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팔프 및 제지공장 폐수의 처리에 관한 미생물학적 연구

1. 폐수 하수 및 썩은 나무에서 분리한 효모의 동정 및 그의 생리적 성질에 관한 연구

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Microbiological Studies on the Treatment of Waste from Pulp and Paper Industries

1. Studies on the Identification and Physiological Characteristics of Yeasts Isolated from the Waste and Decayed Trees and Sewage

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ABSTRACT

1. To get the suitable yeasts for the treatment of waste liquor from pulp and paper industries, the 162 yeasts were isolated from the waste liquor, decayed trees and sewage (1, 7, 8, 9, 1971). 17 species were chosen by its ability to assimilate the carbon compounds and indentified.

2. All of the strain was increased its growing ability by agitation. In particular, the strain 912, strain 613, strain 100, strain 732 showed excellent high productive ratio(A/A_0).

3. The optimum temperature of the strains ranged 27°C and 30°C.

4. Most of the strain was grown actively in 10C/5N-composition and strain 113, strain 432, strain 735, strain 936, and strain 912 showed its optimum growing in 15C/5N-composition and 5C/5N-composition, respectively.

5. The optimum *p*H of the strains lay within range *p*H 4.5~6. Effect of the variation of *p*H on the growth was nearly negligible within this range.

6. The strain 912, strain 100, strain 613, strain 311, strain 235, and strain 732 were expected for the utilization to the treatment of the waste liquor from pulp and paper industries:

INTRODUCTION

There has been tremendous volume of research works to solve the problem of water contamination, but a satisfactory solution still remains to be found. Especially treatment of the waste from pulp and paper industry has been emphasized its importance because of their great amount of various sugar contents. In a broad sence, two general lines of approach have been used; one is a treatment of the waste to minimize the deoxygenating effect of its wood sugar contents on receiving streams; the other is utilization of the raw materials present in the waste.

The original practices had been the discharge of the waste liquor without any pretreatment. Simple modification of the principle of the discharge to stream had been desinged with the local and industrial

situations.

During the world war II, the industrial process could be achieved to about 75% reduction of the biochemical oxygen demand (B.O.D) with precipitation and fermentation with *Candida utilis*. After Delbruek(1922) had demonstrated the possibility of usage of *Candida utilis* as a foodstaff, the production of food yeast from the waste was realized by Kihara(1948), Yamaguchi(1952) and Wily(1954). Other yeast have been applied to this object; *Saccharomyces cerevisiae* by Eweson(1936), *Hansenula anomala* by Peterson(1945), *Hansenula suovoolens* by Kurth(1946), etc., but their yields were not satisfactory.

For studing on the recovery of yeasts which could be performed on these two subjects, the desposition and the utilization of the waste from pulp and paper industries, various kinds of yeasts were isolated from waste of the pulp and paper industries and the other sources such as decaying wood or sewage etc. These were identified and the effects of physiological conditions on the growth were also carried out in this experiment.

MATERIALS AND METHODS

The samples were collected in sterile cap-tube aseptically. Immediately about 1 ml samples were introduced on the yeast extract-malt extract agar(YM. agar). Their environmental circumstances were shown in table 1.

After 2~3 days, colonies were appeared, then transferred on YM. agar. and streaked on YM. agar 3~4 times. For the pure isolation, the modification of the moist chamber method, slightly different from Lindner method, was introduced.

Table 1. Place and date of collected samples and numbers of isolate.

Date	Place	Source	Number of the isolates
1971. 1 7	An yang-ri Sihyung district southern part in Seoul city.	S,H paper Ind.	32
		H.A paperInd.	19
		H.K paper Ind.	14
1971. 7 8 9	Chang-dong Sungbuk district in Seoul.	S.A pulp Ind.	17
	Euy chong boo city.	D.H pulp Ind.	28
1971. 7 8 9	Mt. Sori at Kwang neung.	Decayed trees.	28
1971. 1 7		Sewage	24

Yeasts were diluted in sterile YM. extract containing about 12% gelatin before liquefaction. Variously sized droplets were deposited on a sterile cover slip with a sterile wire and it was placed, culture side down, over the concavity of a sterile hollow ground-glass slide after a drop of sterile water was placed in the chamber. It was sealed with vaselin and the droplets were observed microscopically, and those containing a single cell were marked.

Cell shape and reproduction form were examined at 30-min. intervals. Morphological variation of cells and their reproduction type could be confirmed.

After incubation at 28°C, those colony might be looked with the naked eyes. The cultures were picked up with the flamed knife and transferred to YM. agar or malt agar.

Identification procedures followed those

recommended by Lodder and Kreger-van Rij (1970).

Physiological experiment; Aseptically 5 loops of active growing yeasts were inoculated in a steriled cap-tube containing 5 ml of medium for each test.

YM broth media were used for the experiment about effect of hydrogen ion concentration(*p*H4.0, 4.5, 5.0, 5.5, 6.0, 7.0 and 8.0). temperatures(20, 24, 27, 30 and 37°C) and shaking(112 rev./min.)

In the experment about effect of carbon and nitrogen source in the medium on the growth of the yeast(C/N-composition), the medium was prepared with adding suitable amout of glucose and ammonium sulfate to the 1000ml of yeast nitrogen base(YNB) medium, substracted its nitrogen source (10 gC/2.5gN, 10gC/3.3gN, 10gC/5gN, 10gC/10gN, 5gC/5gN, 15gC/5gN, 20gC/5gN). On the shaking experiment, 0.5 ml of each cell suspensions were inoculated to the 250 ml Erlenmyer flask containing 50 ml of YM. medium.

On the experiments about the effects of *p*H, temperature and C/N ratio, 0.1 ml of cell suspension were transferred to each test tube containing 9.9 ml medium. The culture were grown in a culture cabinet at 29±1°C. In order to measure degree of growth, each culture was measured in terms of optical density using Coleman electric colorimeter (655 mμ) at 4 hour intervals.

RESULTS AND DISCUSSIONS

1) Identification;

17 isolates was chosen from the 162 isolates on the basis of the ability to assimilative carbon compounds.

The taxonomical properties of each strains were agreed with the standard description, but a little difference was found in the assimilative characteristics.

*A sign in the parenthesis shows physiological property of standard description.

Debaryomyces castelli capriotti(strain 100) The strain was isolated from waste of paper industry.

Growth in malt extract: After 3 days at 25°C the cells are spherical to short-oval, (4.0−6.5)×(4.8−7.5)μ; single, in pairs or in groups. (Fig; P-1) A sediment and thin pellicle were formed. After one month at 17°C a sediment and a pellicle were present.

Growth on malt agar: After 2 days at 25°C the cells were spherical, oval to cylindrical, (3.6−7.2)×(4−13)μ; single or in pairs.

After one month at 17°C the streak culture was yellowish-white, smooth.

Slide culture on potato and corn meal agar: No formation of pseudomycelium.

Formation of ascospore: The spores were spherical, one to three, usually one spores per ascus. Spores were obserbed on Gorodkowa agar, and McClary's acetate agar.

Fermentation:

Glucose+	Maltose+(weak)
Galactose−	Lactose−
Sucrose+	Melibose+(weak)
	Raffinose+

Assimilation of carbon compounds:

Glucose+	L-Arabinose+
Galactose+	D-Ribbose−
Sorbose+	Rhamnose+
Sucrose+	Ethanol−(+)
Maltose+	Mannitol+
Lactose+	α-Methyl-d-glucoside+
Melibiose+	Salicin+
Raffinose+	Lactic acid+
Inulin+	Succinic acid+
Soluble starch+	Citric acid+
D-Xylose+	Inositol−

Splitting of arbutin: Positive

Assimilation of potassium nitrate: Negative

Growth in vitamin-free medium: Negative

Growth on 50%(w/w) glucose-yeast extract agar: Positive

Growth at 37°C: Negative

The strain was resembled to strain 235 morphologically but showed distinct differences in lactose assimilation and ability to grow on vitamin free medium.

