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Rapid growth of a thermotolerant yeast on palm oil

C. Lee,* T. Yamakawa and T. Kodama

A thermotolerant and rapidly-growing yeast for production of single cell protein from palm oil was isolated and identified as *Candida tropicalis* F129. The optimum temperature and pH for growth were 38°C and 6.0, respectively. The yeast grew with a high specific growth rate, of 0.92/h in 2% (v/v) palm oil medium, compared with other oil-assimilating yeasts or hydrocarbon-utilizing thermophilic yeasts. The overall cell yield was 1.01 g dry cells/g palm oil after 12 h.

Key words: Palm oil, rapid growth, specific growth rate, thermotolerant yeast.

Palm oil is an economically-important oil in many tropical countries. Recently, the rapidly increasing production of palm oil has caused a decline in its world market price. Several possible solutions to the serious problem of palm oil over-production, in tropical countries such as Malaysia, are based on microbial utilization of the oil. Nakahara *et al.* (1982) applied *Candida blankii* for biomass production from palm oil. Montet *et al.* (1983) used palm oil stearin (the solid fraction of palm oil) as a carbon source for biomass production by several yeast strains. Koh *et al.* (1983, 1985) isolated *Torulopsis candida* and *Acinetobacter calcoaceticus* from palm oil for single cell protein (SCP) production. Kawashima *et al.* (1983) used palm oil to produce biosurfactant. Laborbe *et al.* (1989) studied batch and continuous cultivation of *Candida rugosa* in a palm oil medium for SCP production. Since the melting point of palm oil is 34 to 45°C and heat liberation in fermentations utilizing lipids as substrates is expected to be higher than that in a traditional carbohydrate fermentation, we tried to isolate thermophilic yeasts with high growth rates by using palm oil as a substrate for microbial cell protein production. The growth characteristics and chemical analysis of the isolated yeast were also studied.

Materials and Methods

Media

The isolation medium for palm oil-assimilating yeasts was composed (w/v) of 2% palm oil (Nippon Oil and Fats Co., Ltd), 0.4% NH_4NO_3, 0.47% KH_2PO_4, 0.03% $Na_2HPO_4.12H_2O$, 0.1% $MgSO_4.7H_2O$, 0.001% $FeSO_4.7H_2O$, 0.001% $CaCl_2.2H_2O$, 0.001% $MnSO_4.4H_2O$, 0.01% yeast extract, and 0.002% chloramphenicol. The medium was adjusted to pH 5.5 and mixed with a homogenizer for 3 min, then sterilized at 121°C for 10 min.

The medium for growth studies contained (w/v) 2% palm oil, 0.4% NH_4NO_3, 0.47% KH_2PO_4, 0.03% $Na_2HPO_4.12H_2O$, 0.1% $MgSO_4.7H_2O$, 0.001% $FeSO_4.7H_2O$, 0.001% $CaCl_2.2H_2O$, 0.001% $MnSO_4.4H_2O$, 0.2% yeast extract, and 0.1% peptone.

Isolation Method

Palm oil-assimilating yeasts were isolated from soil or plant samples collected at various places around Tokyo, by means of shaking cultures in L-shaped test tubes and plate cultures at 43°C.

Cultivation in Jar Fermenters

A 2-l jar fermenter (Model MB-C, Iwashiya K. Sawada Co., Ltd) was used to study the effects of temperature, pH and surfactant on growth of the yeast. pH was automatically controlled by addition of 2.25 M H_2SO_4 or 2.5 M NH_4OH. Air was sparged into the fermenter at 1.0 vol/vol.min and the agitation speed was kept at 1000 rev/min. The inoculum was grown in 50 ml of the palm oil medium in a 500-ml shake flask for 12 h at 43°C on a reciprocal shaker (120 rev/min) and all of this was inoculated into 1 l of the same medium in a jar fermenter.

Cultivation in Test Tubes

The other growth studies were examined in test tubes (21 × 200 mm) containing 10 ml of the medium inoculated with 0.1 ml of preculture broth and incubated at 38°C for 7 to 15 h with shaking.

Analytical Methods

Cell concentration was estimated as described below. Six ml of ethyl acetate was added to 3 ml of the culture broth, to remove the residual palm oil from the culture broth. The mixture was shaken gently and the water layer separated off. Ethylene glycol

C. Lee is with the Department of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan. T. Yamakawa and T. Kodama are with the Department of Agricultural Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan; fax: (886)(2) 363 3123. * Corresponding author.

