Isolation and Determination of Yeasts Utilizing Kerosene as a Sole Source of Carbon

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In order to produce microbial cell substances from petroleum, 83 strains of kerosene-utilizing yeasts, as a sole source of carbon, were isolated from 37 materials in contact with petroleum in the petroleum refinery. They could be distributed in either of 15 cultural groups with their colony appearances. Fifteen representative strains in 15 cultural groups were served for determination and identified with the following species: *Candida tropicalis*, 9 strains; *C. guilliermondii*, 2 strains; *C. intermedia*, 2 strains; *C. pulcherrima*, 1 strains; *Torulopsis pinus*, 1 strain.

In order to clarify what the ability of hydrocarbon utilization means biologically, 46 standard strains were served for test, of which the following 5 strains could utilize kerosene as a sole source of carbon: *Candida albicans* IAM 4888; *C. arborea* IAM 4147; *C. lipolytica* IAM 4947; *C. tropicalis* IAM 4862 and IAM 4924. Considering the result, the ability of utilizing kerosene would seem to characterize the genus, but it was not evident that it would characterize the species.

C. tropicalis Pk-233 gave the best cell yield among the above strains when kerosene was employed as a sole source of carbon and moreover, in the production of the cells of Pk-233, employing kerosene as a carbon material was compared with employing glucose.

INTRODUCTION

It has been known that some microorganisms could utilize hydrocarbon as a sole source of carbon and that they could not be differentiated from other common microorganisms without the ability of utilizing hydrocarbon as a sole source of carbon.¹⁾ Besides, they were isolated not only from soils and waters in contact with hydrocarbon in oil-fields and petroleum refineries, but also from common materials such as field soils, garden soils, sea waters and river waters.²⁾

It must be very important for the fermentation industry to employ hydrocarbons as a carbon source material instead of saccharides, which are more costly than hydrocarbons and have been employed as a carbon source

material for many years. The production of microbial cell substances from hydrocarbons has been studied by some investigators in order to produce protein and other valuables on commercial base. In 1963, Champagnat et al. reported that the petroleum fractions had been used as substrates for the cultivation of hydrocarbon-utilizing yeasts, from which protein-vitamin concentrates had been produced.³⁾ It would be considered more favorable to employ yeasts in order to produce microbial cell substances. The yeasts which have been found to utilize hydrocarbons as a sole source of carbon are as follows: *Candida albicans* (Komagata et al., 1963⁴⁾), *C. krusei* (Komagata et al., 1963⁴⁾); *C. lipolytica* (Just et al., 1951¹⁾; Komagata et al.,

3) A. Champagnat, C. Vernet, B. Lainé and J. Filosa, *Nature*, 197, 13 (1963).

4) K. Komagata, T. Nakase and N. Katsuya, presented at the annual Meeting of the Aricultural Chemical Society of Japan in 1953. Abstracts, p. 65.

1) G. W. Fuhs, *Arch. Microbiol.*, 39, 374 (1961).

2) R. Yamada, J. Takahashi and K. Kobayashi, *This Journal*, 27, 390 (1963).

1963⁴⁾; *C. rugosa* (Iizuka et al., 1963⁵⁾; Komagata et al., 1963⁴⁾; *C. tropicalis* (Just et al., 1951¹⁾; Komagata et al., 1963⁴⁾; Kawabata et al., 1965⁶⁾; *Debaryomyces* sp. (Tausson, 1936¹⁾); *Endomyces* sp. (Tausson, 1936¹⁾), *Hansenula anomala* (Komagata et al., 1963⁴⁾); *Hansenula* sp. (Rahn, 1906¹⁾); *Monilia* sp. (Tausson, 1936¹⁾); *Mycotorula japonica* (Aida and Yamaguchi, 1964⁷⁾); *Torula rossa* (Rahn, 1906¹⁾).

In this report, the isolation and determination of the yeasts, which were isolated from soils and waters in contact with petroleum in Chiba Refinery, Maruzen Oil Co., Ltd., and which could utilize kerosene as a sole source of carbon, were studied in order to produce proteins, fats and nucleic acids, and, in addition, the distribution of the ability of hydrocarbon-utilization in the yeasts was examined with the standard strains.