The strain agreed with standard description except for ethanol assimilation

Debaryomyces phaffii capriotti(strain. 235); The strain was isolated from the sewage.

Growth in malt extract: After 3 days at 25°C the cells were oval, long-oval or cylindrical, $(2.0-5)\times(4-13.5)\mu$; single, in pairs, in groups (Fig; P-2). A sediment a thin pellicle, ring were formed.

After one month at 17°C a sediment and pellicle was present.

Growth on malt agar: After 2 days at 25°C the cells were oval to long-oval and cylindrical, $(2-4)\times(3-16)\mu$; single or in pairs.

After one month at 17°C the streak culture was yellowish, shiny, smooth, with a slightly sinuous margin.

Slide culture on potato-and corn meal agar; The formation of pseudomycelium was very scanty. Short branched chains of cells was observed.

Formation of ascospores: The spores were spherical. One or two, generally one spore per ascus. The strain sporulated on Gorodkowa agar.

Formentation:

Glucose+	Lactose−
Galactose+ (week)	Melibiose+ (weak)
Sucrose+	Raffinose+
Maltose+ (weak)	

Assimilation of carbon compounds:

Glucose+	L-Arabinose+
Galactose+	D-Ribose+
Sorbose+	Rhamnose−
Sucrose+	Ethanol+
Maltose+	Mannitol+
Lactose−	α-Methyl-D-glucoside+
Melibiose+	Salicin+
Raffinose+	Lactic acid+
Inulin+	Succinic acid+
Soluble starch+	Citric acid+
D-Xylose+	Inositol−

Spritting of arbutin: Positive.

Assimilation of potassium nitrate: Negative.

Growth in vitamin free medium: Positive.

Growth on 50% glucose-yeast extract agar: Positive.

Growth at 37°C: Positive.

The properties of the strain was agreed well with standard description.

Endomycopsis capsularis(strain 613) The strain was isolated from sewage.

Growth in malt extract: After 2 days at 25°C the cells were oval to elongate, cylindrical $(2.5-7)\times(7-25)$. Hyphae with cross walls was present in addition to pseudomycelium. After a few days 1~2 Ascospores were observed in oval shape cell and many ascospores in elongated or cylindrical Ascus. A sediment, tight pellicle were formed. After one month at 17°C a sediment and a thick, dull wrinkled pellicle were present.

Growth on malt agar: After 3 days at 25°C the cells were oval to elongate, $(3-5)\times(12-23)\mu$; many mycelial hyphae occur.

After one month at 17°C the streak culture was yellowish-white dull, wrinkled.

Slide culture on potato and corn meal agar: True, branched mycelium was formed.

Blastospores were oval and elongated; they occur singly or in chain pseudomyceliums also were present.

Formation of ascospores: The ascospores were spherical or oval two to four spores were formed in the ascus. Ascospores was observed on acetate agar, corn meal agar and YM agar.

Fermentation:

Glucose+(weak)	Lactose−
Galactose−	Maltose+
Sucrose−	

Assimilation of carbon compounds:

Glucose+	L-Arabinose+(−)
Galactose−	D-Ribose+
Sorbose−	Rhamnose−
Sucrose−	Ethanol+
Maltose+	Mannitol+
Lactose−	α-Methyl-D-glucoside+
Melibiose−	Salicin+
Raffinose−	Lactic acid−
Inulin−	Succinic acid+
Soluble starch+	Citric acid−
D-Xylose+(−)	Inositol+(−)

Splitting of arbutin: Positive.

Assimilation of potassium nitrate: Negative,

Growth in vitamin-free medium: Negative.

Growth on 50% (w/w) glucose-yeast extract agar: Variable.

Growth at 37°C: Negative.

There had no special different properties from standard description except for assimilation of D-xylose, arabinose, inositol.

Kluyveromyces bulgaricus(Santa Maria) van der Walt(strain 912) The strain was isolated from decayed tree.

Growth in malt extract: After 3 days at 28°C the cells were subglobose spheroidal or ellipsoidal to cylindrical, $(3-6.0)\times(3-12)\mu$, and occur singly or in pairs(Fig; P-3). An incomplete ring and sediment were formed. After one month at 17°C an incomplete ring and sediment were present.

Growth on malt agar: After 3 days at 28°C the cells spheroidal, subglobose, ellipsoidal to cylindrical, $(3-6)\times(3-10)\mu$, and occur singly or in pair. The culture was cream-colored to greyish-cream. The margin was entire, undulating.

After one month at 17°C the culture was creamish-brown. The margin was undulating.

Dalmau plate culture on corn meal: pseudomycelium is produced. It was well ramified and was usually better developed in anaerobic areas.

Formation of ascospores: The asci contain one to four spheroidal. Sporulation was observed on YM agar and on kleyn's, McClay's acetate agar.

Fermentation:

Glucose+	Melibiose−
Galactose+	Raffinose+
Sucrose+	Inulin+
Maltose−	Soluble starch−
Lactose+	α-Methyl-D-glucoside−

Assimilation of carbon compounds:

Glucose+	L-Arabinose+
Galactose+	D-Ribose−
Sorbose−	Rhamnose−
Sucrose+	Ethanol+
Maltose−	Mannitol+
Lactose+	α-Methyl-D-glucoside−
Melibiose−	Salicin+
Raffinose+	Lactic acid+
Inulin+	Succinic acid+
Soluble starch−	Citric acid−
D-Xylose+	Inositol−

Splitting of arbutin: Positive.

Assimilation of nitrogen compounds:

Potassium nitrate; negative.

Ethylamine hydrochloride; positive.

Growth in vitamin-free medium: Absent.

Growth on 50% (w/w) glucose-yeast extract agar: Absent.

Growth at 37° C: Positive.

Cycloheximide resistance: Positive.

The properties of the strain was agreed with standard description well.

Kluyveromyces cicerisporus Van der Walt, Nel et van kerken (strain 936).

The strain was isolated from decayed trees.

Growth in malt extract: After 3 days at 28°C the cells were spheroidal, subglouse ellipsoidal to cylindrical, $(2.0-6.0)\times(3.0-11.0)\mu$, and occur singly or in pairs, occasionally in small cultures. Elongate cells which measure up to 20 μ in length may be present. A sediment was formed and frequently an incomplete ring After one month at room temperature a sediment was present and usually a ring.

Growth on malt agar: After 3 days at 28°C the cells were spheroidal, subglobose, ellipsoidal to cylindrical, $(1.5-5.0)\times(3.0-12.0)\mu$, and occur singly or in pairs, occasionally in small clusters. The streak culture was butyrous, brownish-cream color, smooth. The margin was undulating.

After on month at room temperature the culture was brownish-cream to creamish-brown, occasionally raised in the center.

Dalmau plate culture on corn meal agar: pseudomycelium was usually better developed in the anaerobic areas. The pseudomycelium was abundantly formed and was usually well ramified.

Formation of ascospores: The asci contain one to four ascospores. The ascospores were spheroidal to prolate-ellipsoidal. Sporulation was observed on YM agar and the common sporulation media.