(1:1, v/v) was added to the water layer to obtain an homogeneous cell suspension, and then this was diluted as necessary so that its optical density at 540 nm could be measured with a Spectronic-20 (Bausch and Lomb). An optical density of 1.0 corresponded to a dried cell weight of 0.435 g/l.

Cellular protein was estimated by the method of Lowry and total nucleic acid by the method of Levine & Cooney (1973). Palm oil concentration was determined by the method of Bligh & Dyer (1959). Amino acids were analysed with an amino acid auto-analyser. Taxonomic studies were by the standard procedure of Lodder (1970).

Results and Discussion

Isolation of Palm-oil-assimilating Yeast

From 236 soil or plant samples, one yeast strain, F129, was isolated as a rapid grower in the palm oil medium at 43°C. The strain was identified as *Candida tropicalis* according to Lodder (1970). When grown on YM medium it had a spherical cell shape with multi-polar budding. Pseudomycelium was observed on corn meal agar. Neither spore nor true mycelium was observed. Stationary cultures in test tubes showed surface growth of pellicle and ring, accompanying powdery sediment formation. The strain did not assimilate KNO_3. The range for growth was 12 to 45°C.

Growth Characteristics

Strain F129 grew well between 30 and 43°C, and was maximal at 38°C at pH 5.5 in a 2-l jar fermenter using 2% (w/v) palm oil as a carbon source (Figure 1). The optimum growth in the shake-tube culture with glucose medium was also at 38°C (data not shown).

These results indicate that strain F129 is a distinctly more thermotolerant strain than the oil-utilizing yeasts described previously (Burkholder *et al.* 1968; Hotinger *et al.* 1974; Montet *et al.* 1983). However, the specific growth rate in the palm oil medium markedly decreased at 30 to 34°C. When glucose was used as a carbon source, the decrease in the growth rate at 30 to 34°C was not so significant (data not shown). This tendency might be caused by the melting point of palm oil, which would not mix well with the liquid medium at the lower temperatures tested. The optimal growth temperature in the glucose medium was still 38°C.

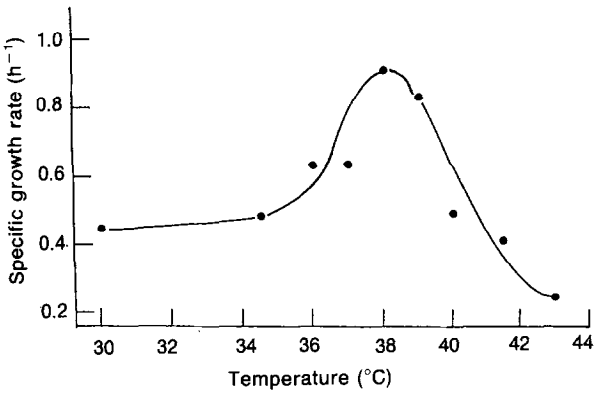

Figure 1. Effect of temperature on the growth of *Candida tropicalis* F-129. The fermenter cultivation was carried out at 30°C to 43°C in palm oil (2%, w/v) medium.

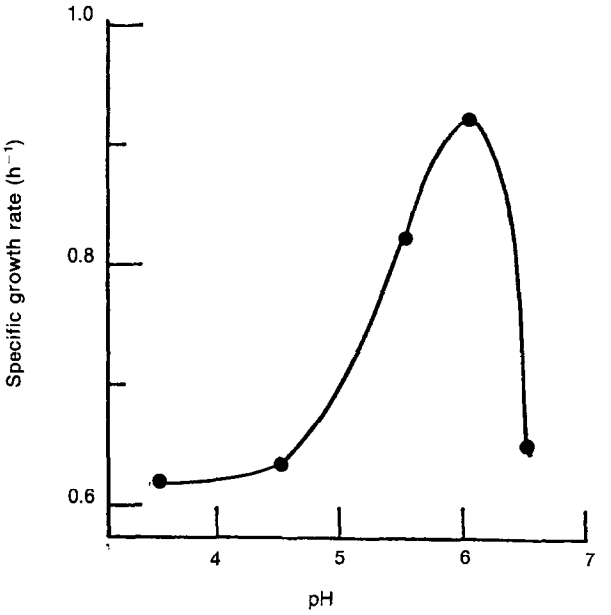

Figure 2. Effect of pH on the growth of *Candida tropicalis* F-129. The fermenter cultivation was carried out at 38°C in palm oil (2%, w/v) medium.