MATERIALS AND METHODS

Isolation. Isolation of hydrocarbon-utilizing yeasts was carried out by means of the enrichment culture in the mineral solution containing kerosene as a sole source of carbon (K_2HPO_4 , 0.8 g; KH_2PO_4 , 0.2 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $NaCl$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.05 g; $FeSO_4 \cdot 7H_2O$, 0.025 g; Na_2MoO_4 , 0.0005 g; $MnSO_4 \cdot 4H_2O$, 0.0005 g; Na_2WO_4 , 0.0005 g; $(NH_4)_2SO_4$, 2 g; yeast extract, 0.1 g; kerosene, 10 g; agar powder, if added, 20 g; distilled water, 1 liter; pH 7.2 or 4.5) by shaking at 25°C, the subsequent enrichment culture and the plating culture as shown in Table II.

Sample materials. Thirty-seven sample materials served for isolation were collected in Chiba Refinery, Maruzen Oil, Co., Ltd. and included oil-soaked soils, waste waters and so on, as shown in Table I.

Microorganisms for determination. Eighty-three isolates were grouped into the cultural groups with their colony appearances on the mineral agar slants containing kerosene, and the representatives of cultural groups were served for identification.

Determination. Determination was carried out

5) H. Iizuka, S. Shiio and N. Seto, presented at the Annual Meeting of the Agricultural Chemical Society of Japan in 1963. Abstracts, p. 64.

6) Y. Kawabata, J. Takahashi and K. Yamada, presented at the Annual Meeting of the Agricultural Chemical Society of Japan in 1963. Abstracts, p. 197.

7) T. Aida and K. Yamaguchi, presented at the Annual Meeting of the Agricultural Chemical Society of Japan in 1963. Abstracts, p. 197.

according to Lodder and Kreger-van Rij's methods⁸⁾ and their modification. A standard inoculum for all

TABLE I. SAMPLE MATERIALS FROM CHIBA REFINERY, MARUZEN OIL CO., LTD.

Sources	Properties	Numbers of Materials
about the topping unit	sand and soil	4
in the waste-treating plant	mud in a ditch	2
	sediment	1
	soil	4
about the storage tank of crude oil	sand	1
in the petrochemical plant	catalyzing sand	2
in a ditch into the waste-treating plant	drain water	4
in an oil-separator (in operation)	waste water	4
in an oil-separator (off operation)	waste water	4
in the storage tank of crude oil	bottom water	3
in the storage tank of cracking oil	bottom water	1
in the petrochemical plant	cooling water	1
in a drain ditch	crude oil	2
crude oil		4
	total	37

TABLE II. ISOLATION OF YEASTS FROM SAMPLE MATERIALS

	1st enrichment culture	2nd enrichment culture	final isolation by plating method
medium	200 ml of solution	20 ml of solution	20 ml of agar medium
substrate	2 g	0.2 g	0.2 g, flooding over agar
pH	4.5 and 7.2	4.5 and 7.2	4.5 to 5.0 and 7.2
vessel	500-ml flask	test tube, 25 mm in diameter	Petri dish
cultivation	rotary shaker	reciprocal shaker	
inoculum	solid materials, 1 to 2 g, or liquid materials, 5 ml	0.1 ml of 1st enrichment culture	1 loop of 2nd enrichment culture (streaked)
temperature	25°C	25°C	25°C
period	7 days	7 days	more than 7 days

8) J. Lodder and N.J.W. Kreger-van Rij, "The Yeasts, A Taxonomic Study" (1952).

tests was one drop of suspension including one loopful of 1-week culture on the mineral agar containing kerosene in 3 ml of sterile water or one loopful of the 1-week culture, and all test media except gelatin stabs were incubated at 25°C.

Morphological characteristics. Shape and size of the cells were measured with 20 cells of 3-day cultures in the malt extract broth and on the malt extract agar and of 1- to 2-week cultures in the mineral solution containing kerosene. Malt extract broth is composed of: malt extract (Difco), 25 g; distilled water, 1 liter; pH not adjusted.

Vegetative reproduction was observed by a phase contrast microscope on a slide culture of Lodder and Kreger-van Rij for a month. Ascospore formation was examined with the cells incubated on Gorodkova agar, on gypsum blocks in water, and in mineral solution without kerosene and ammonium sulphate which was shaken, on 3 days, 7 days, 14 days, 21 days and 30 days, by means of phase contrast microscope and also examined with the cells stained by Schaeffer-Fulton's modification of Wirzt's method.⁹⁾

Cultural characteristics. Appearances of cultures were observed in malt extract broth, on malt extract agar slant, on potato glucose agar slant and on mineral agar slant containing kerosene after 3 days' and 7 days' cultivation.

