Microbial Utilization of Palm Oil

I. Screening and Cultural Conditions of Yeast for Cell Production

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微生物에 의한 palm oil의 利用

菌体蛋白質生産을 위한 우수균주의 選定과 培養條件

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Summary

The microbial utilization of palm oil for single cell protein production can provide a protein source for human consumption and animal feed from the safe raw material. It is desirable to resolve the problem of overproduction of the oil in near future. Therefore many strain capable of assimiliatng palm oil were isolated from various natural sources. Among these strains, the strain Y—128 which was identified with *Torulopsis candida* TAMY128, assimilated crude and refined palm oil effectively.

Substrate was emulsified using nonionic surfactant. The addition of surfactant and emulsification with homogenizer were effective on yeast cell growth. In shaking culture of this strain, corn steep liquor as a natural nutrient was good for yeast growth, and ammonium sulfate was more effective than any other nitrogen sources.

Introduction

A growing concern for the acute food needs of the world's exploding population has led to the examination of a variety of potential food resources. Among these, the single cell protein probably presents the best chance for the development of a unique unagriculturally based food supply. There are, in this viewpoint, many reports and reviews on the production of single cell protein on agricultural by-products, sulfite waste liquor, wood hydrolysates, gas oils and hydrocarbons (Litchfield 1977, Mateles et al. 1968, Tannenbaum et al. 1975, Minoda 1976).

However the fact that the biological activity of the products of microbiological synthesis is thus far completely unknown precludes their direct utilization either for human food or even as animal feed until there is conclusively evidence as to harmlessness (Mateles et al. 1968). A number of problems associated with microbial protein have to be solved to make it an acceptable, safe and inexpensive source of protein for human consumption. Accordingly we have made an attempt to produce yeast cell protein from palm oil which is high-yielding tropical products, nontoxic substrate. Landon(1975), Davis et al.(1979), Mason and

Ginar(1980) reported that palm oil production had been increasing steadily over recent years and was currently in excess of 4 million metric tons per annum, much of which was incorporated in edible products. The World Bank estimated that 4.6 million metric tons of palm oil would be produced throughout the world last year, increasing to between 10.5 million and 11 million metric tons by the year 2000. The share of palm oil in the international fats and oils markets will leap nearly 9% during last year to reach 25.5% by 1990.

Microbial utilization of palm oil has a significance to supply protein resource for feed and human consumption, and to resolve the over-production of palm oil in near future. In this study, we isolated an efficient yeast strains capable of assimilating palm oil from natural sources, and carried out studies on the taxonomy and cultural conditions of this strain.

Materials and Methods

Materials

Palm oil was supplied by Kao Soap Co. Ltd. crude palm oil was consisted of 76.40% carbon 11.73% hydrogen and 0.04% nitrogen(by CHN automatic analyzer),

Media and Culture

Isolation medium contained 2% palm oil as a sole carbon source, 0.4% NH_4NO_3, 0.47% KH_2PC_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, 0.002% chloramphenicol and 0.1% surfactant, The medium was treated with homogenizer for 3 minutes, and adjusted to pH 5.5. In plate culture medium, it was difficult to differenciate colony from palm oil droplets, so carbon source was changed to 2% dextrose. Stock culture medium was consisted of 1% meat extract, 0.5% peptone, 1% malt extract, 2% palm oil and 1.5% agar. Chlormpahenicol was used to restrain the bacterial growth.

50 ml of the medium in 500 ml shaking flask was inoculated with 1 ml of precultured broth, and incubated at 30°C on a reciprocal shaker.

Isolation of yeasts capable of assimilating palm oil

yeasts capable of assimilating palm oil were isolated from soil and plant samples collected from various places in Tokyo, using enrichment culture techniques shown in Fig. 1.

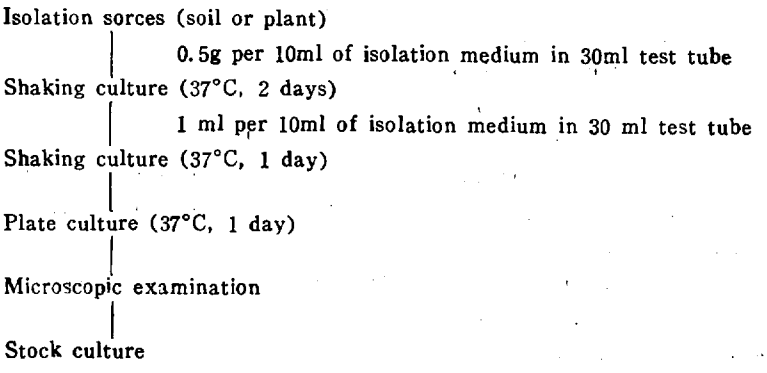

Fig 1. Method of yeast isolation

Assay of yeast

After adquate cultivation, 10 ml from the culture broth was taken and added 1 ml of 11 N NaOH solution, and then heated at 100°C for 10 min and filtered after cooling. Protein was determined by modified Lowry method (Lowry et al. 1951).

