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Isolation of Ethanol-, Acetic acid- and Acetaldehyde-assimilating yeast, *Candida* sp. JY-5

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Ethanol, Acetic acid 및 Acetaldehyde의 동화능력을 가진 *Candida* sp. JY-5 의 분리

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An yeast assimilating ethanol, acetic acid and acetaldehyde among C_2-compounds was isolated. The morphological, cultural, physiological and biochemical properties of the isolate were examined. As the results of taxonomical researches, the isolate was identified as the genus *Candida*.

The numerous investigations on the ethanol-assimilating yeasts have been conducted by authors since a report from Hernandez and Johnson[1] in 1967. The ethanol-assimilating yeasts reported so far included *Candida brassicae*[2,3], *Candida utilis*[4,5,6], *Candida methanolica*[7] *Candida* sp.[8], *Hansenula miso*[9], *Pichia guilliermondii*[10], *Saccharomyces cerevisiae*[1,11,12], and *Torulopsis methanolovescens*[7] indicating 8 species belonging to 5 genera. It was likely that the acetic acid-assimilating yeasts growing on acetic acid as substrate were scarcely researched since the study of Sandor *et al.*[1]

The known yeasts were classified as *Candida utilis*[1,13,14] *Candida methanolica*[7], *Candida intermedia*[1], *Candida lipolytica*[1], *Candida tropicalis*[15], *Hansenula miso*[9] *Scharomyces cerevisiae*[16], and *Torulopsis methanolovescens*[7] and contained only 8 species belonging to 4 genera. In spite that many strains of yeasts were reported and recorded as the ethanol-, or acetic acid-assimilating yeasts[17], only 12 species belonging to 5 genera were practically discussed through their cultivations. Furthermore, the researches on the assimilating yeasts were very few, so only 2 species belonging to 2 genera including *Saccharomyces cerevisiae*[18], and *Candida utilis*[1] became known until the present time. But it is true that *Candida utilis, Candida methanolica, Hansenula miso, Saccharomyces cerevisiae* and *Torulopsis methanolovescens* could possess the capability to assimilate simultaneously both ethanol and acetic acid, and that these strains were frequently used for the careful research on their physiological properties through cultivations. From these collective backgrounds, only 2 species within 2 genera could concurrently assimilate ethanol, acetic acid and acetaldehyde. The experiments on the isolation and cultivation of the yeasts possessing the simultaneous assimilation-ability of the three C_2-compounds were very rarely made. Therefore it was assumed that the other yeasts capable of assimilating the three compounds from any origins could be find. Accordingly, the authors made an attempt for the isolation of yeasts concurrently assimilating ethanol-, acetic

acid-, and acetaldehyde-substrate, selected the powerful strain, examined her microbiological properties and compared her with the existing yeasts.

MATERIALS AND METHODS

Isolation of yeast.

Firstly the enrichment cultures in 500 ml of shaking flasks were exerted with the compositions as shown in Table 1.

Water, soil or fresh-water mud sample collected was added to the shaking flask and the cultivation was carried out on the reciprocal shaker at 30°C for about 7 days (110 Rev. at 6 cm stroke). The inocula from the enrichment culture were streaked on the Medium A, B and C (Table 2), respectively and incubated aerobically at 30°C for 5 to 7 days. After incubation of the plates, the cultures showing good growth on the 3 media were transferred to the medim D and maintained for the stock culture. Ethanol, acetic acid, or acetaldehyde was aseptically added to the already sterilized medium. (Table 2)

Identification of yeast.

For the identification of yeast isolated, the methods and comparisons were mainly referred to "The yeast, taxonomic study (2 ed.) by Lodder".[7]

Chemicals.

To test the assimilation of some compounds by the yeast as sole source of carbon, or nitrogen, chemicals used were guaranteed reagents. The other chemicals employed through this work were products of the certified reagent grade such as extra pure reagent.

Table 1. The composition of the enrichment culture media used for the isolation of ethanol−, acetic acid−, and acetaldehyde−assimilating yeast(pH 5.5).

Chemical	Amount
Ethanol	1.0 g
Acetic acid	0.2 g
Acetaldehyde	0.05 g
Ammonium sulfate	0.3 g
Chloramphenicol	0.03 g
Basal salts	
Potassium phosphate monobasic	0.15 g
Magnesium sulfate	0.05 g
Ferrous sulfate	0.30 mg
Calcium chloride	0.30 mg
Manganese sulfate	0.50 mg
Cupric sulfate	0.05 mg
Distilled water	100 ml

Table 2. The formulas of the media[a] used for the isolation and stock culture of yeast capable of assimilating ethanol, acetic acid and acetaldehyde.