Physiological characteristics. Fermentation was observed on gas and acid production everyday for 10 days in Wickerham's test broth containing each one of sugars tested, with a Durham tube for fermentation. The medium without sugar was served for comparison.¹⁰⁾ The sugars tested were D-glucose, D-galactose, saccharose, maltose, lactose, raffinose, D-xylose, L-arabinose and D-mannitol. Assimilation of carbohydrates was observed on turbidity in Wickerham's test broth for assimilation after 1, 2, 3, and 4 weeks. The medium without sugar was served for comparison.¹⁰⁾ The carbohydrates tested were D-glucose, D-galactose, saccharose, maltose, lactose, D-xylose, L-arabinose, ethanol, D-mannitol, sodium citrate and sodium acetate. Production of acid was observed on Lodder and Kreger-van Rij's test agar slant (yeast extract, 1.5 g; Polypepton, 2.5 g, D-glucose, 50 g, calcium carbonate, 5 g; agar 20 g; distilled water, 1 liter) after 10 days' cultivation. In the case of assimilation of nitrate, growth in Wickerham's test

broth with potassium nitrate was compared with that in the broth containing ammonium sulfate as source of nitrogen, which was observed at intervals for 4 weeks.¹⁰⁾ For the presence of amylase, cultures were tested with a drop of iodine solution for the presence of starch breakdown products, after 1 and 2 weeks' incubation in Wickerham's test broth for assimilation, containing 2 g of soluble starch in 1 liter of the mineral solution. For the presence of protease, microorganisms were examined for gelatin liquefaction in gelatin stab, the appearance and the extent of which were noted at regular intervals for a month at 20°C. Lipase formation was detected on the triolein agar plate and tributyrin agar plate by means of Anderson's simple triglyceride technique¹¹⁾ and on beef suet agar plate of Lodder and Kreger-van Rij on 3 days, 7 days or 14 days. Production of starch-like compounds was determined by appearance of violet color on 10 days or 14 days streak culture by flooding Lugol's solution over.⁸⁾ Splitting of arbutin was determined by appearance of brown color around streak cultures within 10 days.⁸⁾ Reaction in litmus milk was determined by changes in appearance for a month.⁸⁾ Kerosene utilization of standard strains was detected with growth in mineral solution containing kerosene as a sole source of carbon, read after incubation for 14 days; strains which showed growth after two serial subcultures were recorded as positive.

TABLE III. SAMPE MATERIALS WHICH YEASTS WERE ISOLATED FROM

Sources	Properties	Numbers of Materials	Strains isolated at	
			pH 7.2	pH 4.5
in the waste-treating plant	soil	1		1
about the storage tank of crude oil	sand	1	5	
about the topping unit	sand and soil	1		2
in a ditch into the waste-treating plant	drain water	3		23
in an oil separator (in operation)	waste water	2	15	15
in an oil separator (off operation)	waste water	2		17
in a drain ditch	crude oil	1		5
		11	20	63
			83	

9) Society of American Bacteriologists, Manual of Methods for Pure Culture Study of Bacteria (1954).

10) L. J. Wickerham, "Taxonomy of Yeasts, U.S. Dept. of Agri., Tech. Bull.", No. 1029 (1951).

11) G. A. de Vries, Contribution to the knowledge of the genus *Cladosporium* Link ex Fr. Baarn, Holland (1952).

RESULTS

Isolation

As shown in Table III, 83 strains of yeasts were isolated from 10 out of 37 sample materials, most of which were waste water samples. In most cases, more strains of yeasts were isolated in pH 4.5 than in pH 7.2 of the medium for isolation, but, from waste water in an oil separator in operation, a large number of yeasts were isolated in pH 7.2 of the medium as well as in pH 4.5. This fact shows that operation of the oil separator would produce a good effect upon multiplication of yeast cells.

Determination

All of isolates were asporogenous yeasts, utilizing kerosene well as a sole source of carbon and showing good growth on malt extract agar, potato-glucose agar etc. Their descriptions are as follows;

Candida tropicalis (Castellani) Berkhout

Strains: Pk-51, 190, 191, 192, 233, 234, 235; 237 and 239.*

These strains were considered to belong to the genus *Candida*, forming no ascospore, having oval cells and developing pseudomycelium, though they could produce a considerable amount of acid transiently during the cultivation. The properties of isolates were a little different from those of the original in respect of such properties as growth in malt extract, ethanol assimilation and reaction in litmus milk. Furthermore, Pk-237 and Pk-239 were differentiated from the others with the streak culture on malt agar and splitting of arbutin. In spite of these facts, they should come within the category of *C. tropicalis*, for these isolates were identical with this species in many other properties important for it.

