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炭素源에 따른 *Acinetobacter calcoaceticus* 菌株의 菌體收率과 脂肪酸組成

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Cell Yield and Fatty Acid Compositions of *Acinetobacter calcoaceticus* on Various Carbon Source

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Summary

Acinetobacter calcoaceticus KB-2, capable of assimilating palm oil efficiently, was cultivated in the medium containing various lipid materials. Specific growth rate of this strain was between 1.02 and 1.10 h^{-1} on palm oil and vegetable oils. About 90% of oils and fats except crude palm stearin used in this study was assimilated by this strain, and cell yields were about 1.0 g of cell/g of substrate. Fatty acid compositions of *A. calcoaceticus* KB-2 varied with growth substrates. Hexadecane-grown cells demonstrates the major fatty acids species to be $C_{16:0}$ and $C_{16:1}$, while others to be $C_{16:0}$ and $C_{18:1}$. Vegetable oils and other chemicals derived petroleum have the same potentials to palm oil as carbon sources for cell production by this strain.

Introduction

The growing concern has been focused on renewable resources such as starchy and cellulosic substances of agricultural wastes as raw materials for fermentation. Among these, palm oil and vegetable oils such as rapeseed and soybean oil seem to be alternative, following the

great increase of the oils production recently. The most exciting idea is to promote non-conventional protein production in the oilseed-producing countries by using oils and by-products as carbon sources for yeasts, bacteria, or fungi.[1]

Few reports, however, have been published on the utilization of oils and fats as raw materials for fermentation by microorganisms.[2] Single cell protein productions were carried out recently as the substrate of animal fats[3], fish oil[4], soapstocks[5], olive oil[6], rapeseed oil and palm oil [1, 7-10].

Authors [7, 8] isolated a yeast strain, *Torulopsis candida* Y-128, and a bacterium, *Acinetobacter calcoaceticus* KB-2, capable of assimilating palm oil efficiently, and investigated cultural conditions for cell production from palm oil. *Acinetobacter calcoaceticus* KB-2 grew with a specific growth rate of 1.10 h^{-1} at 39 ℃ on refined palm oil, and the cell productivity of this strain was high in a short cultivation time.

In this study, this strain was cultivated on various carbon sources of lipid materials, and compared with the cell yields each other for cell production. Fatty acid compositions of this strain grown on various carbon sources were also analyzed.

Materials and Methods

Materials. Crude palm oil and the fractions of the oil were supplied by Kao Soaps Co. Ltd. Refined palm oil (commercial, Nippon Oil and Fats Co. Ltd.), soybean (Hayashi Co. Ltd.) and rapeseed (Nakarai Chemical Ltd.) were used as the substrate, and the fatty acid compositions of the oils were analyzed as shown in Table 1. Other substrates used in this study were in reagents grade.

Table 1. Percentage of fatty acid compositions of oils and fats*

Compound	Crude palm olein	Crude palm stearin	Crude palm oil[8]	Refined palm oil[8]	Soybean oil	Rapeseed oil
C 12:0	0.2	0.1	0.2	0.3	-	-
C 14:0	1.0	1.2	1.0	1.0	0.1	-
C 16:0	39.5	50.1	45.7	39.9	10.9	5.2
C 16:1	0.1	0.1	0.1	0.1	-	0.2
C 18:0	4.7	4.9	4.5	4.8	5.5	2.1
C 18:1	43.0	33.6	38.5	42.6	25.3	55.4
C 18:2	10.7	8.8	9.7	11.0	51.0	25.2
C 18:3	0.4	0.3	0.3	0.3	6.8	0.4
C 20:0	0.4	0.3	-	-	0.2	11.5

* Fatty acid composition is expressed in terms of percentage of total fatty acids.

Organism. The organism used was *Acinetobacter calcoaceticus* KB-2. Taxonomic characteristics and procedures for maintaining stock cultures were described previously[8].

