Microbial Utilization of Tropical Agricultural Products

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熱帶性 農業生產物의 微生物에 의한 有效利用

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Summary

Tropical plants have a merit in productivity among agricultural products. Among these, palm oil production had been increasing steadily over resent years, and much of which was incorporated in edible products. The microbial utilization of palm oil for single cell protein production can provide a protein source for human consumption from the safe raw material. Furthermore it is desirable to resolve the problem of over production of the oil in near future.

In this study, yeast strain capable of assimilating palm oil was isolated from various soils samples in Jeju island. The isolated yeast strain Y-35 was identified with *Torulopsis candida* JNUA 35, and this strain assimilated crude palm oil effectively.

The addition of nonionic surfactant and emulsification with homogenizer were effective in some degree on yeast cell growth. In shaking culture of this strain, yeast extract as a natural nutrient was good for yeast growth, and ammonium phosphate was more effective than any other nitrogen sources. Lower than 2% of palm oil concentration was available to convert the substrate to cell mass effectively in shaking culture, considering the biophysical properties of crude palm oil.

Introduction

Man is losing to feed himself as population increases faster than food supplies. There are some limits in improvement of agricultural techniques. augmentation of cultivated acreage, and promotion of fisheries for solving this problems. Therefore a growing concern for the acute food needs 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 many reports and revlews on SCP production from hydrocarbons or chemicals derived from them, sulfite waste liquor, wood hydrolysates and agricultural by-products (Litchfield 1977, Mateles et al. 1968, Tannenbaum et al. 1975, Cooney et al. 1980) . However for human use, SCP product must be free from toxic substances in the cells themselves or introduced into the cells through residues in the substrate such as polyaromatic hydrocarbons and from contamination by pathogenic microorganisms or their toxins (Litchfield 1977) .

Tropical plants have a merit in productivity amoag agricultural products. Ginar(1980) reported that palm oil production mainly in Malaysia had been increasing steadily oevr resent years and currently in excess of a 4 million metric tons per annum, much of which was incorporated in

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edible products. The World Bank estimated that palm oil production would be increasing to 11 million metric tons by the year 2000. The share of palm oil in the international fats and oils markets will leap nearly 9% in 1980 to reach 25. 5% by 1990 (28 % of soybean) .

Microbial utilization of tropical agricultural products (palm oil) has a significance to supply protein resource for human consumption from cheap and safe law material, and to resolve the overproduction of palm oil in future. In this study, an efficient yeast strain capable of assimilating crude palm oil was isolated from field soil, and carried out studies on the taxonomy, cultural conditions and chemical analysis of this strain.

Materials and Methods

Materials

Malaysian 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(Koh 1981).

Media and Culture

Isolation medium contained 2% palm oil as a sole carbon source, 0.4% NH_4NO_3, 0.47% KH_2PO_4 0.03% NaH_2PO_4. $12H_20$, 0.05% $MgSO_4$. $7H_20$, 0.001% $FeSO_4$. $7H_20$, 0.01% yeast extract and 0.002% chloramphenicol in tap water. The medium was adjusted to pH 5.5 and sterilized for 10 min at 110°C. In plate culture medium, carbon source was changed to 2% dextrose to differenciate colony from palm oil droplets. Stock culture medium was consisted of 1% palm oil, 1% meat extract, 0.5% polypeptone, 1% malt extract and 1.5% agar. Chloramphenicol was used to restrain the bacterial growth. 50ml of the medium in 500ml shaking flask was inoculated with 1 ml of precultured broth, and incubated at 37°C on a rotary shaker.

Isolation of yeasts capable of assimilating palm oil yeasts capable of assimilating palm oil were isolated from soil samples collected from various places around Jeju island, using enrichment culture techniques shown in Fig.1.

Isolation sources(soil)
↓ 0.5g per 10㎖ of isolation medium in 30㎖ test tube
Shaking culture(37°C, 2 days)
↓ 1㎖ per 10㎖ of isolation medium in 30㎖ test tube
Shaking culture(37°C, 1 day)
↓
Plate culture(37°C, 1 day)
↓
Microscopic examination
↓
Stock culture

Fig 1. Method of yeast isolation

Assay of yeast

After adquate cultivation, 15 ml from the culture broth was taken and added 1ml of 6 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). After screening of yeasts, cell growth was determined as follows : cell suspension was prepared by adding a solvent (dioxane : ethylacetate=3 : 2), and the optical density at 540 nm was measured by spectrophotometer.