Growth in malt extract: Cells are short-oval to oval, sometimes round, $(3\sim10)\times(4\sim10)\mu$. Besides pseudomycelium, some true mycelia

are produced. A sediment and a membrane are formed.**

Streak culture on malt agar: Cream-colored to yellowish, tough, surface hirsute or smooth,* smooth margin surrounded by mycelium.

Slide cultures: Pseudomycelium develops abundantly, true mycelium formed, verticils developed, blastoconidia develop along pseudomycelial cells.

Ferment D-glucose, D-galactose, saccharose and maltose. Assimilate D-glucose, D-galactose, saccharose, maltose, D-xylose, L-arabinose, ethanol,** D-mannitol, acetate and citrate.

Production of acid: Positive.

Assimilation of potassium nitrate: Negative.

Enzymes: Amylase negative; Protease negative; Lipase positive with beef suet and tributyrin, and negative with triolein.

Production of starch-like compounds: Negative.

Splitting of arbutin: Positive or negative.*

Reaction in litmus milk: No change.**

Source: Soil and water in Chiba Refinery, Maruzen Oil Co., Ltd., 1964.

Candida guilliermondii (Castellani) Langeron et Guerra

Strains: Pk-236 and 241.

These strains were similar to *C. guilliermondii* except ethanol assimilation and were identified with this species.

Growth in malt extract: Cells are short-oval, small, $(2\sim4)\times(2.5\sim5)\mu$. A sediment is formed.

Streak culture on malt agar: Cream-colored, soft, smooth and glistening.

Slide cultures: Pseudomycelium consists of chains of stalagmoid cells.

Ferment D-glucose and saccharose. Raffinose weakly fermented. Assimilate D-glucose, D-galactose, saccharose, D-xylose, L-arabinose, acetate and citrate.

Production of acid: Positive.

Assimilation of potassium nitrate: Negative.

Enzymes: Amylase negative; Protease nega-

* asterisked strains have asterisked properties.

** double-asterisked properties are different from those of the original.

tive; Lipase positive with beef suet and tributyrin, and negative with triolein.

Production of starch-like compounds: Negative.

Splitting of arbutin: Positive.

Reaction in litmus milk: No change.

Source: Drain water in a ditch into the waste-treating plant in Chiba Refinery, Maruzen Oil Co., Ltd. 1964.

Candida intermedia (Ciferri et Ashford) Langeron et Guerra

Strains: Pk-238 and 243.*

These strains were similar to *C. intermedia* except ethanol assimilation and there are considerable variations between these strains in respect of streak cultures on malt extract, growth in malt broth and maltose fermentation.

Growth in malt extract: Cells are oval, usually in short chains, $(2\sim5.5)\times(2.5\sim7.5)\mu$. A sediment or a sediment a ring and a pellicle* are formed.

Streak culture on malt agar: White-yellowish or whitish, soft, glistening and smooth or wrinkled.

Slide cultures: Pseudomycelium is not very well developed, and there is not much differences between cells of pseudomycelium and blastospores.

Ferment D-glucose, D-galactose, saccharose and raffinose for one third. Maltose fermented or not.* Assimilate D-glucose, D-galactose, saccharose, maltose, lactose, D-xylose, L-arabinose, ethanol,** D-mannitol, acetate and citrate.

Production of acid: Positive.

Assimilation of nitrate: Negative.

Enzymes: Amylase negative; Protease negative; Lipase positive with tributyrin and negative with beef suet and triolein.

Production of starch-like compounds: Negative.

Splitting of arbutin: Positive.

Reaction in litmus milk: No change.

Source: Soil, water and crude oil in a drain ditch in Chiba Refinery, Maruzen Oil Co., Ltd., 1964.

Candida pulcherrima (Lindner) Windisch

Strain: Pk-229.

This strain was similar to *C. parapsilosis* in the points of formation of blastoconidia in a slide culture and cream-colored streak culture on the malt extract, but, agreeing physiologically with *C. pulcherrima*, this microorganism was identified with *C. pulcherrima*.

Growth in malt extract: Cells are round to short-oval, $(2\sim4.5)\times(2\sim6)\mu$. A sediment is formed.

Streak culture on malt agar: Cream-colored, soft, glistening and smooth.