Fermentation:

Glucose+	Melibiose−
Galactose+	Raffinose+
Sucrose+	Inulin+
Maltose+(−)	Soluble starch−
Lactose+	α-Methyl-D-glucoside−

Assimilation of carbon compounds:

Gluccose+	L-Arabinose+
Galactose+	D-Ribose+
Sorbose+	Rhamnose−
Sucrose+	Ethanol+
Maltose−	Mannitol+
Lactose+ (weak)	α-Methyl-D-glucoside−
Melibiose−	Salicin+
Raffinose+	Lactic acid+
Inulin+	Succinic acid+
soluble starch−	Citric acid+
D-Xylose+	Inositol−

Splitting of arbutin: Positive.

Assimilation of nitrogen compounds:

Potassium nitrate: Negative.

Ethylamine hydrochloide: Positive.

Growth in vitamin-free medium: Absent.

Growth on 50% (w/w) glucose-yeast extract agar: Absent.

Growth at 37°C: Positive.

Cycloheximide resistance: Positive.

The properties of the strain was agreed with well standard description.

Pichia guilliermondii Wickerham (strain 201) The strain was isolated from the sewage.

Growth in malt extract: After 3 days at 25°C the cells were short-oval, oval, $(2.5-5)\times(2.5-7)\mu$, or cylindrical, up to 14 μ long, single, in psirs or in chains.

A sediment and a incomplete ring and islets were formed. After one month at 17°C a sediment and a ring were present.

Growth on malt agar: After 3 days at 25°C the cells were short-oval, oval or cylindrical, $(2.2-5)\times(3-14)\mu$, single, in pairs or in chains. After one month at 17°C

the streak culture was yellowish to cream-colored, soft, smooth and shiny.

Slide culture on potato and corn meal agar: It was rather delicate and consists of primitive pseudomycelial cells.

Formation of ascospores: one to four were formed per ascus. They were easily liberate from the ascus. sporulation was observed on McClary's acetate agar and malt extract agar.

Fermentation:

Glucose+	Lactose−
Galactose+	Raffinone+
Sucrose+	Melibiose−
Maltose−	

Assimilation of carbon compounds:

Glucose+	L-Arabincse+
Galactose+	D-Ribose+
Sorbose+	Rhamnose+
Sucrose+	Ethanol+
Maltose+	Mannitol+
Lactose−	a-Methyl-D-glucoside+
Melibiose+	Salicin+
Raffinose+	Lactic acid+ (weak)
Inulin+	Succinic acid+
Soluble starch−	Citric acid+
D-Xylose+	Inositol−

Splitting of arbutin: Positive.

Assimilation of potassium nitrate: Negative.

Growth in vitamin free medium: Negative.

Growth on 50% (w/w) glucose-yeast extract agar: Positive (weak).

Growth at 37°C: Positive.

There have been no special difference from standard description except for some morphological properties.

Pichia scolyti (Phaff et Yoneyama) Kreger-van Rij (strain 772).

The strain was isolated from paper industry.

Growth in malt extract: After 3 days at 25°C the cells were oval to long-oval, $(2-4) \times (4.2-10.5)\mu$, single, in pairs often in chains. A sediment was formed.

After one month at 17°C a sediment and a ring present.

Growth on malt agar: After 2 days at 25°C the cells were oval, $(1.5-2.8) \times (2.5-9.5)\mu$, single or in pairs.

After one month at 17°C the streak culture was creamcolored to white, pasty glistening.

Slide cultures on potato and corn meal agar: Pseudomycelium was abundant and well developed. Blastospores were arranged in small verticils along the pseudomycelial cells.

Formation of ascospores: The spores were hat-shaped; one to four were formed per ascus. They were easily liberated from the ascus.

Fermentation:

Glucose+	
Galactose+	Lactose−
Sucrose+ (very weak)	Raffinose+
Maltose+	

Assimilstion of carbon compoands:

Glucose+	L-Arabinose+
Galactose+	D-Ribose+
Sorbose−	Rhamnose+
Sucrose+	Ethanol+
Maltose+	Mannitol+
Lactose+ (weak)	α-Methyl-D-glucoside+
Melibiose+	Salicin+
Raffinose+	Lactic acid−
Inulin−	Succinic acid+
Soluble starch−	Citric acid+
D-Xylose+	Inositol−

Splitting of arbutin: Positive.

Assimilation of potassium nitrate: Negative.

Growth in vitamin-free medium: Variable.

Growth on 50% (w/w) glucose-yeast

extract agar: Weakly positive.

Growth at 37°C: Positive.

Morphological properties of the cell and its strong glucose and galactose fermentative abilities were different from standard description but no special discrepancy was found.

Saccharomyces cidri Legakis (strain 735)

The strain was isolated from pulp industry.

Growth in malt extract: After 3 days at 28°C the cells were spheroidal, subglobose to ellipsoidal, (2.5-5.0) × (3.0-6.5) μ, and occur singly or in pairs. A sediment was formed.

After one month at room temperature a sediment and a ring were present.

Growth on malt agar: After 3 days at 28°C the cells were spheroidal, subglobose to ellipsoidal, (2.0-6.5) × (2.5-8.0) μ, and occur singly or in pairs. The streak culture was cream-colored to yellowish-cream, usually raised along the center, smooth. The margin was entire to undulating.

After one month at room temperature the culture was cream-colored, smooth.

Dalmau plate cultures on corn meal agar: No pseudomycelium was formed.

Formation of ascospores: The asci contain one to four spheroidal ascospores. Sporulation was observed on YE agar, corn meal agar.

Fermention:

Glucose+	Melibiose+
Galactose+ (weak)	Raffinese+ (complete)
Maltose+	Inulin−
Sucrose+	Soluble starch−
Lactose−	α-Methyl-D-glucoside+

Assimilation of carbon compounds:

Glucose+	L-Arabinose+ (−)
Galactose+	D-Ribose+ (−)
Sorbose+	Rhamnose−
Sucrose+	Ethanol+
Maltose+	Mannitol+
Lactose−	α-Methyl-D-glucoside+
Melibiose+	Salicin−
Raffinose+	Lactic acid+
Inulin+	Succinic acid+
Soluble starch−	Citric acid−
D-Xylose+	Inositol−

Splitting of arbutin: Absent.

Assimilation of nitrogen compounds:

Potassium nitrate: Negative.

Ethylamine hydrochloride: Positive.

Growth in vitamin-free medium: Absent.

Growth on 50% (w/w) glucose-yeast extract agar: Absent.

Growth at 37°C: Absent.

Cycloheximide resistance: Positive.

It had been reported that this species had been isolated only from Cider (1961). but the properties of the strain which was isolated from waste from pulp industry were agreed with well standard description.

Saccharomyces italicus Castelli (strain 903)

The strain was isolated from the decayed trees.

Growth in malt extract: After 3 days at 28°C the cells were spheroidal, subglobose, ellipsoidal, occasionally cylindrical, (3.0-7.0) × (4-18) μ, and occur singly, in pairs. A sediment, incomplete ring was formed.

After one month at room temperature a sediment was formed and occasionally a ring and islets.

Growth on malt agar: Cell shape and size were same as in malt extract culture. The streak culture was brownish-cream. The margin was entire to undulating.

After one month at room temperature the culture was light-brown, shiny to rather dull. The margin was undulate.

Dalmau plate cultures on corn meal agar: pseudomycelium was better developed under anaerobic conditions. This pseudomycelium

was strongly branched. The blastospores arranged in compact clusters.

Formation of ascospores: Usually vegetative cells were directly transformed into asci with one to four ascospores per ascus. The ascospores were spheroidal. Sporulation was observed on YM agar and on acetate agar.