Taxonomic study of the strain Y-35

Diagnostic tests of strain Y-35 were carried out according to the method of Lodder(1970).

Methods of analysis

The dried cell was prepared by the followling procedure; centrifuged(3000 rpm, 20 min) after cultivation, and removed residual palm oil with acetone and diethylether, and then filtered and dried under vacuum at 30°C.

Total nitrogen was estamited by the method of Kjeldall, and crude fat by the method of Soxhlet ether extraction. The method of Montgomery(1961) was used to determine the carbohydrate content.

Results

Isolation of yeasts capable of assimilating palm oil

More than one hundred strains were isolated from 220 soil samples. However only some of the strains assimilated crude palm oil effectively, most isolates were poor in growth. Among these, the strain Y-35 shows higher protein yield on crude palm oil than any other strains. The strain Y-35 was isolated from field soil in Jeju-Si.

Taxonomic study of the strain Y-35

Microscopic appearence of strain Y-35 is shown in Fig.2.

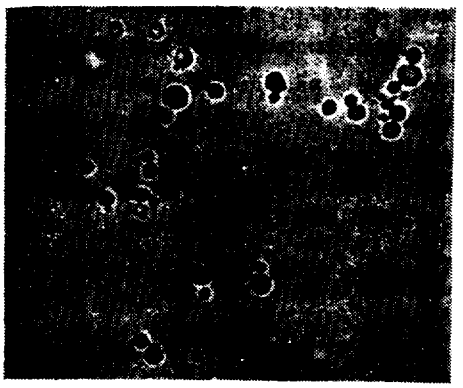

Fig. 2. The yeast strain *Torulopsis candida* JNUA 35 grown on YM-media agar (X 400).

Growth in YM media : After 3 days at 25°C, cells are round, 2−5.5μ. A sediment and grey, thin, smooth and creeping pellicles are formed.

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.

Sporulation : Not formed.

Fermentation : absent.

Sugar assimilation : glucose + galactose +

L-sorbose	−	α-methyl-D-glucoside	+
salicin	−	sucrose	+
maltose	+	melebiose	+
cellobiose	−	trehalose	+
lactose	−	raffinose	+
melezitose	+	inulin	+
soluble starch	+	D-xylose	+

L-arabinose	−	D-ribose	−
L-rhamnose	−	ethanol	+
glycerol	+	adonitol	+
D-manitol	+	D-sorbitol	+
inositol	−	citric acid	+
DL-lactic acid	+	succinic acid	+

Assimilation of potassium nitrate : negative.
Splitting of arbutin ; positive.
Production of compounds like starch : negative.
Growth in vitamin-free medium : increase observed.
Osmo-tolerence : 14%.

Urease test : negative.

According to these taxonomic studies, the morphological and physiological properties of this strain were very similiar to those of *Torulopsis candida* by J. Lodder(1970) in "The Yeast" except that the strain did not assimilate cellobiose and salicin. Furthermore this strain can grow in pH 1.5 and the growth is recognized at 41°C. Therefore the strain Y-35 was identified with *Torulopsis candida* JNUA 35.

Cultivation of the yeast

The addition of nonionic surfactants and then emlusification with homogenizer(max. speed for 3 min) stimulated the yeast growth as shown in Tabe 1.