Taxonomic study of the strain Y-128

Diagnostic tests of strain Y-128 were carried out according to the method of Lodder(1970).

Results

Isolation of yeasts capable of assimlating palm oil

More than two hundred strains were isolated from 240 samples. However only some of the strains assimilated palm oil effectively, most isolates were poor in growth. Fig. 2 shows the comparision of crude palm oil with refined one as the carbon source in shaking culture of some isolates. Most strains could assimilate crude palm oil better than refined one. Among these, the strain Y-128 shows higher protein yield on crude and refined palm oil than any other strains. The srtain Y-128 was isolated from field soil in Tokyo. The amount of protein in culture broth after 24 hr cultivation at 30°C was 3.76 mg/ml, when we used crude palm oil

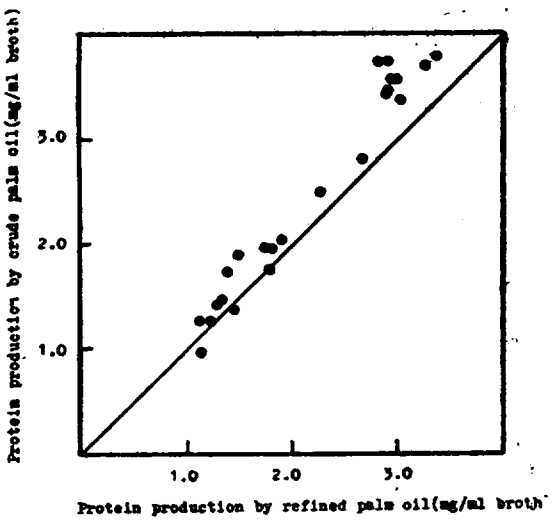

Fig 2. Comparison of crude palm oil with refined palm oil as carbon soures in shaking culture.

Taxonomic study of the strain Y-128

Microscopic appearance of strain Y-128 is shown in Fig. 3. Growth in YM media(peptone. yeast extract. malt extract medium):

After 3 days at 25°C, cells are round, 2.8-5.6 μ size. A sediment and pellicles are formed. The pellicles are gray, thin, smooth and creeping.

Streak culture on YM media agar: After one month at 25°C, the streak culture is cream colored, raised, undulated, dull and smooth.

Liquefaction of gelatin: Negative.

Slide culture: No psudomycelium is formed.

Fermentation: Absent.

Sugar assimilation:

		glucose	+	galactose	+
L-sorbose	—	maltose	+	sucrose	+
cellobiose	—	trehalose	+	lactose	—
melibiose	+	raffinose	+	melezitose	+
inulin	—	soluble starch	+	D-xylose	+
D-arabinose	—	L-arabinose	—	D-ribose	—
L-rhamnose	—	ethanol	+	glycerol	+
D-mannitol	+	D-sorbitol	+		
α-methyl-D-glucoside			+		
salicin	—	DL-lactic acid	+	succinic acid	+
citric acid	+	inositol	—	galactitol	+

Assimilation of KNO_3: Negative.

Splitting of arbutin: positive.

Production of compounds like starch: Negative.

Growth in vitamin-free medium: Increase observed.

Osmo-tolerence: 13-14%.

Urease test: Negative.

Fig 4. The yeast strain *Torulopsis candida* TAMY128 grown on YM-media agar (x 400).

According to these taxonomic studies, the morphological and physiological properties of this strain were very similiar to those of *Torulopsis candida* described by J. Lodder (1970) in "The Yeast", except that the strain did not assimilate some carbon compounds, such as cellobiose, salicin and L-arabinose. Futhermore this strain can grow below pH 2.0 and the growth is recognized at 42°C. Therefore the yeast strain Y-128 was identified with *Torulopsis candida* TAMY128.

Cultivation of the yeast

Fig. 4 shows the growth curve of *Torulopsis candida* TAMY128 in shaking culture at 30°C. The special characteristics of this strain is the change of pH in the culture broth, and this lowered rapidly in the course of yeast growth. This strain grew well even in acidic condition of culture broth.

