J. Korean Soc. Food Nutr.
19(6), 641~645(1990)

Studies on Fungal Lipids Containing γ-Linolenic Acid 2. Influence of Cultural Conditions on The Production of γ-Linolenic Acid by *Mucor* sp.

Geun-Seoup Song, Choong-Ki Kim, Yong-Ju Kwon, Hee-Cheon Yang* and Tae-Kyoo Lee*

Dept of Food Science and Technology, Chonbuk National University, Chonju 560-756, Korea
**Dept of Food Science and Technology, College of Chonju Woosuk, Samrye-Eub 565-800, Korea*

Abstract

In order to study the influence of culture conditions on cell growth and lipid formation by *Mucor* sp., various carbon and nitrogen sources, initial pH, and C/N ratio of medium were investigated. Glucose was found to be suitable carbon source in terms of lipid yield and γ-linolenic acid(GLA) content. When NH_4Cl and $(NH_4)_2SO_4$ were used as nitrogen source, lipid content was high(19~21%) but GLA content was low(15~17%). On the other hand, when $NaNO_3$ and KNO_3 were used, lipid content was low(about 13%) but GLA content was high(22~23%). The highest production of lipid was obtained at a C/N ratio of 40 using glucose and $(NH_4)_2SO_4$ as carbon and nitrogen source, respectively. It was found that lipid yield was high at pH 4.6. Also this fungus did not grow at 35℃, and lipid yield was higher at 15℃ than 25℃.

Introduction

γ-linolenic acid(6, 9, 12-cis, cis, cis-octadecatrienoic acid, GLA) showed some medicinal effects on the cardiovascular diseases, tumor, hypercholesterolemia, etc.[1,2].

Oil containing this compound has been produced from plant seeds(*Oenothera* sp. or *Boraginaceas*) but GLA is also synthesized by some fungi[3,4]. Microbial production of GLA may have certain advantages, and thus recently fungal oil containing GLA has been produced commercially from *Mortierella* sp. in Japan[5].

Thus, in this paper, we report on the effects of culture condition such as carbon and nitrogen sources, initial pH, temperature, and C/N ratio on growth and lipid formation of *Mucor* sp..

Materials and Methods

Microorganism

The fungal strain used in this study was *Mucor* sp. FA-007 described in the previous paper[6].

Medium and culture conditions

Carbon sources were added to a concentration of carbon equivalent to 3% glucose and nitrogen sources were added to a concentration of nitrogen equivalent to 0.3%$(NH_4)_2SO_4$. C/N ratio was controlled by varying the amount of $(NH_4)_2SO_4$ in the medium keeping 3% glucose as carbon source. Different initial pH was adjusted with 0.1N NaOH and 0.1 N HCl.

The basal medium and culture condition was the same as in the previous paper[6].

Analytical methods

Measurement of dry cell weight(DCW) and total lipid(TL), and fatty aid analysis were performed as described previously[6]. Glucose concentration of the culture broth was measured by the DNS method[7].

All the presented data express average values of three individual series of cultivation and analytical experiments.

Results and Discussion

Change of dry cell weight, total lipid and fatty acid composition during incubation

As shown in Fig. 1, DCW and TL were increased up to 7th day and then decreased. Glucose as carbon source was consumed rapidly up to 5th day. pH of culture broth was decreased to pH 2.3 on 3rd day, and then was maintained at pH 2.2 -2.3. DCW on 5th day compared to 3rd day and DCW on 7th day comapred to 5th day were increased about 30㎎ and 15㎎, respectively. Also TL increase in the same periods were about 17㎎ and 8㎎, respectively. From these results, we could know that neutral lipid was significantly From