Table 1. Effect of surfactant addition on yeast growth (0.1% addition and homogenization)

Kind of Surfactant		HLB-Value	Shape	Cell Concentration (g/l of broth) 12 hrs	24 hrs
Tween	20	16.7	liquid	4.5	7.9
//	40	15.6	paste	5.0	8.6
//	60	14.9	paste	4.6	7.9
//	80	15.0	paste	4.5	8.2
//	85	11.0	liquid	6.2	8.8
Emulgen	903	7.8	liquid	5.2	7.5
//	913	14.5	liquid	5.7	8.5
//	931	17.2	powder	4.7	7.9
//	950	18.2	crystal	3.2	5.7
Sucrose fatty acid ester					
	F-140	13.0	powder	6.3	8.3
//	F-160	15.0	powder	3.9	7.6
//	OW-1570	15.0	paste	5.0	8.5
//	OW-1670	16.0	powder	4.5	7.9
Tryton	100		liquid	5.2	5.7
Control(no addition)				3.9	7.7

as the palm oil was dispersed into the broth in small droplets. In the initial state of growth, small drops of palm oil were attached by the yeasts and assimilated more rapidly than large ones. However the effect of surfactants shows no significant difference in the late state compared with initial state of growth. The main function of the surfactant is to aid the formation

and stabilization of palm oil emulsion. In this experiment, the concentration of surfactant was enough in the range of 0.05 to 0.1%. Lower concentration of surfactant presented low effect of palm oil dispersion in the broth, and higher concentration the inhibition effect in cell growth and separation of crude palm oil between olein and stearin phase.

The effect of natural nutrients on cell growth was investigated, and the results are presented in Table 2. The effect of natural nutrients addition shows no significant difference each other. As shown in Table 3, ammonium phosphate was more effective than other nitrogen

Table 2. Effect of growth factor addition on yeast growth

Growth Factor(0.01%)	Cell Concentration(g/l of broth)
Control(no addition)	7.2
Yeast extract	7.8
Peptone	7.4
Meat extract	7.7
Malt extract	7.5

Cell concentration was determined after shaking culture at 37°C for 24 hr.

Table 3. Effect of nitrogen source on yeast growth

Nitrogen Source(1.4 N %)	Cell Concentration(g/l of broth)
$(NH_4)_2SO_4$	7.9
NH_4Cl	8.2
NH_4NO_3	7.7
$NaNO_3$	1.5
$(NH_2)_2CO$	8.3
$(NH_4)_2HPO_4$	8.5

Cell concentration was determined after shaking culture at 37°C for 24 hr.

sources. Nitrogen of nitrate group was hard to be assimilated. In shaking culture of this strain, phosphate source shows similiar results in cell growth. Higher concentration of palm oil more than 2% decreased cell yields. It was considered that physical properties of the broth, such as emulsification of the substrate, oxygen transfer and adhesion of palm oil on the wall of flask might affect the cell growth.

Chemical compositions of dried cell is shown in Table 4. Protein content was over 40% and fat content was scanty.

Table 4. Compositions of dried cell

Moisture	5.4%
Protein	46.1
Total Carbohydrate	36.7
Crude Fat	1.5
Ash	9.2

Protein was calculated by total nitrogen X 6.25.

Discussion

Fatty acid composition of crude palm oil mainly consists of oleic acid and palmitic acid, 43.7% and 39.9% respectively on the average (Tan et al. 1981). Solid fat content of crude palm oil at 35°C is 6.6% and this fraction could not be assimilated completely by the yeast strain *Torulopsis candida* JNUA 35 in shaking culture. Therefore the selection of thermophilic yeast strain, the emulsification of palm oil, and useful fermentor in cultivation seemed to be recommended.

The important thing for production of single cell protein from palm oil was the physical properties of substrate. This strain utilized small droplets of palm oil easily rather than large ones. Therefore the emulsification of palm oil is recommended, but at 37°C, the cultivation temperature of this strain, palm oil was not emulsified fully and adhered on the wall of flask and mini-fermentor during cultivation. The adhesion of palm oil prevented the yeast from utilizing the substrate completely. The addition of surfactant and homogenization were effective in some degree. However small amount of surfactant addition was not enough in emulsification of palm oil into the broth, compared with hydrocarbon fermentation (Whitworth et al. 1973), and large amount of surfactant addition inhibited the cell growth. In this study, nonionic surfactants were added and treated with homogenizer, palm oil was emulsified enough at room temperature. However palm oil was softened and adhered during cultivation, because the melting point of crude palm oil was in the range of 27°C to 42.5°C. In the problem of palm oil emulsification, commercial refined palm oil seemed to be better substrate than crude one. The melting point of commercial refined palm oil is 34 to 45°C, and that is higher than that of crude one. However crude palm oil is the cheaper in cost, so it seemed to be important thing to select the thermophilic yeast strain. Futher studies would be necessary to isolate useful strains from native samples in tropical area or to mutate the strain capable of assimilating the crude palm oil.