The addidition of surfactants and then emulsification with homogenizer stimulated the yeast growth as shown in Table 1, as the palm oil was dispersed into the broth in small droplets. Among the surfactants used in this study, sucrose fatty acid esters were more effective than other ones. In Fig. 5, the

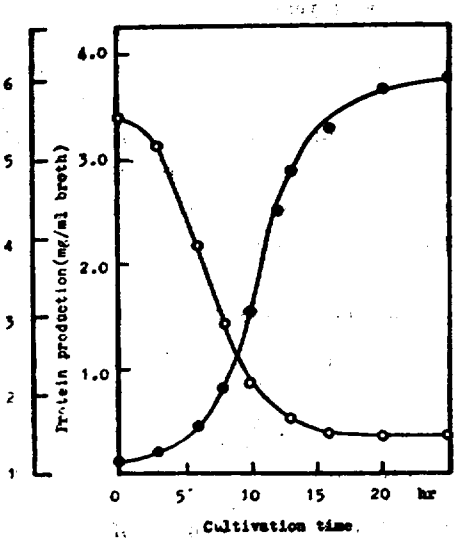

Fig 3. Time course of yeast growth in shaking culture at 30°C. ○—○ pH, ■—■ Yeast protein. Cell growth was determined by the method of Lowry et al .

assimilation of oil by the adsorbed cells is clearly demonstrated. In initial state of growth, small drops of palm oil were attached by the yeasts and assimilated more rapidly than large ones. The main function of the surfactant is to aid the formation and stablization of oil emulsion. In this experiment, the concentration of surfactant was enough in the range of 0.05 to 1.0% Lower concentration of surfactant presented low effect of palm oil dispersion in the broth, and higher concentration the inhibition effect in cell growth.

The effect of natural nutrients on cell growth was investigated, and the results are presented in Table 2. In this experiment, rapid drop of pH in culture broth was supposed to inhibit the cell growth as shown in Fig. 4. Therefore phosphate sources among the constituents of isolation medium were increased by two times to compensate the pH of the broth, and then

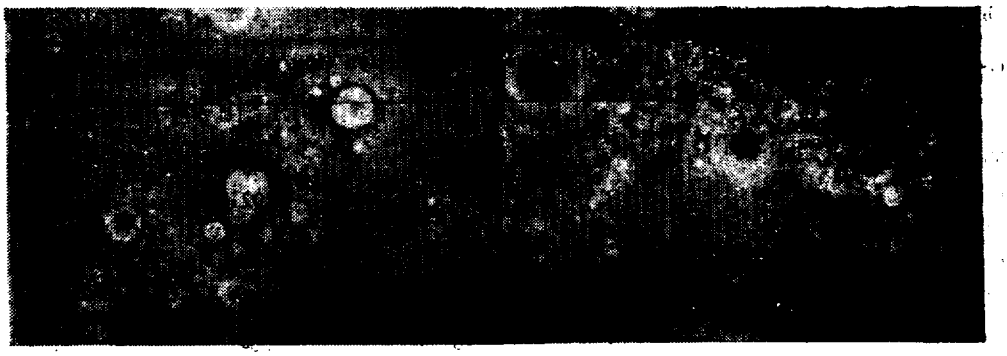

Fig 5. Photomicrograph of the oil-yeast dispersion showing the assimilatien of oil drops. (a) 4 hr from start, palm oil was dispersed in the broth and the yeast attached small droplets of oils. (10 hr from start, small droplets were assimilated by the yeast but large ones of palm oil were still remained (x 400).

seven natural nutrients were separately added in the medium. In initial state of cultivation, much more cells were obtained in the case of natural nutrients added. The addition of carnitine was some effective in initial state of growth, however the effect was not so much in palm oil fermentation. Among the natural nutrients, corn steep liquor and yeast extract were good in cell yield. The addition of natural nutrients made lag time shorter and growth rate greater.