Fermentation:

Glucose+	Melibiose−
Galactose+	Raffinose−
Sucrose+	Inulin−
Maltose+	Soluble starch−
Lactose−	α-Methyl-D-glucoside−

Assimilation of carbon compounds:

Glucose+	L-Arabinose+
Galactose+	D-Ribose−
Sorbose−	Rhamnose−
Sucrose+	Ethanol+
Maltose+	Mannitol−
Lactose−	α-Methyl-D-glucoside+
Melibiose−	Salicin−
Raffinose−	Lactic acid+
Inulin−	Succinic acid−
Soluble starch−	Citric acid−
D-Xylose−	Inositol−

Splitting of arbutin: Absent.

Assimilation of nitrogen compounds:

Potassium nitrate: Negative.

Ethylamine hydrochloride: Negative.

Growth in vitamin-free medium: Variable.

Growth on 50% (w/w) glucose-yeast extract agar: Variable.

Growth at 37°C: Positive.

The properties of the species showed good agreement with standard description.

Saccharomyces rosei (Guilliermond) Lodder et Kreger-van Rij (strain 432).

The strain was isolated from the sewage.

Growth in malt extract: After 3 days at 28°C the cells were spheroidal to short-ellipsoidal, (2.5-6.5) × (2.5-7.0) μ, and occur singly, in pairs or in small cruster cells contain lipid globules (Fig; P-4). A sediment was formed and occasionally incomplete ring as well.

After one month at 17°C a sediment present and frequently a ring as well.

Growth on malt agar: Cell size and shape were same as culture in malt extract. The streak cultur was white to greyish-cream color, rather flat, smooth, shiny. The margin was entire to undulating.

After one month at 17°C the culture was greyish-cream, flat, smooth, shiny. The margin was entire to undulating.

Dalmau plate culture on corn meal agar: Blastospores were arranged in small tree-like aggregates.

Formation of ascospores: Ocassionally diploid cells were directly transformed into asci. The asci usually contain one to three or four ascospores. Sporulation was observed on YM agar and McClary's-acetate agar, gorodkowa-agar.

Fermentation:

Glucose+	Melibiose−
Galactose+	Raffinose+
Sucrose+	Inulin+ (weak)
Maltose−	Soluble starch−
Lactose−	a-Methyl-D-glucoside+

Assimilation of carbon compounds:

Glucose+	L-Arabinose+ (−)
Galactose−	D-Ribose+ (−)
Sorbose+	Rhamnose−
Sucrose+	Ethanol+
Maltose+	Mannitol+
Lactose−	α-Methyl-D-glucoside+
Melibiose−	Salicin−
Raffinose+	Lactic acid+
Inulin+ (weak)	Succinic acid−
Soluble starch−	Citric acid−
D-Xylose+ (weak)	Inositol−

Splitting of arbutin: Absent.

Assimilation of nitrogen compounds:

Potassium nitrate: Negative.

Ethylamine hydrochloride: Negative.

Growth in vitamin-free medium: Variable.

Growth on 50% (w/w) glucose-yeast extract agar: Positive.

Growth on 60% (w/w) glucose-yeast extract agar: Absent.

Cycloheximide resistance: Growth at 37°C.

The species showed strong sucrose fermentative ability and L-Arabinose, D-Ribose assimilative ability. other properties were good agreed with standard description.

Candida humicola (Daszewska) Diddens et Lodder (strain 732).

The strain was isolated from pulp industry.

Growth on glucose-yeast extract-peptone water: After 3 days 25°C the cells were short-oval, drop-like, lemon-shaped, cylindrical and measure (3.5-5) × (8-30) μ.

Growth on glucose-yeast extract-peptone agar: After one month at 25°C the streak culture was yellow, glistening, smooth.

Dalmau plate culture on corn meal agar: Pseudomycelium was usually well developed and ramified to laminaria-shaped. Ocassionally true mycellium was observed.

It was well developed aerobic condition and in anaerobic condition primitive pseudomycelium was formed.

Fermentation: Absent.

Growth of carbon compounds:

Glucose+	L-Arabinose+
Galactose+	D-Ribose+
Sorbose+	Rhamnose+
Sucrose+	Ethanol+
Maltose+	Mannitol+
Lactose+ (weak)	α-Methyl-D-glucoside+
Melibiose+	Salicin+
Raffinose+	Lactic acid+
Inulin−	Succinic acid+
Soluble starch+ (very weak)	
D-Xylose+	Citric acid+
	Inositol+

Assimilation of potassium nitrate: Absent.

Growth in vitamin-free medium: Weak.

Sodium chloride tolerance: 7-12% (w/v).

Maximum temperature of growth: 34-37°C.

Starch formation: Positive.

Hydrolysis of urea: Positive.

This species showed active assimilation of glucose and xylose shape of pseudomycelium was slightly different from standard discription, but other properties was agreed well.

Candida macedoniensis (Cast. et Chalmers) Berkhout (strain 190).

The strain was isolated from paper industry.

Growth in glucose-yeast extract-peptone water: After 3 days at 25°C the cells were mostly short-ovoid, (2.5-6) × (4.5-9) μ, cylindrical cells up to 15 μ also occur (Fig; P-5).

Growth on glucose-yeast extract-peptone agar: After one month at 25°C the streak culture was cream colored to yellowish, semidull, soft, almost smooth.

Dalmau plate cultures on corn meal agar: Pseudomycelium branched chains of blastospores.

Fermentation:

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

Assimilation of carbon compounds:

Glucose+	L-Arabinose+
Galactose+	D-Ribose+ (−)
Sorbose−	Rhamnose−
Sucrose+	Ethanol+
Maltose−	Mannitol+
Lactose+	α-Methyl-D-glucose−
Melibiose−	Salicin+

Raffinose+	Lactic acid+
Inulin+	Succinic acid+ (weak)
Soluble starch−	Citric acid−
D-Xylose+	Inocitol−

Assimilation of potassium nitrate: Absent.

Assimiliation of potassium nitrate: Absent.

Growth in vitamin-free medium: No growth.

Sodium chloride tolerance: 10%—13% (w/v).

Maximum temperature of growth: 43—47°C.

The properties of this species were agreed with well standard description.

Candida melinii Diddens et Lodder (strain 131).

The strain was isolated from paper industry.

Growth in glucose-yeast extract-peptone water: After 3 days at 25°C the cells were ovoid, (2-4) × (3-7) μ, single or in pairs, in groups (Fig; P-6). Sediment, and jelly like pellicle were present.

Growth on glucose-yeast extract-peptone agar: After one month at 25°C the streak culture was greyish to cream-colored, soft, smooth.

Dalmau plate cultures on corn meal agar: The pseudomycelium consists of ramified chains of coarse pseudohyphae bearing clusters and chains of globose and ovoid blastospores in verticillated positions.

Fermentation: Absent.

Assimilation of carbon compounds:

Glucose+	L-Arabinose+ (−)
Galactose−	D-Ribose+ (−)
Sorbose−	Rhamnose+
Sucrose+	Ethanol+
Maltose+	Mannitol+
Lactose−	α-Methyl-D-glucoside+
Melibiose−	Salicin+
Raffinose−	Lactic acid+
Inulin−	Succinic acid+
Soluble starch−	Citric acid+
D-Xylose+	Inositol−

Assimilation of potassium nitrate: Absent.

Growth in vitamin-free medium: No growth.

Sodium chloride tolerance: 10—13% (w/v).

Maximun temperature of growth: 43—47°C.

The properties of this species were agreed with standard description except for the L-Arabinose and D-Ribose assimilative characters.