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 liquid phase were separated by Kreulen 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.

Fermentor which is able to prevent from adhering on the wall, physiological properties of this strain during cultivation, and scaling up to cell production, now we are under study.

Among the composition of dried cell, fat content was scanty. It was difficult to remove the residual palm oil from yeast cells after cultivation. Therefore we used acetone and diethylether in the recovery of yeast cells, at that time some amount of cellular lipids seemed to be lost.

Conclusively microbial utilization of palm oil seem to be promising for supplying the protein source and the resolution of overproduction in near future.

Literatures cited

Cooney, C.L., ChoKyun Rha, and Tannenbaum. S.R. 1980. Single Cell Protein : Engineering. Economics, and Utilization in Foods. Adv. in Food Res., 26 : 1−52, Academic Press Inc.

Ginar, L. and Mason, I., 1980. Palm oil production keeps pace with would demand. Food Engineering INT'L, June, 24-27.

Jacobsberg, B. and Ceria, O.C.H., 1976. Studies in palm oil crystallization. J. Am. Oil Chem. Soc., 53 : 609−617.

Koh, J.S., 1981. Microbial Utilization of Palm Oil. Jeiu National Univ. J. 12 : 71−78.

Kreulen, H. P., 1976. Fractionation and winterization of edible fats and oils. Am. Oil Chem . Soc., 53 : 393−396.

Litchfield, J.H., 1977. Comparative technical and economic aspects of single-cell protein processes. Adv. in Appl. Microbiol., 22 : 267−305.

Lodder, J., 1970. "The Yeast, A Taxonomic Study" 2ed., North-Holland Pub. Co., Amsterdam, London.

Lowry, O.H., Rosebrough, N.I., Farr, A.L. and Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193 : 265−275.

Mateles, R.I, and Tannenbaum, S.R., 1968. Single Cell Protein, MIT Press, Cambridge, MA.

Montgomery, R., 1961. Futher studies of the phenol-sulfuric acid reagent for carbohydrates. Biochem. Biophys., 48 : 591−593.

Tan, B.K. and Flingoh, C.H. OH, 1981. Malaysian Palm Oil, Chemical and physical characteristics. PORIM technol., 3 : 1−5.

Tannenbaum, S.R, and Wang, D.I.C., 1975. "Single Cell Protein II," MIT Press, Cambridge, MA.

Whitworth, D.A., Mou-Young, M. and Viswanatha, 1973. Hydrocarbon fermentation, oxidation mechanism and nonionic surfactant effects in a culture of ***Candida lipolytica***. Biotech. Bioeng., 15 : 649−675.

國 文 抄 錄

熱帶性作物은 他作物에 비해 生産性이 매우 높아 인구증가에 따른 資源難의 解決方案으로서 관심을 끌고 있다. 熱帶性 農業生産物인 palm oil의 生産量은 最近 매년 급격한 증가추세를 보이고 있으며 國內 食品工業界에 油脂資源으로서 소비가 증대되고 있으므로써 有効한 活用方案이 요구되고 있는 實情이다.

本硏究는 값싸고 安全한 原料로부터 蛋白質源을 生産하기 위해 palm oil을 유일한 炭素源으로 하여 資化할 수 있는 有用한 酵母를 제주도의 토양중에서 분리선발한 후 形態的, 生理的인 實驗을 통해 이를 *Torulopsis candida* JNUA 35로 同定하였다.

菌体蛋白質의 生産을 위한 培養條件을 設定하는데 있어서 基質의 特性을 고려하여 非이온性 界面活性劑를 첨가하여 palm oil을 培地에 分散시키므로써 균체수율을 向上시켰으며, 分離한 酵母의 진탕배양의 諸條件을 검토하였으며 酵母菌体의 化學的 成分을 分析하였다.