At 38°C, strain F129 had an optimal pH, for growth in the 2% (w/v) palm oil medium in a 2-l jar fermenter, of 6.0 (Figure 2). The maximum specific growth rate of the strain under these conditions was 0.92 h^{-1}. Since the melting point of palm oil is above 34°C, the relatively high temperature of 38°C is advantageous for the fermentation of palm oil. The isolated yeast grew best on palm oil at 38°C, and the specific growth rate (0.92 h^{-1}) of the strain is higher than those reported for hydrocarbon-assimilating and methanol-assimilating yeasts and other oil or fat-assimilating yeasts (Montet *et al.* 1983; Koh & Minoda 1984). Nakahara *et al.* (1982) selected a thermotolerant strain of *Candida blanki* which was capable of growing at 40°C for utilizing palm oil but the specific growth rate (0.37 h^{-1}) was lower than that of strain F129 at the same temperature.

The effect of various nutrients on the yeast cell growth is shown in Figure 3. The results indicated that yeast extract (Difco Laboratories, Detroit, MI, USA), Casamino acid (Difco) and peptone (Difco) were effective in increasing cell yield. Although thermophilic yeasts often need growth factors such as biotin or a vitamin complex (Levine & Cooney 1973; Minami *et al.* 1978), the addition of 9 vitamin mixtures (Table 1) had no effect on the growth of this strain. Addition of sucrose esters of fatty acids (Nitto ester F-140; Dai-Nippon Sugar MFG Co. Ltd, Tokyo, Japan) did not improve cell production nor its growth rate, although it caused better emulsification of palm oil.

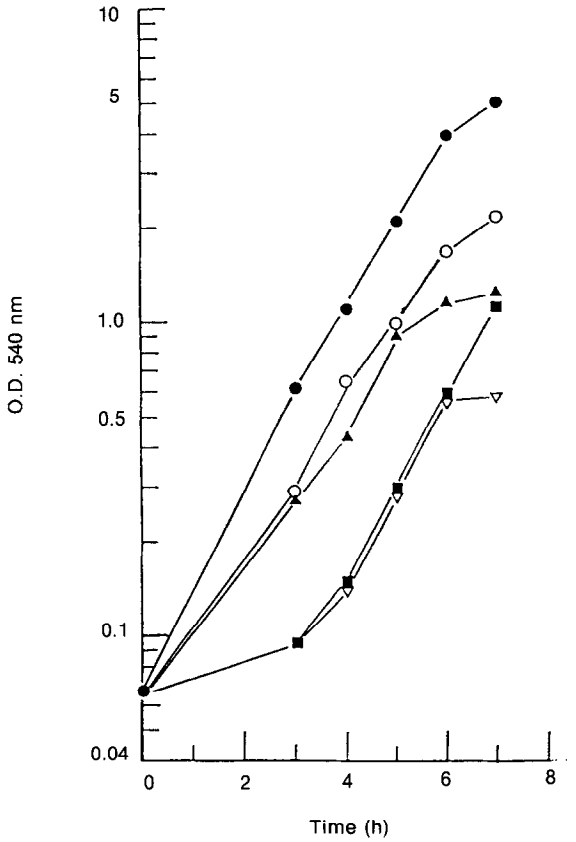

Figure 3. Effect of various nutrients on cell growth. Shaking culture was carried out in a test tube at 38°C using the palm oil medium without yeast extract and peptone. Composition of the vitamin mixture added (■) was described in Table 1. Yeast extract (●), casamino acid (○) and peptone (▲) were tested at 0.2%, 0.2% and 0.1% (w/v), respectively. An O.D. value of 1.0 was equivalent to 0.435 mg cells/ml. ▽—Control, with no additional nutrients.