Candida rhagii (Diddens et Lodder) Jurzitza, Kühlwein et Kreger-van Rij (strain 864). The strain was isolated from pulp industry.

Growth in glucose-yeast extract-peptone water: After 3 days at 25°C the cells were globose to short ovoid, (2.5-5) × (3.5−6) μ, single, in pairs and in groups (Fig; P-7).

Growth on glucose-yeast extract-peptone agar: After 3 days at 25°C shape and size of cells were same as on extract culture.

After one month at 25°C the streak culture was cream-colored, not mucor, glistening, soft, smooth.

Dalmau plate curtures on corn meal agar: The pseudomycelium consists of ramified chains of long pseudohyphae bearing groups of oval blastospores in verticillated positions.

Fermentation:

Glucose+	Lactose−
Galactose+	Melibiose−
Sucrose+	Raffinose+ (weak)
Maltose−	Inulin−

Assimilation of carbon compounds:

Glucose+	L-Arabinose+
Galactose+	D-Ribose+
Sorbose−	Rhamnose−
Sucrose+	Ethanol+
Maltose+	Mannitol+
Lactose−	α-Methyl-D-glucoside+

Melibiose−	Salicin+
Raffinose+	Lactic acid+ (weak)
Inulin−	Succinic acid+
Soluble starch−	Citric acid+
D-Xylose+	Inositol−

Assimilation of potassium nitrate: Absent.

Growth in vitamin-free medium: Weak to good growth.

Vitamins stimulating growth: The strains were not stimulated by thiamine.

Maximum temperature of growth: 33-37°C.

There have been no special discrepancy to the standard description between the properties of this species, except for the properties of vitamins stimulating growth.

Cryptococcus luteolus (Saito) Skinner (strain 311).

The strain was isolated from paper industry

Growth in malt extract: After 3 days at 25°C cells were ovoidal to elongate, (3.0-6.0) × (5.5-9.5) μ, occurring single, ocassionally in pairs. An incomplete ring a little sediment was present (Fig; P-8).

After one month there was a fair amount of sediment, a moderate ring.

Growth on malt agar: Cell morphology was similar to that in malt extract. In young cultures the growth was cream-like.

After one month at 25°C the streak culture was cream colored to yellowish and semiglossy surface. The growth was soft and slimy. The border was entire.

Dalmau plate cultures potate-glucose agar: Pseudomycelium was absent, ocassionally primitive cells chain were appeared on its physiological condition.

Fermentation: Absent.

Assimilation of carbon compounds:

Glucose+	L-Arabinose+
Galactose+	D-Ribose+
Sorbose+ (weak)	Rhamnose+
Sucrose+	Ethanol−
Maltose+	Mannitol+
Lactose−	α-Methyl-D-glucoside+
Melibiose+ (weak)	Salicin+
Raffinose+	Lactic acid+
Inulin−	Succinic acid+
Soluble starch+ (weak)	Citric acid+
D-Xylose+ +	Inositol+

Assimilation of nitrogen compounds:

Potassium nitrate: Negative.

Ethylamine hydrochlolide: Negative.

Growth in vitamin-free medium: Very weak.

Growth on 50% (w/w) glucose-yeast extract agar: Absent.

Maximum temperature of growth: 30-32°C.

Acid production on chalk agar: Absent.

Starch formation: Positive.

Gelatin liquefaction: Absent.

The properties of this species were good agreement with standard description.

Torulopsis molischiana (Zikes) Lodder (strain 113).

The strain was isolated from paper industry.

Growth in glucose-yeast extract-peptone water: After 3 days at 25°C the celle were ovoid or ellipsoidal or spherical, (2−7) × (3−9) μ, singly in pairs ocassionally elongate, long-ovoid cells occured (Fig; P-9). Beside a sediment, an incomplete ring were present.

Growth on glucose-yeast extract-peptone agar: After one month at 25°C the streak culture was greyish-white strains have a butyrous streak culture.

Dalmau plate culture on corn meal agar: No pseudomycelium was formed.

Fermentation:

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

Assimilation of carbon compounds:

Glucose+	L-Arabinose+ (weak)
Galactose−	D-Ribose+
Sorbose+	Rhamnose−
Sucrose−	Ethanol+
Maltose+	Mannitol+
Lactose−	α-Methyl-D-glucoside−
Melibiose−	Salicin+
Raffinose−	Lactic acid−
Inulin−	Succinic acid−
Soluble starch+	Citric acid−
D-Xylose+	Inositol−

Assimilation of potassium nitrate: Positive.

Growth in vitamin-free medium: Absent.

Sodium chloride tolerance: 3-4% (w/v).

Maximum temperature of growth: 44-45°C.

There was no special discrepancy between the properties of the strain and standard description, but the morphological cell shape was varied slightly.

Torulopsis candida (Saito) Lodder (strain 330) The strain was isolated from paper industry.

Growth in glucose-yeast extract-peptone water: After 3 days at 25°C the cells were almost spherical to ovoid. The cells were varied in size, $(2.3-5.5)\times(3-6.5)\mu$, single, in pairs (Fig; P-10). Thin pellicle and sediment were formed.

Growth in glucose-yeast extract-peptone agar: After one month at 17°C the streak culture was cream-colored to yellowish-white, smooth, slightly wrinkled.

Dalmau plate cultures on corn meal agar: No pseudomycelium was formed.

Assimilation of carbon compounds:

Glucose+	L-Arabinose+
Galactose+	D-Ribose+
Sorbose+	Rhamnose+
Sucrose+	Ethanol+ (weak)
Maltose+	Mannitol+
Lactose+	α-Methyl-D-glucoside+
Melibiose+	Salicin+
Raffinose+	Lactic acid+
Inulin+	Succinic acid+
Soluble starch+	Citric acid+
D-Xylose+	Inositol+

Assimilation of potassium nitrate: Negative.

Ethylamine hydrochloride: negative.

Growth in vitamin-free medium: Good growth.

Sodium chloride tolerance: 10-21%.

Maximum temperature of growth: 32-33°C.

The properties of this strain were agreed well with the standard discription.

2. Physiological experiment

Effect of shaking: It has been generally accepted that aeration is the most important factor to accelerate the growth of yeasts. A great deal of research works for the aeration effect on the growth of yeasts has been performed by Kurth (1946), Haeris (1960), etc. But the investigation of agitation effect on the growth has been very few. As shown in table 2, ratio of growth rates (Ra/Rc) and productive ratio (A/A_0) of all the strains increased 1.3−3 and 2−7.5 folds, respectably. Furthermore, their adaptation periods were markedly shortened in comparison with unagitated control groups.

Growing ability of the yeasts was increased by the shaking. However, effect of shaking on the growth of each strain was shown characteristically and all the strain might to roughly grouped into following four types (Ea, Eb, Ec and Ed)

Ea type: Growth rate was increased markedly and its period to reach the stationary phase was shortened largely by the shaking.

Eb type: Period to reach the stationary phase was shortened by the shaking but

growth rates were increased very slightly.

Ec type: Growth rate was increased markedly but its period to reach the stationary phase was not shortened by the shaking.

Ed type: Growth rate was increased very slightly and its period to reach the stationary phase was shortened negligiblly by the shaking.

strain 311 (Ea type) was shortened its period to reach the stationary phase about 24 hrs. and growth rate was increased about 3.3 times to the control (Fig. 1).

strain 131 (Eb type) was shortened its period about 10 hrs but growth rate was increased about 1.4 times. (Fig. 2.)

strain 732 (Ec type) was increased its growth rate instinctly in the same period to reach the stationary phase to compare with control group. (Fig. 3).

strain. (Ed type) 864 was effected slightly by the shaking (Fig. 4). Shaking effect on this strain was only to increase growing time.