Medium A (pH 6.0)		Medium C (pH 6.0)	
Ethanol	0.5 %	Acetaldehyde	0.05%
Ammonium sulfate	0.3 %	Ammonium sulfate	
Sodium phosphate monobasic	0.1 %	Sodium phosphate monobasic	0.1 %
Chloramphenicol	0.03%	Chloramphenicol	0.03%
Agar	1.5 %	Agar	1.5 %
Medium B (pH 6.0)		**Medium D (pH 6.0)**	
Acetic acid	0.5 %	Glucose	1.0 %
Ammonium sulfate	0.3 %	Ammonium sulfate	0.3 %
Sodium phosphate monobasic	0.1 %	Sodium phosphate monobasic	0.1 %
Chloramphenicol	0.03%	Magnesium sulfate	0.05%
Agar	1.5 %	Agar	1.5 %

[a]Medium A, B and C were empolyed for the isolation of ethanol−, acetaldehyde-assimilating yeasts, respectively. Medium D was used for the stock culture of the yeast.

RESULTS AND DISCUSSION

The yeast assimilating ethanol, acetic acid and acetaldehyde was isolated from water, soil or fresh-water mud samples.

The morphological and cultural properties of isolate.

The isolated yeast, strain JY-5, showed a slightly ellipsoidal and sausage shape on malt agar as shown in Table 3.

Table 3. The morphological and cultural properties of the strain of JY-5.

Shape and size of cell (After 3 days of incubation at 25℃ on malt agar)	Spheroidal to slightly ellipsoidal, (3.5−6.0) × (6.0−14.0) μm
Occurrence of cell	Singly or in pairs
Growth in malt extract (After 3days of incubation at 25℃)	

Table 3. 계속

Surface growth	Pellicle
Subsurface growth	Slightly granular, transparent
Sediment/Amount of growth	Granular/Moderate
Growth on malt agar	
Colony property	Butyrous
Colony color	Cretaceous, white
Elevation/Form	Convex/Circular
Margin	Erose
Surface/Characteristic aroma	Smooth/Produced
Growth in malt extract (After 40days of incubation at 22℃)	
Surface growth	Sediment
Subsurface growth	Transparent
Sediument/Amount of growth	Granular/Abundant
Growth on malt agar (After 40days of incubation at 22℃)	
Colony property	Butyrous
Colony color	Cretaceous, white
Elevation/Form	Umbonate/Circular
Margin	Erose or slightly filamentous with well-developed pseudomycelium
Surface	Smooth
Growth on 2% glucose-yeast extract-peptone water (After 3days of incubation at 25℃)	
Surface growth	Ring
Subsurface growth	Granular, transparent
Sediment/Amount of growth	Viscous/Abundant
Growth on 2% glucose-yeast extract-peptone agar (After 3days of incubation at 25℃)	
Colony property	Butyrous
Colony color	Dull, white
Elevation	Pulvinate
Form/Margin	Circular/Entire
Surface	Smooth
Growth on 2% glucose-yeast extract-peptone agar (After 40days of incubation at 22℃)	
Colony size (diameter)	2−11mm

Table 3. 계속

Colony property	Butyrous
Colony color	Glistening, cretaceous white
Elevation/Form	Pulvinate/Circular
Margin	Border entire with Pseudomycelium
Surface	Smooth
Characteristic aroma	Produced
Budding	Multilateral
Pseudomycelium formation	Formed from slide culture and Dalmau plate culture on potato-dextrose agar or 2% glucose-yeast extract-peptone agar
Spore formation (observed 3 or 7days intervals for 5 weeks incubation at 25℃)	
Grodkowa agar/Malt agar	Not formed
Fowell's acetate agar	〃
YM agar/Potato-dextrose agar	〃
Starkey's ethanol medium	〃

The growth of strain JY-5, after 3 days at 25°C in malt extract was pellicle-type in surface and granular in sedimentation. This strain reproduced by multilateral budding didn't form ascospores on some testing media. Fig. 1 pesented the vegetative cells of this strain grown in 2% glucose-yeast extract-peptone water. The microscopic photograph of strain JY-5 was also presented in Fig. 2 showing the presence of pseudomycelium from slide culture on potato-dextrose agar.

The physiological properties of isolate.

It is well known that the ability or inability to ferment carbohydrates to ethanol and carbon dioxide is a most useful characteristic for differentiating species. Of the 19 sugars tested, only glucose was possible in fermentative utilization as shown in Table 4. As assimilation of sugars as sole source of carbon, of 19 sugars tested, fructose, galactose, glucose, maltose, mannose, soluble starch, xylose and dextrin were assimilated by the strain JY-5. And this strain showed the ability to utilize nitrate depending upon assimilatory nitrate reduction. Growth on ethylamine hydrochloride appeared to be negative.

In spite that the assimilation on creatine as sole source of nitrogen seemed not to be general application as diagnostic

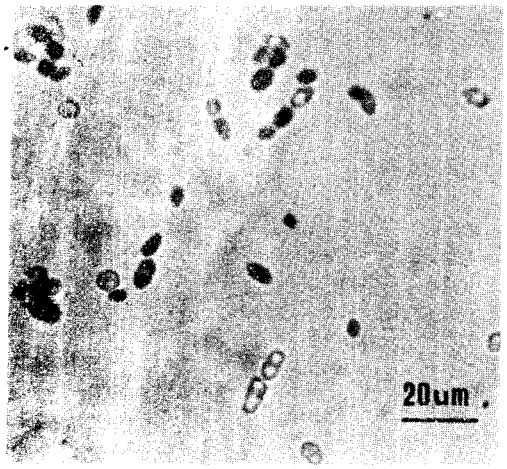

Fig. 1. Vegetative cells of strain JY-5 grown in 2% glucose-yeast extract-peptone water for 3 days at 25℃.