Fermentation. The medium contained 2% carbon source, 0.6% $(NH_4)_2SO_4$, 0.6% Na_2HPO_4, 0.4% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$, 0.001% $CaCl_2 \cdot 2H_2O$ and 0.1% corn steep liquor (Nisshoku). The experiments were carried out in a 2 liter jar fermentor, and the working volume was 1 liter. Temperature was maintained at 39 ℃, and pH was controlled at 6.8 by the addition of 5% NH_4OH solution. Aeration and agitation speed were 1.0 vvm and 1,000 rpm respectively. Starter culture of 50 ml in a shake flask inoculated for 12 h at 37 ℃ on a reciprocal shaker (120 strokes/min), and was used to inoculate jar fermentor cultures at an inoculum size of 5%.

Analytical methods. Cell mass was determined by optical density or dry weight measurement as described previously[8]. The growth rate defined to be the time necessary for the population to double. It was determined during the exponential growth phase.

Residual oils were extracted by the method of Bligh and Dyer[11], and dried and weighed. Fatty acid compositions of the oils were determined by GLC analysis of the methyl esters of the oils with a GLC instrument (Hitachi model-163) equipped with a flame ionization detector and a glass column (2 *m* long, 3 *mm* diameter), packed with 10% DEGS (diethylglycol-succinate) on chromosorb W. The separation was done isothermally at 180℃. The oxygen requirement was calculated according to Mateles.[12]

Results

The growth and cell yield of *A. calcoaceticus* KB-2 on various carbon sources for cell production is shown in Table 2. Acetic acid and ethanol were sterilized separately by filtration, and added. The initial concentration of acetic acid was 1%, and added periodically to 20 g/l finally. Compared with water-soluble substrates such as ethanol and acetic acid, better growth and higher yield were recorded for lipid materials. When crude palm oil was used as the substrate, some amount of oil was adhered to the wall and baffles of the fermentor, and cell yield was lowed compared with that obtained with refined palm oil. Specific growth rate and cell yield for refined palm oil were 1.07 h^{-1} and 1.03 g of cell/g of substrate respectively after 8 h of cultivation. Hexadecane was also a good substrate for cell production by this strain. After 24 h of cultivation, about 90% of the substrate was utilized, and cell yield was 1.10 g of cell/g of substrate. When oils and fats except crude palm stearin was used as a carbon source for cell production, about 90% of the substrate was assimilated by this strain, and cell yields were about 1.0 g of cell/g of substrate. The oxygen requirement on oils and fats fermentation calculated theoretically was almost similiar to that on hydrocarbon fermentation.

This strain could assimilate in general unsaturated and saturated fatty acids effectively as carbon sources. However, unsaturated fatty acids such as oleic acid would be assimilated more easily and rapidly than saturated acids such as palmitic acid by this strain as shown in Figure 1. Linoleic acid which is a main fatty acid of vegetable oils was not assimilated easily, compared with other fatty acids. When

Table 2. Growth of *Acinetobacter calcoaceticus* KB-2 on various carbon sources*

Carbon source (2%)	Cultivation time, hr	Specific growth rate, hr^{-1}	Substrate consumed, %	Yield factor g cell/g substrate	Oxygen requirement O_2 g/g cell
Crude palm olein	7	1.04	92.4	1.00	1.63
Crude palm stearin	7	1.04	81.8	1.07	1.55
Crude palm oil	8	1.02	86.5	0.97	1.72
Refined palm oil	8	1.07	91.0	1.03	1.54
Triolein	8	1.10	90.8	1.04	1.55
Soybean oil	7	1.04	88.4	1.01	1.60
Rapeseed oil	7	1.03	92.3	0.98	1.67
n-Hexadecane	24	0.27	84.0	1.10	1.92
Ethanol	10	0.63	ND**	0.59	-
Acetic acid	8	0.66	ND	0.42	-

* Each fermentation was at pH 6.8 and 39 ℃. The cells were harvested at the stationary phase, and the final cell concentrations were 10-19 g/liter (dry basis).