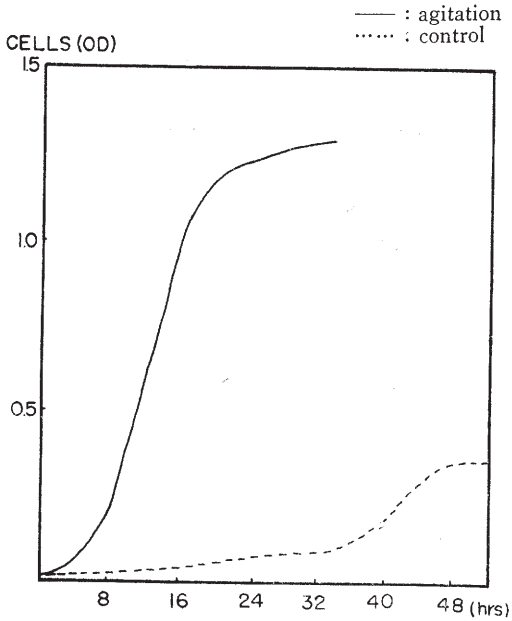
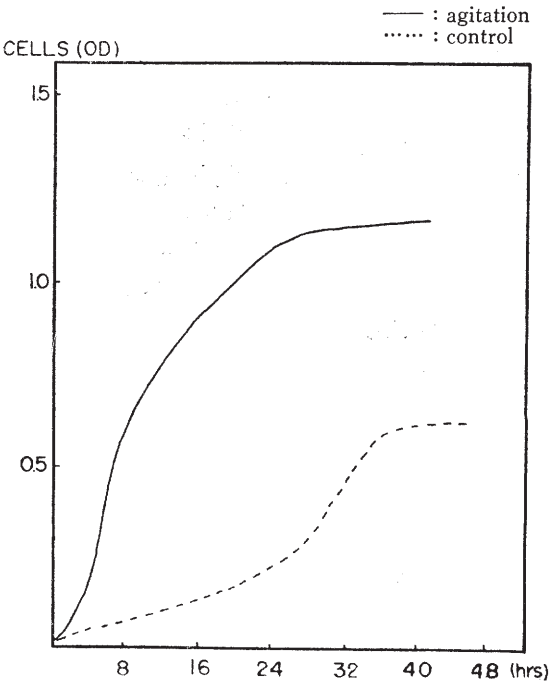


Fig. 1. Effects of agitation on the growth of strain 311 (Ea type).

Fig. 2. Effects of agitation on the growth of strain 131 (Eb type)



Effect of temperature: The optimum temperature of the most strains was shown in the range of 27°C~30°C

Above results were agreed with well Inskeep (1951) and White (1954).

As shown in table 3, the growth of the yeasts were effected slightly or markedly by the fluctuation of the temperature.

It might be grouped into 3 types roughly (Ta, Tb and Tc).

Ta type shows more remarkable variation on the growth. Tb type shows very slight variation on the growth. Tc type shows between Ta type and Tb type.

Fig 5. shows growth curve of the representative type (strain 936) in Ta type. Growth of strain 936 increased linearly as rising the temperature and its optimum temperature was 30°C. The strain was grown moderately at 37°C but the growth was suppresed heavily under 20°C.

Fig. 3. Effects of agitation on the growth of strain 732 (Ec type)

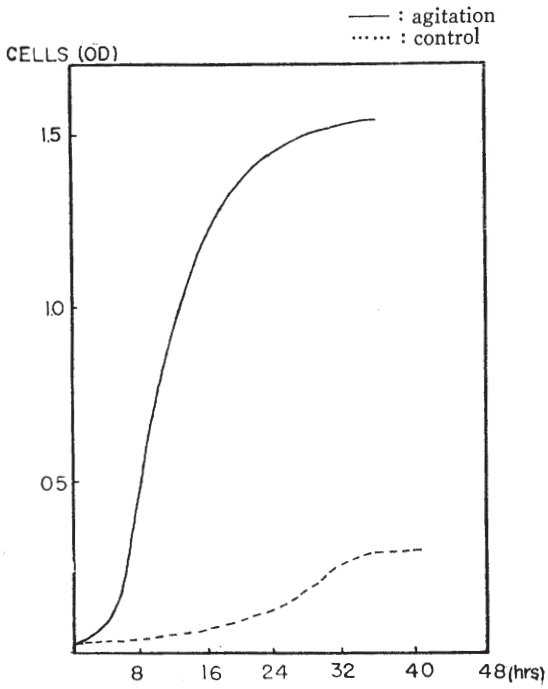


Fig. 4. Effects of agitation on the growth of strain 732 (Ed tyde)

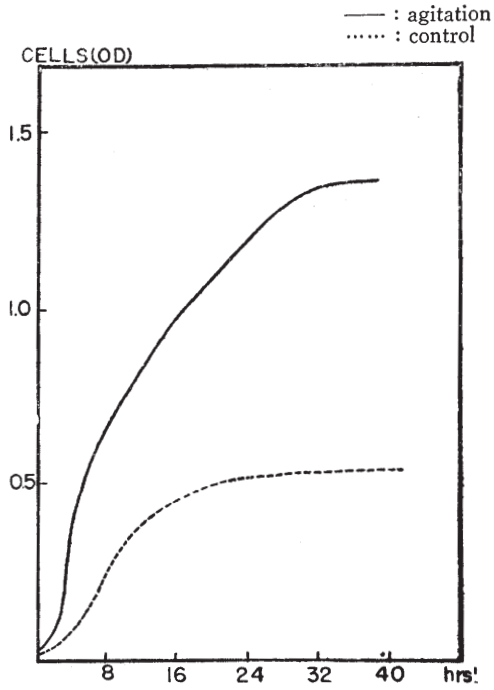


Table 2. Effects of agitation on the growth.

No	Type	S.N. ($\times 10^7$)	Tc-s	St	A/Ao	Rc	Rs	Rs/Rc
311	Ea	9.1	32	24	3.43	0.061	0.204	3.3
100	Ea	6.8	6	16	6.69	0.070	0.178	2.5
613	Ea	4.0	14	24	6.9	0.072	0.191	2.7
735	Ea	13.0	2	21	3.8	0.077	0.326	4.2
903	Eb	6.8	2	12	3.37	0.097	0.185	1.9
131	Eb	6.5	2	10	1.95	0.103	0.141	1.4
330	Eb	9.4	4	13	2.14	0.075	0.115	1.5
235	Ec	13.0	2	4	3.17	0.105	0.201	1.9
772	Ec	5.4	2	4	4.00	0.072	0.152	2.1
732	Ec	17.0	6	0	6.15	0.063	0.157	2.5
912	Ec	5.3	6	−4	7.47	0.091	0.191	2.1
864	Ed	6.2	2	−18	2.8	0.094	0.108	1.1
201	Ed	5.2	2	−4	2.8	0.104	0.161	1.6
113	Ed	5.8	2	−8	2.59	0.087	0.143	1.6
936	Ed	10.0	8	−8	4.92	0.093	0.116	1.2
190	Ed	14.0	2	−4	2.00	0.092	0.131	1.4
432	Ed	6.6	2	−12	2.36	0.110	0.184	1.7

S.N; Seeding cells number.
Ts; Adaptation period(Shaking)
Tc; Adaptation period(Control)
Tc-s; Tc-Ts
Sc; Time to reach the Stationary phase(Control)
Sa; Time to reach the Stationary phase(Shaking)
St; Sc-Sa
Rc; Growth rates of control
Rs; Growth rate of Agtation
Ao; Cell number at stationary phase(Control)
A; Cell number at stationary phase.(Shaking)