Table 1. Effect of Surfactant Addition on Yeast Growth
0.1% addition and homogenization

Kind of surfactant	HLB-value	Shape	Protein production
Sucrose fatty acid ester F- 140	13	Powder	3.85 mg/ml of broth
〃 F- 160	15	〃	3.78
〃 P-1670	16	〃	3.85
〃 OW-1570	15	Paste	3.62
Emulgen - 902	12.4	Liquid	3.78
〃 - 910	12.2	〃	1.88
〃 - 911	13.7	〃	3.78
〃 - 920	15.5	Powder	3.80
〃 - 930	15.1	〃	2.68
〃 - 931	17.2	〃	3.78
〃 - 985	18.9	〃	3.80
〃 - 707	10.5	Liquid	2.73
〃 - 709	15	〃	2.68
Control(no addition)			2.48

Protein was determined after 24 hours cultivation in shaking flask at 30°C

Table 2. Effect of Growth Factor Addition on Yeast Growth

Growth factor (0.01%)	Protein production (mg/ml of broth) 8 hours	12 hours	24 hours
Control (no addition)	0.46	1.98	3.90
Yeast extract	0.78	2.48	4.40
Carnitine	0.94	2.02	3.77
Malt extract	0.37	2.38	4.27
Corn steep liquor	0.90	2.52	4.40
Meat extract	0.90	2.52	4.40
Peptone	0.46	2.64	4.13
Sodium acetate	0.58	2.10	3.70

As shown in Table 3, ammonium sulfate was more effective than other nitrogen sources. This strain seemed to assimilate ammonium nitrogen sources more easily than nitrate or urea. Nitrate group of nitrogen source was hard to be assimilated, and the culture broth was decolored from yellow to white.

Table 3. Effect of Nitrogen Source on Yeast Growth

Nitrogen source(1.4N%)	Final pH of broth	Protein production
$(NH_4)_2SO_4$	1.7	2.94 mg/ml of broth
NH_4Cl	1.65	2.72
NH_4NO_3	1.8	2.10
$NaNO_3$		0.14
$(NH_2)_2CO$		1.16

* Protein was determined after shaking culture at 30°C for12 hours.

Discussion

In this study, we used crude palm oil, refined one and crude olein as a carbon source. Jacobsberg and Ceria(1976) reported that crude palm olein was the palm oil fraction of which main component was oleic acid. Among these, most strain could assimilate crude palm oil better than refined one. Crude palm oil is the cheaper in cost, however, solid fat content at 30°C is 0.1 to 10.4% and this fraction could not be assimilated completely by the yeast strain *Torulopsis candida* TAMY128 in shaking culture. Therefore the emulsification of palm oil and useful fermentor in cultivation seemed to be recommended.

In selection of surfactants consideration was given to their nontoxic properties. Sucrose fatty acid esters have been regarded as safe and used in food or food additives. Consequently the inclusion of these surfactants in single cell protein production would be free of any toxicity harzards. Sucrose fatty acid ester(commercial name "Nitto p-1670") consist of 30% stearic

acid and 70% palmitic acid in coupling fatty acids. Wada et al. (1979) reported that the main compositions of fatty acid in crude Sabah palm oil were 46.6% palmitic acid, 36.6% oleic acid and 4.4% stearic acid. Whitworth et al. (1973) also reported the role of surfactants in hydrocarbon fermentation using nonionic surfactants. It was not possible to determine the mechanism of the assimilation of surfactants used in this study. However this strain could grow in the medium of sucrose fatty acid ester as a sole carbon source. The constituent linear fatty acids in this surfactant might be rendered available by the action of esterases. When we used surfactants in the culture media, it was considered that the surface active agent replaced the oil phase on the surface of the crystal as reported by Kreulen(1976). The crystals and the lipuid phase were separated and then oil droplets were assimilated by the yeasts easily. After 24 hr cultivation, there were much amount of solid fat remained. Jacobsberg(1976) reported that the main component of solid fat was stearin.

The addition of natural nutrients stimulated the cell growth in initial state of cultivation. Among these, carnitine was some effective in initial state of growth. It is considered that carnitine has the property of increasing oxygen consumption and catalytically stimulating long chain fatty acid oxidation as reported by Fritz (1963) and Sakaguchi(1962) .

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국 문 초 록

새로운 蛋白質源으로서 微生物의 菌体蛋白質을 生産하여 家畜의 사료나 食品源으로서 利用하기 위한 硏究의 일환으로 palm oil을 資化하는 有用한 酵母를 토양에서 分離하고, 이를 *Torulopia candida* TAMY128로 同定하였다. 유일한 炭素源으로서 사용한 palm oil을 非이온性 界面活性劑를 첨가하여 培地에 分散시키므로써 菌体生産을 높였으며 分離酵母의 振盪培養條件을 검토한 結果 질소원으로 $(NH_4)_2SO_4$, 生育因子로서 Corn steep liquor가 유수하였다.