** ND, not determined.

this strain was cultivated in a shake or jar fermentor culture, palm oil was dispersed into small droplets in the culture broth at log phase, and shape and size of cells were varied according to cultivation conditions. The strain was usually appearing short rod-shaped in exponential phase but nearly sperical in stationary phase on the medium containing water-soluble substrates[9], but long rod-shaped like fungi on the medium containing linoleic acid or palm oil in a shake culture as shown in Figure 2. In jar fermentor culture, long rod-shaped of cells began to appear partly in log phase and predominantly in late stages of log phase, but disappeared in the stationary phase.

The fatty acid composition of *A. calcoaceticus* KB-2 varied with growth substrates as shown in Table 3. The analysis of hexadecane-grown cells demonstrated the major fatty acids species to be

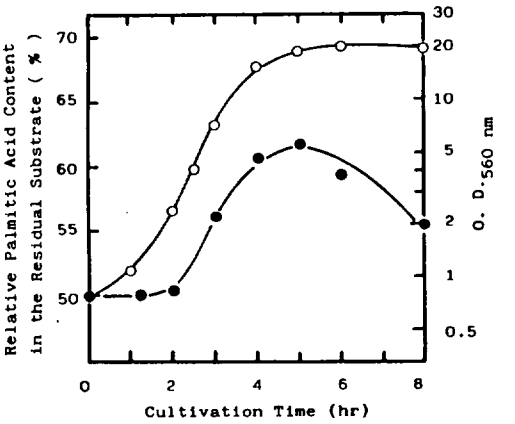
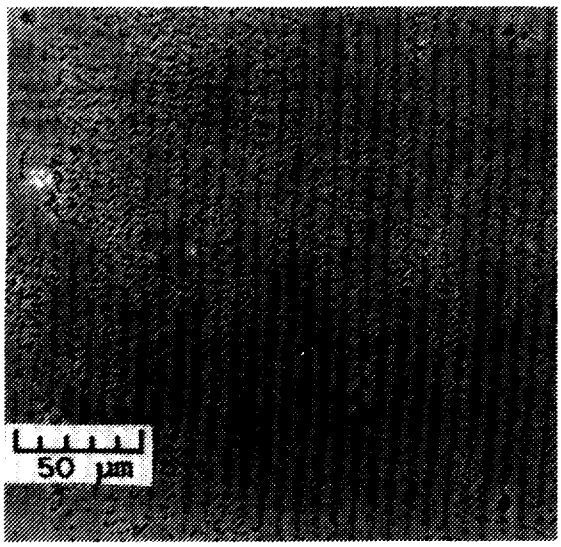
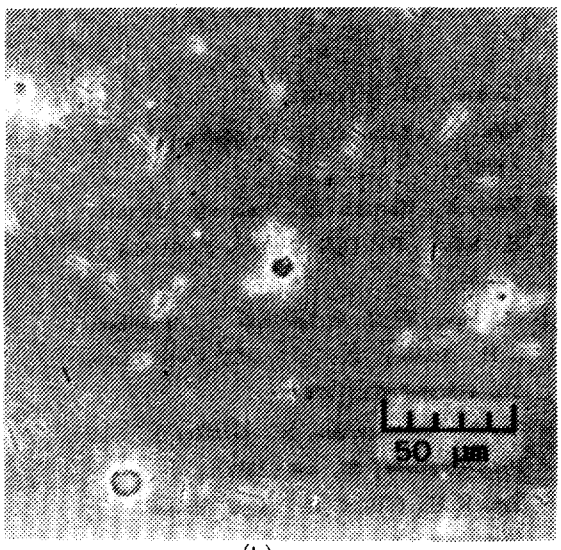


Fig. 1. Assimilation of saturated and unsaturated fatty acids by *A. calcoaceticus* KB-2. Growth (○) and relative palmitic acid content in the residual substrate of culture broth (●). Palmitic (0.5%) and oleic acid (0.5%) were used as carbon sources. Fermentation was carried out at 39℃, pH 6.8 in a jar fermentor.



(a)



(b)

Fig. 2. (a) *Acinetobacter calcoaceticus* KB-2 grown on nutrient broth-medium agar after 24 hr at 37℃ (x600).
(b) *A. calcoaceticus* KB-2 grown in a medium containing 2% linoleic acid in a shake culture (x600).
Cells are shown as long-shaped and attached to droplets of linoleic acid.