Fig. 5. Effects of temperature on the growth of strain 916 (Ta type)

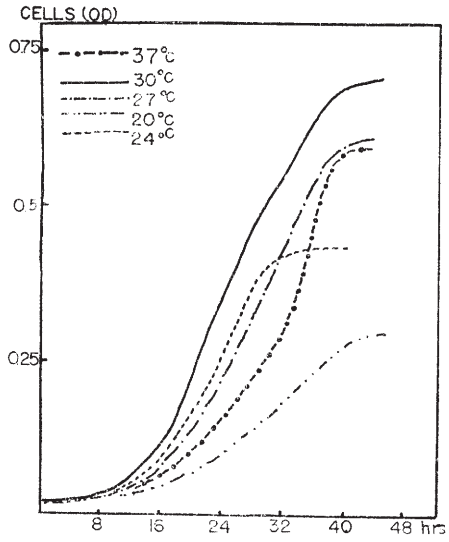


Fig. 6. Effects of temperature on the growth of strain 432 (Tb type)

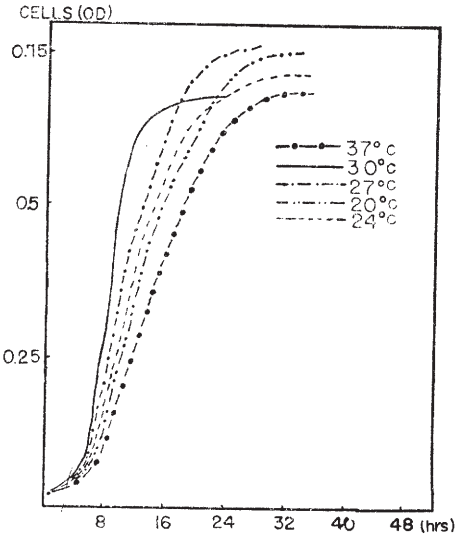


Table. 3. Effects of temperature on the growth.

No.	Type	S.N	Se	R	A					Op
					20	24	27	30	37	
916	Ta	5×10^7	40	0.093	10	22	31	39	23	30
732	Ta	28.5	32	0.063	5	9	16	13	7	27
912	Ta	5.3	36	0.091	7	23	30	36	23	30
903	Ta	6.8	30	0.097	5	10	16	18	12	30
131	Ta	5.5	32	0.103	10	18	22	27	16	30
201	Tb	16.0	30	0.104	5	12	18	24	20	30
190	Tb	1.9	52	0.092	10	14	19	16	12	27
772	Tb	11.0	40	0.072	13	16	18	16	10	27
295	Tb	19.0	30	0.105	7	16	25	23	20	27
113	Tb	7.7	36	0.087	5	11	16	23	10	30
735	Tb	17.0	32	0.077	6	7	10	10	16	30
100	Tb	20.0	36	0.070	7	8	11	10	8	27
432	Tc	1.6	32	0.110	20	23	26	24	23	27
864	Tc	8.3	32	0.094	17	18	20	18	16	27
613	Tc	8.0	28	0.072	12	14	16	14	13	27
311	Tc	8.2	40	0.061	7	8	9	11	8	30
330	Tc	7.2	44	0.075	6	15	23	16	5	27

S.N; Seeding number.
Se; Time to reach the stationary phase.
R: Growth rate.
A: Total cells number/seeding cells number
OP: Optimum temperature.

Fig. 6 showed growth curve of strain 432 in Tb type. The strain was grown nearly indepententent of the variation of temperature.

C/N composition (various composition of carbon and nitrogen compound in the medium.); the effects of carbon source on the growth of the yeasts was studied by Sperber(1945) and Schultz(1949) and effects of nitrogen source was investigaed by Thorone(1944) and Wilson(1954).

The poor literatures of the C-N compound as nutrient have been performed.

In this experiment the strains were grown actively in the 1000ml medium containing 10 gC/5 gN, (Rc type) Except for the strain 113, strain 432, strain 735, strain 916, and strain 912

The strain 113 was grown actively in the 15 C/5 N medium(Rg type) and growth of the strain was heavily suppresed in the 5 C/5 N composition and 20 C/5 N composition medium.

The strain 432 was grown actively only in the medium containing 15 C/5 N composition but strain 735 was grown moderately all of the other composite medium.

In the case of strain 912(Re type), growth was increased in 5 C/5 N medium. But in the 20 C/5 N composition its growing ability was heavily suppressed.

Effect of hydrogen ion concentration; The optimum *p*H of the most yeasts lay within the range *p*H 4.5~6.

Effect on the growth by the variation of *p*H was nearly negligible within this range. This results were agreed with Menzinsky (1950)

In the physiological experiments, the growth of yeasts were effected mainly by shaking, secondarlly by the variation of temperature, C/N-composition and *p*H. In table 4, the growth type and the optimum conditions of 17 strains are listed.

In the view of total reproductive ability, the strains which were expected for excellent production are strain 912, strain 735, strain 100, strain 613, strain 235, and strain 732.

To perform the object, minimizing the deoxygenating effect of wood sugar contents in the waste and producing the suitable fodder yeasts in the waste, many yeasts will be isolated from various source and chosen by its composition and abilities to increase its cell number, to assimilate carbon compounds and to reduce the BOD of the waste.

Table. 4. The growth type and the optimum conditions of 17 strains.

No	Type of agitation	Temperature		C/N-type	*p*H
		Type	OP		
100	Ea	Tb	27	Rc	4.5
235	Ec	Tb	27	Rc	5.0
613	Ea	Tc	27	Rc	4.5
912	Ec	Ta	27	Re	5.0
936	Ed	Ta	30	Rg	4.5
201	Ed	Tb	30	Rc	6.0
772	Ec	Tb	27	Rc	5.5
735	Ea	Tb	27	Rg	4.5
903	Eb	Ta	27	Rg	4.5
432	Ed	Tc	27	Rg	5.0
732	Ea	Ta	27	Rc	5.0
190	Ed	Tb	27	Rc	5.5
131	Eb	Ta	30	Rc	6.0
864	Ed	Tc	27	Rc	5.5
311	Ea	Tc	30	Rc	4.5
330	Eb	Tb	27	Rc	5.0
113	Ed	Tb	27	Rg	5.0

摘 要

1. 팔프 및 제지공장의 폐수를 처리하기위한 적당한 효모를 개발하기 위하여 5개의 팔프 및 제지공장 폐수와 썩은 나무 및 하수에서 162점을 분리하고 당류에 대한 자화능력이 좋은 17점을 선택하여 동정하였다.

동정된 균주는 다음과 같다.

1. *Debaryomyces castelli*
2. *Debaryomyces phaffii*
3. *Endomycopsis capsularis*
4. *Kluyveromyces bulgaricus*
5. *Kluyveromyces cicerisporus*
6. *Pichia guilliermondii*
7. *Pichia scolyti*
8. *Saccharomyces cidri*
9. *Saccharomyces italicus*
10. *Saccharomyces rosei*
11. *Candida humicola*
12. *Candida macedoniensis*
13. *Candida melinii*
14. *Candida rhagii*
15. *Cryptococcus luteolus*
16. *Torulopsis candida*
17. *Torulopsis malischiana*

2. 모든 균주는 교반으로써 그 생장이 왕성해지며 특히 균주 strain 912, strain613, strain 100, strain 732는 아주 높은 효율을 나타냈다.

