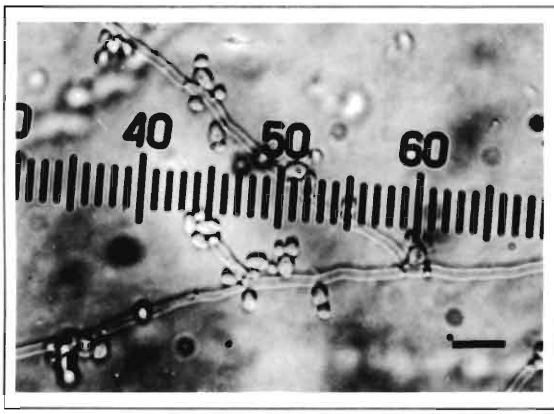

Fig. 11 C. albicans
Pseudomycelium formation on slid culture.

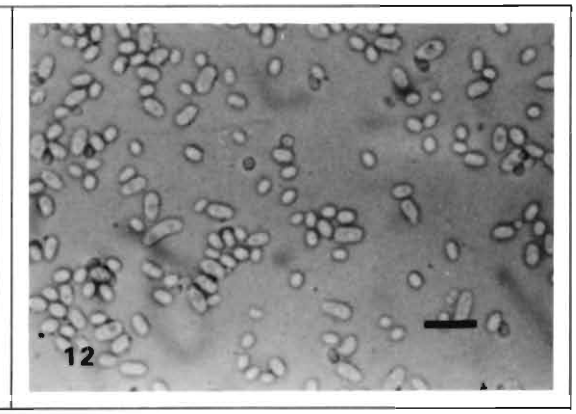

Fig. 12 T. etchellsii
After 3 days in malt extract.