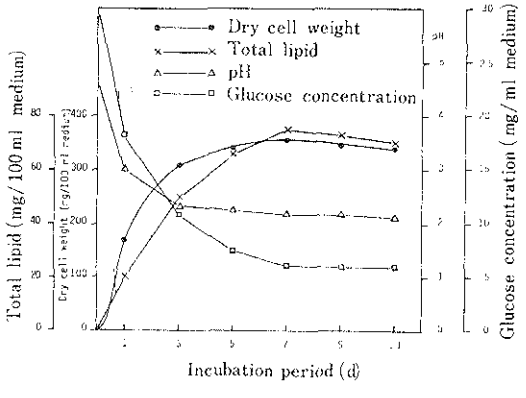

Fig. 1. Growth and total lipid formation of *Mucor* sp. FA-007.
Carbon source : Glucose, 30g /1
Nitrogen source : $(NH_4)_2SO_4$, 3g /1

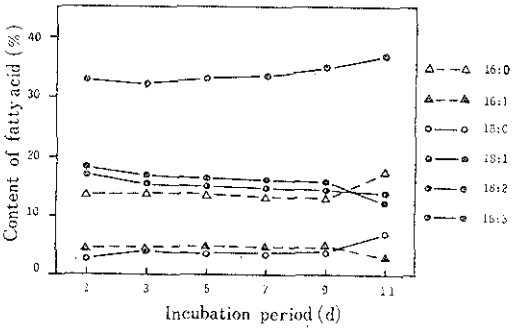

Fig. 2. Change in faty acid composition of total lipid in *Mucor* sp. FA-007 during incubation.
Carbon source : Glucose, 30g /1
Nitrogen source : $(NH_4)_2SO_4$, 3g /1

these results, we could know that neutral lipid was significantly synthesized during the periods.

Table 1. Influence of carbon sources on cell growth and lipid formation by *Mucor* sp. FA-007

		Carbon sources				
		Glucose	Xylose	Sucrose	Lactose	Soluble starch
Fatty acid (%)	16 : 0	13.1	20.4	14.7	–	18.6
	16 : 1	4.8	4.2	4.9	–	3.8
	18 : 0	4.3	7.9	5.0	–	7.8
	18 : 1	33.5	36.0	33.8	–	34.7
	18 : 2	17.5	11.8	14.2	–	12.8
	18 : 3[a]	15.6	13.7	16.4	–	17.6
DCW[b](㎎/100㎖)		355	285	350	0	147
TL[c](㎎/100㎖)		75	35	21	–	22
TL/DCW(%)		21.1	12.3	6.0	–	15.0
pH[d]		2.2	2.3	2.3	–	2.3

a : γ-linolenic acid, b : Dry cell weight, c : Total lipid d : pH after cultivation, Carbon Nitrogen source : $(NH_4)_2SO_4$, 3g/1, C/N ratio : 18.8

On the basis of DCW and TL, 7 days was suitable as cultivation time. Thus we carried out 7 day-cultivation for further studies.

Fig. 2 shows the change in major fatty acid composition of total lipid during incubation. GLA content was maintained as about 15~16% from 3rd to 9th day. Interesting fact was that saturated fatty acids(16 : 0 and 18 : 0) were increased after 9th day but unsaturated fatty acids(18 : 3 and 16 : 1) were decreased after 9th day.

Influence of carbon and nitrogen sources

DCW, TL and major fatty acid composition of *Mucor* sp. FA-007 grown on different carbon sources were summarized in Table 1.

Among the carbon sources glucose showed the highest cell growth and lipid content, which resulted in the highest productivity of GLA. This result was in good agreement with other reports[8,9]. Although there are papers showing lactose to be used for microbial growth[8,9], *Mucor* sp. FA-007 did not use lactose as carbon source.

Various nitrogen sources also affected the cell growth, lipid content and GLA content of *Mucor* sp. FA-007(Table 2)

Nitrate type nitrogen sources such as $NaNO_3$ and KNO_3 gave good cell growth, low lipid content and high GLA content. On the other hand, ammonia type nitrogen sources such as $(NH_4)_2SO_4$ and NH_4Cl gave poor cell growth, high lipid content and low GLA content. Among the nitrogen sources tested, $(NH_4)_2SO_4$ and $NaNO_3$ were found to be suitable nitrogen sources in terms of lipid yield and GLA production.