3. 각균의 최적온도는 27°C—30°C 였다.

4. 대부분의 균주는 10 C/5 N의 조성을 갖는 배지에서 잘 자라며 strain 113, strain. 432 strain 735, strain 916 은 15 C/5 N 배지에서 균주 st.912 는 5 C/5 N 배지에서 왕성하게 생장했다.

5. 각균의 최적 *p*H 는 4.5—6.0 이었으며 이 정도 범위에서 변화에 의한 생장 차이는 극히 적었다.

6. 폐수 처리에 이용이 가능한 균은 strain 912, strain 100, strain 613, strain 311, strain 235, strain 732, 였다.

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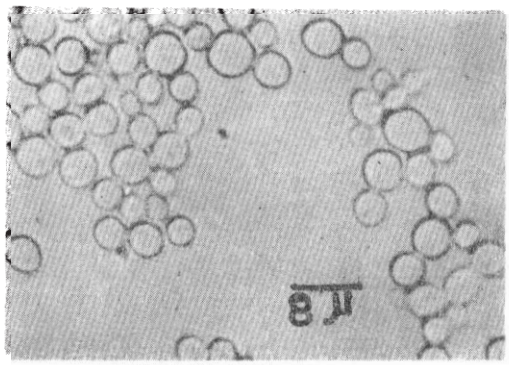
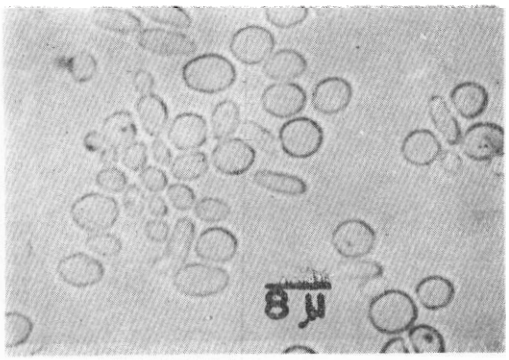
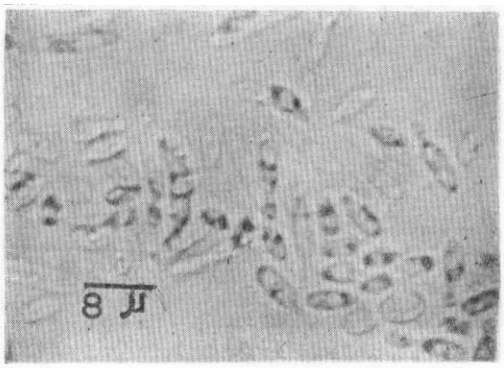
Plate 1.



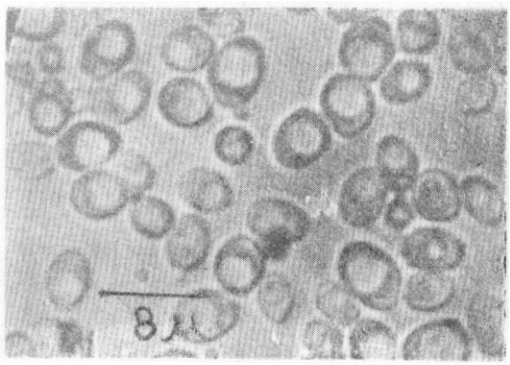
Fig; P-1. *Debaryomyces castelli*



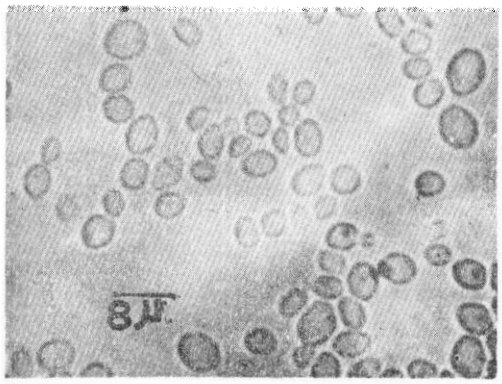
Fig; P-2. *Debaryomyces phaffii*



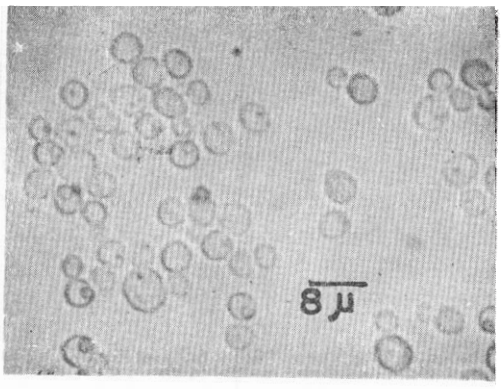
Fig; P-3. *Kluyveromyces bulgaricus*



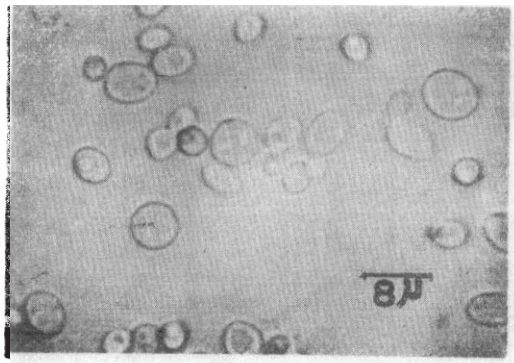
Fig; P-4. *Saccharomyces rosei*



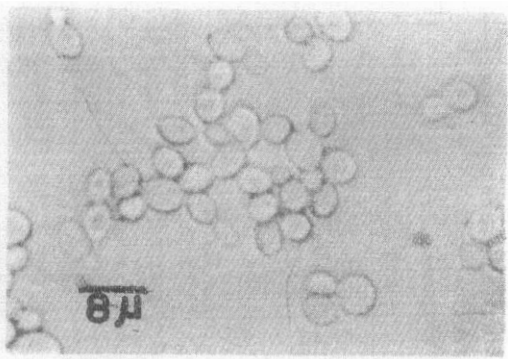
Fig; P-5. *Candida Macedoniensis*



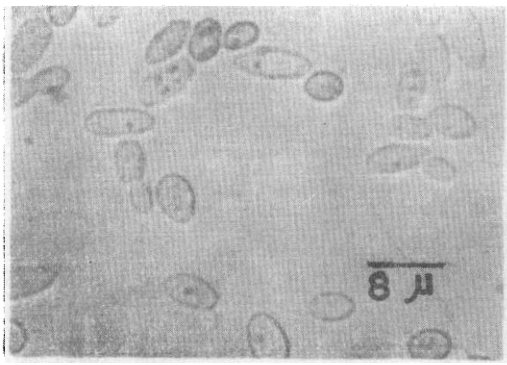
Fig; P-6. *Candida melinii*



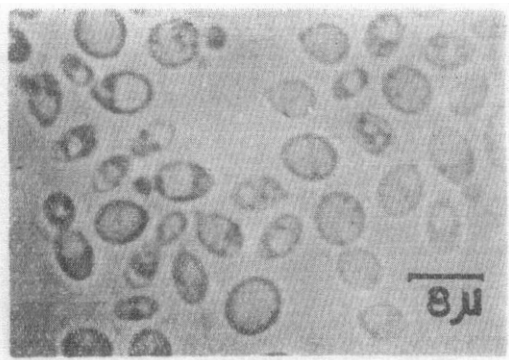
Fig; P-7. *Candida rhagii*



Fig; P-8. *Cryptococcus luteolus*



Fig; P-9. *Torulopsis molischiana*



Fig; P-10. *Torulopsis candida*