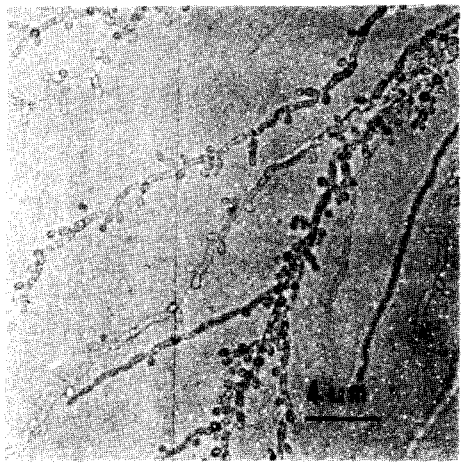

Fig. 2. Pseudomycelium of strain JY-5 grown on a slide culture for 10 days at 25 ℃.

Table 4. The physiological properties of the strain JY-5.

Fermentation					
L(+)-Arabinose	−	Maltose	−	L(−)-Sorbose	−
Cellobiose	−	D(+)-Mannose	−	Soluble starch	−
D(−)-Fructose	−	D(+)-Mellibiose	−	Sucrose	−
Galactose	−	Raffinose	−	D(+)-Trehalose	−
Glucose	+	L(−)-Rhamnose	−	D(+)-Xylose	−
Inulin	−	D-Ribose	−		
Lactose	−	Salicin	−		
Assimilation					
L(+)-Arabinose	±	D(+)-Mannose	+	Sucrose	+
Cellobiose	−	D(+)-Melibiose	±	D(+)-Trehalose	−
D(−)-Fructose	+	Raffinose	±	D(+)-Xylose	+
Galactose	+	L(+)-Rhamnose	±	Dextrin	+
Glucose	+	D-Ribose	−	Potassium nitrate	−
Inulin	±	Salicin	−	Ethylamine·HCl	−
Lactose	−	L(−)-Sorbose	−	Creatine	+
Maltose	+	Soluble starch	+		

Splitting of arbutin	Positive
Growth in vitamin-free medium	Positive
Growth on 50 % (w/v) glucose-yeast extract agar	Positive
Growth at 37℃/40℃	Positive/Positive
Formation of ester	Positive
Catalase activity	Positive
Hydrolysis of urea	Positive
Splitting of fat/stearic acid	Positive/Positive
Formation of pseudomycelium on potato-dextrose agar	Positive
Production of starch-like compounds	Negative

Table 4. 계속

Production of acids	Negative
Production of carotenoid pigments	Negative
Liquefaction of gelatin (50 days at 25℃)	Negative
Cycloheximide resistance (100μg/ml)	Negative
Tolerance to sodium chloride	Ca. 8 %

criterion, the tested result was positive. The result on arbutin splitting showed also positive reaction. This strain showed negative reactions in the production of carotenoid pigments, acids and starch-like compounds, gelatin-liquefaction and cycloheximide resistance.

Naming of the strain JY-5.

From the morphological, cultural, and physiological properties of this strain, data obtained were integrated, compared and discussed referring to "The yeast a taxonomic study (2 ed.)". This isolated strain presented cell spheroidal, reproduction by multilateral budding, formation of pseudomycelium, absence of ascospore and other spores, absence of visible pigmentation and a negative iodine reaction to extracellular polysaccharide. As these comprehensive results, the strain JY-5 was considered to be the genus *Candida*. The strain JY-5 seemed to be concerned with *Candida parapsilosis* as a relative of this species, but the slightly significant differences between two were also found. The differences indicated that *Candida parapsilosis* was positive in sorbose- and trehalose-assimilation and showed the poor growth in vitamin-free medium, but the strain JY-5 was negative in sorbose- and trehalose-assimilation, and showed rich growth in vitamin-free medium. Therefore this strain was thought to be a relative belonging to *Candida parapsilosis*.

Neverthless, the authors didn't exactly record this isolated strain *as Candida parapsilosis* because the discussion on basis of G+C content and coenzyme Q was required with this yeast cells. Accordingly the authors named this isolate as *Candida* sp. JY-5 strain.

요 약

C_2화합물 가운데 Ethanol, acetic acid 및 acetaldehyde을 공통적으로 동화할 수 있는 효모를 토양 및 하수 진흙으로부터 분리한 후 형태학적, 배양적 및 생리학적 특성을 고려하여 그 분류학적 위치를 검토하였다. 그 결과 분리효모는 다극 출아법에 의한 영양 증식을 하였으며 자낭포자를 형성하지 않고 또한 색소가 형성되지 않는 점으로 보아 *Candida*속에 속하였으며 *Candida* sp. JY-5로 명명하였다.

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