J. Korean Soc. Food Nutr.
19(6), 636~640(1990)

한국영양식량학회지

Studies on Fungal Lipids Containing γ-Linolenic Acid 1. Fatty Acid Composition of *Mucor* sp.

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Abstract

For a study on the production of γ-linolenic acid(GLA) by fungi, 3 fungal strains were isolated from soil. Their cell growth, lipid content and fatty acid composition were compared in shake flask culture. Among these fungi, the fungus, designated as FA-007, has high lipid content(21.1%) and GLA content(15.6% of total fatty acids). The fungal strain FA-007 was tentatively identified as *Mucor sp.* on the basis of morphological characteristics. Fungal oil produced by this fungus was composed of 75.2% neutral lipid, 5.3% glycolipid and 19.5% phospholipid. Although the GLA content in phospholipid was higher than it in neutral lipid, the GLA content in neutral lipid was high as 15.5%.

Introduction

Lipid production from microorganisms has been an object of research interest for many years. Most of the work has been carried out with lipid accumulating yeast strains. Filamentous fungi, however, may also be suitable for lipid production because of their high lipid content and unusual fatty acids. One fatty acid which has achieved increased interest in recent years is γ-linolenic acid(6, 9, 12-cis, cis, cis-octadecatrienoic acid, GLA), a prostaglandin precursor.

Microbial production of GLA may have certain advantages, and thus many fungi such as *Rhizopus arrhizus*[1] and *Mortierella* stains[2] have been investigated as an alternative producer. In our country, a few works on the production of fungal oil containing GLA have been carried out[3,4]. But still there are much rooms for the isolation of higher GLA producing fungi and for the improvement in their GLA productivities.

In this work, we had done a screening of fungi as the producer of GLA and isolated some fungal strains producing GLA.

Materials and Methods

Isolation of fungi

One gram of each soil sample from various places were suspended in 10㎖ of sterilized distilled water. After a proper dilution of the suspension, supernatant were spread on the malt yeast extract agar(MYEA) plates and incubated at 30℃ for 3 days. Single colony of fungi was isolated and transferred repeatedly into a new MYEA plate until ensure the pure culture, and then the pure cultures of fungi were stocked on MYEA slants. The isolated fungi were kept in refrigerator until used.

Medium and cultivation

The culture medium contains per liter, glucose 30g, $(NH_4)_2SO_4$ 3g, KH_2PO_4 3g, $MgSO_4 \cdot 7H_2O$

0.3g NaCl 0.1g, $FeSO_4$, $7H_2O$, 10mg, $CaCl_2 \cdot 2H_2O$ 10mg, $CuSO_4 \cdot 5H_2O$ 0.2mg, $ZnSO_4 \cdot 7H_2O$ 1mg, $MnSO_4 \cdot 4H_2O$ 1mg and Thiamine-HCl 2mg, The initial pH of the medium was adjusted to 4.6 with 0.1N NaOH.

Each isolated fungus was inoculated into a 250mℓ shaking flask contalning 100mℓ of medium. Liquid cultures were conducted in a rotatory shaking incubator(Korea Manhatan Co.) at 25℃, 120rpm for 7 days.

Measurement of dry cell weight and lipids

For the measurement of dry cell weight(DCW), fungal mycella were harvested from each 100mℓ of culture broth by suction filtration and washed with distilled water. The DCW was determined by drying the harvested mycelia at 100℃ overnight.

For the extraction of lipids, the wet mycelia from each 100mℓ of culture broth were suspended in 50mℓ of chloroform-methanol solution(2 : 1, V/V), homogenized for 3min with homogenizer, and then filtered. The residue was extracted two times more as described above. The collected filtrate was extracted and washed accroding to the method of Folch[5]. After the solvent was distilled by evaporation, the resultant lipid was vacuum-dried to a constant weight.

Presented data express average values of three individual series of cultivation and analytical experiments.

Analysis of fatty acid composition

According to the method of Firestone et al.[6], the fungal lipid was esterified with methanolic BF_3 solution(14%, W/V). The methyl esters of obtained fatty acids were analyzed by gas liquid chromatography(GLC) in 2m×4mm glass column packed with GP 10% SP-2330 on 100 to 120 mesh chromosorb ® WAW. GLC was operated at 200℃ with N_2 as the carrier gas at a flow rate of 40mℓ/min. Flame ionization detector(FID) was used and its temperature was 250℃. The esters were identified by comparing their retention times with those of standards.

Also identification of fatty acids was confirmed by using a fused silica Supelcowax 10 capillary column(30m×0.32mm ID). The column temperature of capillary gas chromatography was held at 210℃ for 4min and then programmed to 240℃ at a rate of 4℃/min. FID was used at 250℃ and nitrogen gas was flowed at a rate of 1.2mℓ/min.

Identification of the fungal strain

The morphological characteristics of the isolated fungus were observed by using slide culture techniques[7].

The identification of the fungal strain was carried out according to the keys to the higher taxa in The Fungi[8].

Fractionation of the fungal lipids

The fungal lipids were fractionated into three classes in an activated 10g silicic acid column(40 cm×2cm ID) by using 200mℓ of chloreform, 400mℓ of acetone and 200cm of methanol to elute neutral lipid, glycolipid and phospholipid, respectively.