Slide cultures: Pseudomycelium is well developed, blastospores sometimes form small chains, and blastoconidia are formed.**

Ferment D-glucose only. Assimilate D-glucose, D-galactose, saccharose, maltose, D-xylose, L-arabinose, ethanol, D-mannitol, acetate and citrate. Production of acid: Positive.

Assimilation of nitrate: Negative.

Enzymes: Amylase negative; Protease negative; Lipase positive with tributyrin, weakly positive with beef suet and negative with triolein.

Production of starch-like compounds: Negative.

Splitting of arbutin: Positive.

Source: Soil in the waste-treating plant in Chiba Refinery, Maruzen Oil Co., Ltd., 1964.

Torulopsis pinus Lodder et Kreger-van Rij

Strain: Pk-150.

This microorganism is not physiologically active and not similar to any species listed in Lodder and Kreger-van Rij's descriptions. However, it would be considered as a variety of *Torulopsis pinus* according to Lodder and Kreger-van Rij's key to the species, for it agrees with *T. pinus* in respect of sugar utilization, though differing from the latter in some important properties, such as nitrate assimilation, capsule formation and cultural characteristics.

Growth in malt extract: Cells are around, slightly oval, $(2.5\sim6)\times(3\sim6)\mu$, not surrounded by a capsule.** A pellicle is formed.**

Streak culture on malt agar: Smooth, grayish olive to olive.**

Slide cultures: No pseudomycelium is formed.

No fermentation. Assimilate D-glucose and acetate. Production of acid: Negative.

Assimilation of nitrate: Positive.**

Enzymes: Amylase negative; Protease negative; Lipase positive with tributyrin, negative; with beef suet and triolein.

Production of starch-like compounds: Negative.

Splitting of arbutin: Negative.

Reaction in litmus milk: No change.

Source: Sand about the storage tank of crude oil in Chiba Refinery, Maruzen Oil Co., Ltd., 1964.

Relation of the isolates with origin

From drain waters in a ditch flowing in the waste-treating plant, *Candida intermedia*, *C. guilliermondii* and *C. tropicalis* were isolated, where the former two species were swarmed in large numbers. However, the latter would turn out to surpass the formers in numbers in the oil separator, into which the drain

was poured, and only *Candida tropicalis* was isolated from the waste waters of the oil separator, as shown in Table IV.

TABLE V. ABILITY OF STANDARD YEASTS STRAINS TO UTILIZE KEROSENE AS A SOLE SOURCE OF CARBON

To Utilize kerosene	Strains employed
able	<i>Candida albicans</i> IAM 4888 <i>C. arborea</i> IAM 4147 <i>C. lipolytica</i> IAM 4947 <i>C. tropicalis</i> IAM 4862 and IAM 4924
not	<i>Brettanomyces bruxellensis</i> IFO 677 <i>Candida albicans</i> IAM 4905 <i>C. guilliermondii</i> IAM 4412 <i>C. krusei</i> IAM 4801 <i>C. mycoderma</i> IAM 4564 <i>C. parapsilosis</i> IAM 4488 <i>C. pseudotropicalis</i> IAM 4829 <i>C. pseudotropicalis</i> var. <i>lactosa</i> IAM 4843 <i>C. utilis</i> IAM 4215, IAM 4220 and IAM 4233 <i>Cryptococcus neoformans</i> IAM 4514 and IAM 4772 <i>Debaryomyces hansenii</i> IAM 4356 <i>Enodmyces magnusii</i> IAM 4754 <i>Endomycopsis capsularis</i> IAM 4307 <i>Eremascus fertilis</i> IFO 691 <i>Hanseniaspora valbyensis</i> IAM 4010 <i>Hansenula anomala</i> IAM 4213 and IAM 4239 <i>Kloeckera apiculata</i> IAM 4018 and IAM 4438 <i>Lipomyces starkeyi</i> IAM 4753 <i>Nadsonia fulvescens</i> IFO 666 <i>Nematospora coryli</i> IFO 658 <i>Pichia membranaefaciens</i> IAM 4025 and IAM 4154 <i>Pityrosporum ovale</i> IFO 656 <i>Rhodotorula glutinis</i> IAM 4757 <i>Saccharomyces cerevisiae</i> IAM 4428, IAM 4702 and IAM PG 1-1 <i>S. chevalieri</i> IAM OG 1-39 <i>S. elegans</i> IAM PG 5-95 <i>Saccharomycodes ludwigii</i> IAM 4380 <i>Schizosaccharomyces pombe</i> IFO 340 <i>Schwanniomyces occidentalis</i> IAM 4332 <i>Sporobolomyces salmonicolor</i> IAM 4764 <i>Torulopsis colliculosa</i> IAM 4426 <i>Trichosporon cutaneum</i> IAM 4450 <i>Trigonopsis variabilis</i> IAM 4443