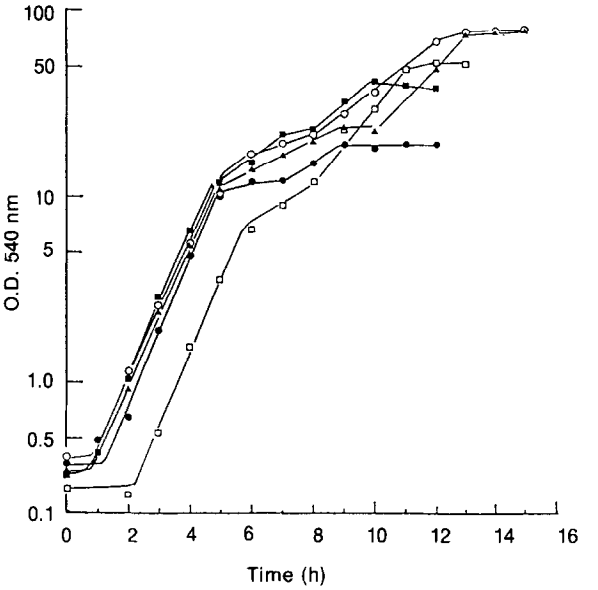

Figure 4. Effect of various concentrations of palm oil on cell growth. The fermenter cultivation was carried out in 1-l of palm oil medium at 38°C. Palm oil concentrations (w/w) tested were 1% (●), 2% (■), 3% (□), 4% (▲) and 5% (○). An O.D. value of 1.0 was equivalent to 0.435 mg cells/ml.

Table 1. The concentration and composition of the mixed vitamin solution used in this study.

Compound	Final concentration in medium (μg/l)
Vitamin B_1	2
Vitamin B_2	1000
Vitamin B_6	200
Vitamin B_{12}	2
Vitamin K	2000
Biotin	2
Choline	4000
Inositol	2000
Folic acid	20
Nicotinamide	200
p-Aminobenzoic acid	200
Pantothenic acid	200

Table 2. Amino acid composition of yeast protein.

Amino acid	FAO reference* (g/100 g protein)	*Candida tropicalis* F-129 (g/100 g protein)
Aspartic acid		8.9
Threonine	4.0	6.4
Serine		5.8
Glutamic acid		15.6
Glycine		4.5
Alanine		6.2
Citrulline		4.0
Valine	5.0	5.7
Cystine		0.5
Methionine	3.5 (Met + Cys)	1.3
Isoleucine	4.0	5.1
Leucine	7.0	7.2
Tyrosine		2.8
Phenylalanine	6.0 (Tyr + Phe)	4.3
Ornithine		0.2
Lysine	5.5	8.4
Histidine		2.2
Arginine		5.4
Proline		3.5

* Levine & Cooney (1973).

The growth yields at various concentrations of palm oil were also investigated. At concentrations between 1% to 4% (w/v), cell mass increased linearly with oil concentration; the maximum yield of 33.9 g/l was obtained at 4% palm oil, while 35.4 g/l dried cells was obtained at 5% (w/v) (Figure 4). Diauxic growth was observed at all concentrations of palm oil (1 to 5%) in shaking culture (Figure 4), as also observed by Laborbe *et al.* (1989). Strain F129 consumed 85% of the palm oil added at 2% (w/v) concentration after 12 h, with a cell yield of 101% on the basis of consumed carbon source.

Analysis of the F129 cells gave a dry cell protein content of 35% (w/w) and a total nucleic acid content 3.1%. The amino acid profile of the yeast protein is shown in Table 2. As with many yeasts (Koh *et al.* 1983; Montet *et al.* 1983), this strain has a low content of methionine and cysteine, while lysine comprised 3% (w/w) of the dry cell weight. The protein content is considered to be well-balanced according to the Food and Agricultural Organization of the United Nations (FAO) reference levels (Levine & Cooney 1973). Sinskey & Tannenbaum (1975) reported that microorganisms with high growth rates had high RNA contents, but this strain had a rather low nucleic acid content [3.1% (w/w) of dry weight], compared with other yeasts (5 to 10%) (Ogata *et al.* 1970; Levine & Cooney 1973; Laborbe *et al.* 1989). The nucleic acid content of this strain corresponds to 1.06% (w/w) of protein, and this is similar to the nucleic acid content of wheat protein.

The results reported here indicate that *Candida tropicalis* strain F129 possesses a very high specific growth rate ($0.92\ h^{-1}$), giving high cell yields (101%) on palm oil at higher temperatures compared with other thermophilic oil-assimilating yeasts, and the strain will therefore be useful for biomass production using palm oil.

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