Results and Discussion

Screening of fungi for GLA production

In order to find fungi producing GLA, about 50 strains of filamentous fungl were isolated from various soil samples and cultured in liquid medium for 7 days

Among them, GLA producing fungi were only 3 strains, and their DCW, lipid yield and fatty acid composition are listed in Table 1. The strain FA-007 produced 21.1% of its DCW as lipids and its GLA content on the total fatty acids was relatively high as 15.6%. Compared with the other 2 strains, the strain FA-007 showed higher lipid content and GLA content. Therefore we selected the strain FA-

Table 1. Dry cell weight, lipid content and fatty acid composition of 3 isolated fungi producing γ-linolenic acid

Organism	DCW[a]	TL[b]	TL/DCW	Fatty acid(%)										
No.	(mg/100mℓ)	(mg/100mℓ)	(%)	14 : 0	14 : 0	15 : 0	16 : 0	16 : 1	17 : 0	17 : 1	18 : 0	18 : 1	18 : 2	18 : 3[c]	18 : 3
FA-007	355	75	21.1	1.0	2.6	1.2	13.1	4.8	1.7	2.5	4.3	33.5	17.5	15.6	2.2
FA-004	230	42	18.3	0.5	1.0	0.3	19.5	2.5	-	-	21.3	45.0	4.3	1.9	3.7
FA-051	325	61	18.8	0.3	0.9	-	27.4	1.1	-	-	4.8	44.2	10.4	9.8	1.1

a : Dry cell weight, b : Total lipid, c : γ-linolenic acid(GLA)

007 for further experiments.

Identification of GLA produced by fungi

In order to confirm the GLA in lipids obtained from isolated fungi, capillary gas chromatographic analysis using fused silica Supelcowax 10 capillary column(30m×0.32mm ID) was applid.

When the packed column(2m×4mm ID, 10% SP-2330 on 100-120 mesh chromosorb ® WAW) was used, α-linolenic acid and γ-linolenic acid were separated, that is, GLA was eluted more fastly than α-linolenic acid. But because arachidic acid and GLA had similar retention times, they were not separated.

In the capillary GC analysis, arachidic acid and GLA showed apparently separated peaks(Fig. 1). Thus we could find out that GLA was contained in the fungal lipids extracted from these 3 fungal strains.

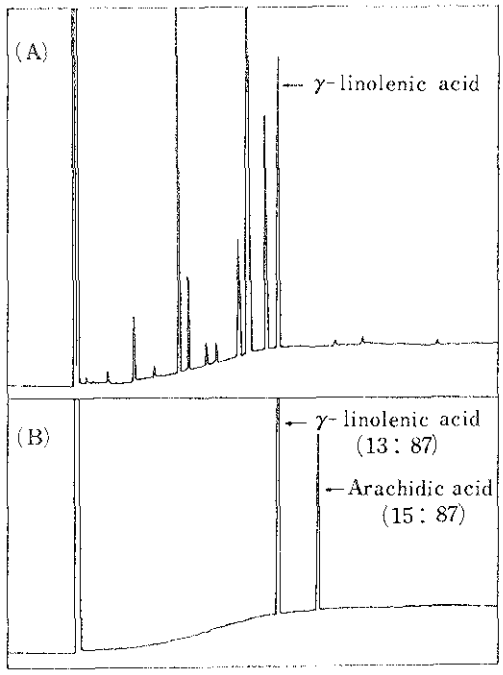


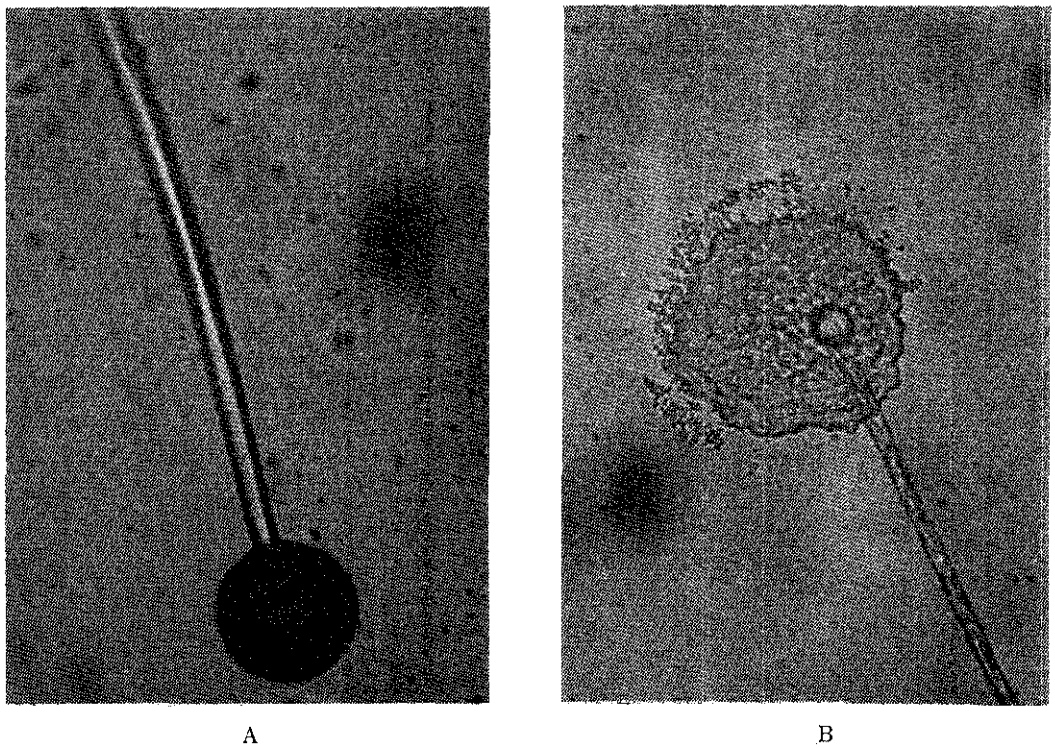
Fig. 1. Capillary GC chromatogram of fatty acid methyl esters obtained from isolated fungal strain FA-007(A), and authentic γ-linolenic acid and arachidic acid methyl esters(B)
Instrument : Hewlett-Packard 5880A capillary GC.
Column : Supelcowax 10 fused silica capillary (30m×0.32mm ID)
Column : 210℃(4 minhold)→240℃(4℃/min)
Detector : FID, 250℃ Carrier gas : Nitrogen, 1.2mℓ/min