$C_{16:0}$ and $C_{16:1}$, while others to be $C_{16:0}$ and $C_{18:1}$.

Discussion

The specific growth rate and yield coefficient of this strain on ethanol were low, compared with other strain of *A. calcoaceticus*(13). These results may be attributed to high concentration of the substrate and cultivation temperature, since initial concentration of ethanol was 2%, and some amount of ethanol would be evaporated at 39℃ during cultivation. Considering these factors, the results are generally agreed with Du Preez et al.(13) When oils and fats were used as carbon sources for cell production, specific growth rates and cell yields were showed to almost same values of between 1.02 and 1.10 h^{-1}, and between 0.97 and 1.07 g of cell/g of substrate respectively. This results show that this strain is characterized in high growth rate and productivity in a short cultivation time on lipid materials. Therefore, vegetable oils and other chemicals derived from petroleum have the same potentials to palm oil as carbon sources for this strain. Especially, the advantage of this strain is that both the saturated and unsaturated fatty acids are assimilated efficiently by this organism, and there is no need to add the surface active agents for emulsification of lipid materials into the culture broth. Oils and fats in the medium were emulsified soon after the growth of this strain during cultivation. The phenomenon is not demonstrated clearly in this study, but the lipase of this strain is under investigation.

According to the literature, the variations in shape and size of cells of *A. calcoaceticus* sp. like fungi have not been reported to now. Although the physiological property of this variations is not known clearly, it is supposed that there may be a correlation between lipase production

Table 3. Percentage of fatty acid composition of *Acinetobacter calcoaceticus* KB-2 grown on various substrates*

Compound	Palm oil	n-Hexadecane	Ethanol	Acetic acid
C 10:0	-	-	-	0.25
C 12:0	1.69	4.96	11.32	3.94
C 14:0	1.05	1.89	0.34	0.44
C 14:1	0.48	2.36	-	0.55
C 16:0	24.42	33.56	31.36	38.53
C 16:1	4.36	38.51	6.62	3.43
C 17:0	0.46	1.76	1.62	0.80
C 18:0	3.99	6.94	3.38	2 48
C 18:1	56.85	8.63	42.60	47.66
C 18:2	6.17	0.86	2.76	1.06
C 18:3	0.53	0.53	-	0.86

* Fatty acid composition is expressed in terms of percentage of total fatty acids.

and variations of cells of *A. calcoaceticus* KB-2 to utilize the fatty materials as carbon sources.

The pattern of fatty acid composition of this strain was generally in agreement with that of Makula et al.[14], and that of the cells grown in palm oil medium was supposed to be derived from the substrate. However, $C_{18:1}$ fatty acid was accumulated in the palm oil-grown cells, compared with that of substrate. Hexadecane-grown cultures exhibited $C_{16:0}$ and $C_{16:1}$ as the predominant fatty acid species, whereas other substrate cultures contained a significantly high concentration of $C_{18:1}$ fatty acid The quantitative differences illustrated the effect that nutritional conditions exerted on fatty acid composition of the cells.

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國文抄錄

Palm oil 資化性菌인 Acinetobacter calcoaceticus KB-2를 여러가지 油脂를 炭素源으로 하여 培養한 후 菌體生産을 檢討한 結果, 本菌株의 比增殖速度가 1.02～1.10 hr^{-1}였으며, 粗 palm stearin을 제외한 油脂의 約 90%를 資化하여 菌體收率은 約 1.0 g cell/g substrate를 얻었다. 本菌株의 菌體脂質의 脂肪酸組成은 基質에 따라 差異가 있었으며, 主脂肪酸組成은 hexadecane을 基質로 培養한 菌體의 경우는 C 16:0 및 C 16:1가, 그 외의 基質을 炭素源으로 하였을 경우는 C 16:0 및 C 18:1이었다. 油脂 및 炭水水素 등도 菌體生産을 위한 炭素源으로서의 利用에 充分한 可能性이 있는 것으로 판단되었다.

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