Influence of C/N ratio

Because C/N ratio is known to have profound effects on cell growth and lipid accumulation in

Table 2. Influence of nitrogen sources on cell growth and lipid formation by *Mucor* sp. FA-007

		Nitrogen sources					
		$NaNO_3$	KNO_3	NH_4NO_3	$(NH_4)_2CO$	NH_4Cl	$(NH_4)_2SO_4$
Fatty acid (%)	16 : 0	12.1	12.5	10.5	15.4	17.1	13.1
	16 : 1	11.5	10.9	11.9	3.3	3.4	4.8
	18 : 0	7.1	6.2	5.8	7.6	6.4	4.3
	18 : 1	16.9	16.7	19.9	16.3	32.5	33.5
	18 : 2	17.8	22.9	25.1	27.3	13.6	17.5
	18 : 3[a]	23.3	22.3	18.2	20.3	17.9	15.6
DCW[b](mg/100mℓ)		545	475	570	450	340	355
TL[c](mg/100mℓ)		73	62	55	42	65	75
TL/DCW(%)		13.4	13.1	9.6	9.3	19.1	21.1
pH[d]		7.4	7.2	6.2	6.0	2.2	2.2

a : γ-linolenic acid, b : Dry cell weight, c : Total lipid, d : pH after cultivation
Carbon source : Glucose, 30g/l, C/N ratio : 18.8

Table 3. Influence of C/N ratio on cell growth and lipid formation by *Mucor* sp. FA-007

		C/N ratio					
		18.8	40	80	120	160	200
Fatty acid (%)	16 : 0	13.1	18.9	21.3	21.0	18.5	18.8
	16 : 1	4.8	3.8	1.9	1.7	1.7	1.8
	18 : 0	4.3	4.3	6.6	6.3	5.4	5.6
	18 : 1	33.5	44.3	42.8	42.6	47.0	47.1
	18 : 2	17.5	11.2	8.9	9.2	10.1	10.5
	18 : 3[a]	15.6	10.2	11.2	11.6	12.1	12.2
DCW[b] (mg/100mℓ)		355	430	300	260	180	105
TL[c] (mg/100mℓ)		75	125	83	64	39	20
TL/DCW (%)		21.1	29.1	27.6	24.6	21.7	19.0
pH[d]		2.2	2.4	2.8	2.8	3.0	3.0

a : γ-linolenic acid, b : Dry cell weight, c : Total lipid
d : pH after cultivation, Carbon source : Glucose, 30g/l
Nitrogen source : $(NH_4)_2SO_4$

Table 4. Influence of initial pH on cell growth and lipid formation by *Mucor* sp. FA-007

		Initial pH			
		2.6	3.6	4.6	5.6
Fatty acid (%)	16 : 0	14.2	13.6	13.1	12.6
	16 : 1	3.3	6.8	4.8	8.1
	18 : 0	—	4.5	4.3	3.4
	18 : 1	34.7	33.9	33.5	37.4
	18 : 2	9.4	17.8	17.5	14.3
	18 : 3[a]	30.9	13.8	15.6	17.4
DCW[b] (mg/100mℓ)		25	305	355	545
TL[c] (mg/100mℓ)		9	61	75	62
TL/DCW (%)		36.0	2.2	2.2	2.3
pH[d]		2.4	2.2	2.2	2.3

a : γ-linolenic acid, b : Dry cell weight,
c : Total lipid, d : pH after cultivation
Carbon source : Glucose, 30g/l
Nitrogen source : $(NH_4)_2SO_4$, 3g/l

microorganisms, we investigated the influence of C/N ratio(Table 3.)