Identification of isolated fungal strain FA-007

The hyphae without stolons and rhizoids were widely extended on the PDA(potato detrose agar) and the hyphae were not septated.

Aerial hyphae were also abundant. The sporangia formed at the ends of sporangiophores were globose type and contained many oval shaped sporangiospores. When the sporangium was broken, we could find a well-defined spherical columella (Fig. 2). Also round and thick walled chlamydospore was formed in the end of hyphae, but sporangiole and merosporangium were not observed.

From these morphological characterisitics and the fatty acid compositon containing GLA(n-6 series), the isolated fungus, FA-007, was identified as *Mucor* sp. belonging to the class Zygomycetes.



A B

Fig. 2. Sporangium, sporangiophore(A), columella and sporangiospore(B) of isolated fungal strain FA-007.

Table 2. Morphological characteristics of isolated fungal strain FA-007

Septate	Nonseptate
Sporangium	Globose sporangia posess spherical columella, Multispored sporangia
Sporangiophore	Not divided
Chlamydospore	Formed in the end of substrate mycelium
Merosporangium or sporangiole	Absent
Rhizoids or stolons	Absent

Table 3. Fatty acid composition of nonpolar and polar fraction of lipid produced by *Mucor* sp. FA-007

Lipid fraction	Content	Fatty acid(%)											
	(%)	>14 : 0	14 : 0	15 : 0	16 : 0	16 : 1	17 : 0	17 : 1	18 : 0	18 : 1	18 : 2	18 : 3[a]	18 : 3<
Neutral lipid	75.2	1.0	2.7	1.3	14.4	4.7	1.9	2.6	4.6	33.3	16.6	15.5	1.4
Glycolipid	5.3	–	–	2.4	11.8	2.4	0.2	1.4	4.4	42.2	24.7	4.5	6.0
Phospholipid	19.5	–	1.5	0.8	10.4	4.8	0.4	1.4	2.6	36.5	20.1	18.3	3.2

a : γ-linolenic acid

Neutral, glyco and phospholipid of *Mucor sp.* FA-007

The content and fatty acid compositions of neutral, glyco and phospholipid of *Mucor sp.* FA-007 are shown in Table 2.

Neutral lipid, glycolipid and phospholipid content were 75.2%, 5.3% and 19.5% of total lipid, respectively. Although a high GLA content(18.3%) was obsedved in phospholipid, the GLA content in neutral lipid was also high as 15.5%. Thus it seems more manageable to induce GLA accumulation by controlling culture conditions.

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(Received August 20, 1990)

γ-Linolenic acid 함유 곰팡이 지질에 관한 연구
1. *Mucor* sp.의 지방산 조성

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요 약

감마-리놀렌산 생산능이 있는 새로운 곰팡이 균주를 탐색하기 위하여 토양으로부터 감마-리놀렌산 생산능이 있는 3개의 곰팡이 균주를 분리하였으며, 이들 균주를 진탕 배양을 행하여 균체성장, 지질생성량 및 지방산조성을 비교 조사하였다. 3가지 균주중에서 FA-007 균주가 균체성장도 양호하였고, 지질함량이 건조균체중 21.1%, 감마-리놀렌산 함량이 총지방산중 15.6%로 높은 특성을 나타내었다. 이 균주는 형태학적 특성을 조사한 결과 *Mucor* sp.로 동정되었다. 이 균주가 생산하는 지질은 중성지질이 75.2%, 당지질이 5.3% 및 인지질이 19.5%로 구성되어 있었고, 중성지질과 인지질에서의 감마-리놀렌산 함량은 각각 15.5% 및 18.3%를 차지하였다.