TABLE IV. ISOLATES AND THEIR ORIGINS

Origins	Isolates-its numbers	
	pH 7.2	pH 4.5
soil in the waste-treating plant		<i>C. pulcherrima</i> -1
sand about the storage tank of crude oil	<i>T. pinus</i> -5	
sand and oil about topping unit		<i>C. intermedia</i> -2
drain water in a ditch into the waste-treating plant		<i>C. guilliermondii</i> -9 <i>C. intermedia</i> -9 <i>C. intermedia</i> *-2 <i>C. tropicalis</i> *-3
waste-water in an oil separator (in operation)	<i>C. tropicalis</i> -15	<i>C. tropicalis</i> -14 <i>C. tropicalis</i> *-1
waste-water in an oil separator (off operation)		<i>C. tropicalis</i> -17
crude oil in a drain ditch		<i>C. intermedia</i> -5

Distribution of the ability of utilizing kerosene as a sole source of carbon in the type culture collection of the yeasts

It happened that most of yeast isolates utilizing kerosene as a sole source of carbon would belong to the genus *Candida*. Therefore, 46 yeast strains of IAM^{*1)} and IFO^{*2)} type culture collections were tested for utilizing kerosene as a sole source of carbon in order to clarify the distribution of the ability of utilizing kerosene in the standard strains of the yeasts as shown in Table V. Considering the result, it seemed that most of yeasts utiliz-

ing kerosene as a sole source of carbon would belong to the genus *Candida* and that the ability of utilizing kerosene would not be such a property as could distinguish a species.

Possibility of producing microbial cell substances

Production of microbial cells, which were gathered by means of centrifugation from 2 weeks cultures of the isolates, in mineral solution containing kerosene is shown in Table VI. There are variations among cell yields of the isolates, which seemed to result from variations in distribution of kerosene and supply of oxygen in cultures. In production of microbial cells with *C. tropicalis* Pk-233, employing glucose as a sole source of carbon, 0.11 g of cells (dried weights) were gained from 1 g of glucose, while, in the case of kerosene, 0.074 g to 0.092 g of cells (dried weights) were gained from 1 g of kerosene, though there were a good deal of remnants not utilized, as shown in Table VII. Therefore, it seemed that employing kerosene for producing microbial cell substances was not always inferior to employing glucose. In addition, *C. tropicalis* Pk-strains, *C. guilliermondii* Pk-236 and *C. pulcherrima* Pk-229 could produce considerable amounts of riboflavin from kerosene as well as from glucose, which was diffusible into the medium.

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TABLE VI. CELL YIELDS, MICROORGANISMS BEING CULTURED WITH KEROSENE AS A SOLE SOURCE OF CARBON

Microorganisms		dry weight*
<i>Candida tropicalis</i>	Pk-51	43.8
	Pk-190	39.4
	Pk-191	54.7
	Pk-192	52.2
	Pk-233	314.0
	Pk-234	45.6
	Pk-235	7.7
	Pk-237	173.0
<i>Candida guilliermondii</i>	Pk-239	66.1
	Pk-236	37.2
<i>Candida intermedia</i>	Pk-238	73.2
	Pk-243	43.0
<i>Candida pulcherrima</i>	Pk-229	46.3
<i>Torulopsis pinus</i>	Pk-150	7.4
<i>Candida albicans</i>	IAM 4888	52.0

* mg of lyophilized cells, gained from 150 ml of cultures in 500-ml flasks for 2 weeks at 25°C.

TABLE VII. PRODUCTION OF THE CELLS OF *Candida tropicalis* Pk-233 WITH GLUCOSE OR KEROSENE AS A SOLE SOURCE OF CARBON

	Glucose	Carbon sources kerosene		
		1	2	3
starting amounts of substrates*	9.274	10.39	10.32	10.39
amounts of substrates consumed for 2 weeks*	9.060	1.88	2.28	1.90
consumed/employed × 100	97.7	18.1	22.1	18.3
cell yields, lyophilized*	0.9558	0.1838	0.1888	0.1748
cell yields/consumed substrates	0.11	0.074	0.083	0.092

* g in 200 ml.

*1) Institute of Applied Microbiology, the University of Tokyo.

*2) Institute for Fermentation, Osaka.

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