The highest DCW and lipid yield were obtained at a C/N ratio of 40, and their content were about 430mg/100mℓ medium and 125mg/100mℓ medium, respectively. But the highest GLA content(about 15% of total fatty acids) was obtained at a C/N ratio of 18.8.

From these results, we could found that optimum C/N ratio for GLA production using this fungus was between 18.8 and 80.

Influence of initial pH and temperature

The initial pH of culture medium greatly affected cell growth and lipid formation of *Mucor* sp. FA-007 as shown in Table 4.

As initial pH increased, DCW was increased and the highest DCW was attained at pH 5.6. But the highest lipid yield was observed at pH 4.6. Thus we thought that optimum pH for GLA production by this fungus would be between pH 3.6 and pH 5.6.

Table 5 shows the influence of temperature. This fungus did not grow at 35℃. DCW, lipid yield and lipid content were better at 15℃ than at 25℃. From this result, we thought that this fungus would be psychrophile.

Reference

1. Horrobin, D. F. : *Clinical Uses of Essential Fatty Acids.* Eden Press, Montreal, London(1982)
2. Murota, S. : Prostaglandins, Leucotrienes, and their biological significance. *J. Jpn. Oil Chem. Soc.*, 30, 717(1981)

Table 5. Influence of temperature on cell growth and lipid formation by *Mucor* sp. FA-007

		Temperature		
		15℃	25℃	35℃
Fatty acid (%)	16 : 0	17.6	13.1	−
	16 : 1	1.1	4.8	−
	18 : 0	11.9	4.3	−
	18 : 1	38.8	33.5	−
	18 : 2	10.3	17.5	−
	18 : 3[a]	14.3	15.6	−
DCW[b](mg/100mℓ)		375	355	0
TL[c](mg/100mℓ)		88	75	−
TL/DCW(%)		23.5	21.1	−
pH[d]		2.3	2.2	−

a : γ-linolenic acid, b : Dry cell weight,
c : Total lipid, d : pH after cultivation
Carbon source : Glucose, 30g/l
Nitrogen source : $(NH_4)_2SO_4$, 3g/l

3. Herbert, R. A. and Kelth, S. M. : Microbiological production of γ-linolenic acid. European Patent, Publication No. 0 153 134(1985)
4. Suzuki, O., Yokochi, T. and Yamashina, T. : Influence of cultural conditions on lipid composition of two strains of *M. isabellina*. *J. Jpn. Oil Chem. Soc.*, 31, 921(1982)
5. Suzuki, O., Yokochi, T., Amano, K., Sano, T., Seto, S., Outu, Y., Ishida, S., Iwamoto, S., Morioka, K., Satoh, A. and Uotani, K. : Development in production of fat containing γ-linolenic acid by fungi and its industialization. *J. Jpn. Oil Chem. Soc.*, 37, 1081(1988)
6. Song, G. S., Kim, C. K., kwon, Y. J., Lee, T. K. and Yang, H. C. : Studies on fungal lipids containing γ-linolenic acid. 1. Fatty acid composition of Mucor. sp.. Submitted to *J. Korean Soc. Food Nutr.*
7. Miller, G. L. : Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.*, 31, 426(1959)
8. Rateldge, C. : Microbial oils and fats : An assesment of their commercial potential. *Prog. Indust. Microbiol.*, 16, 119(1982)
9. Kang, H. S. and Shin, H. K. : Influence of medium composition on the production of γ-linolenic acid by *Mucor* sp. KCTC 8405P. *Kor. J. Appl. Microbiol. Bioeng.*, 17, 568(1989)

(Received August 20, 1990)

γ-Linolenic acid 함유 곰팡이 지질에 관한 연구
2. *Mucor* sp.의 γ-Linolenic Acid 생산에 미치는 배양조건의 영향

송근섭 · 김충기 · 권용주 · 양희천* · 이태규*

전북대학교 식품공학과, *전주우석대학 식품공학과

요 약

탄소원과 질소원, 배양온도, 배지의 초기 pH 및 C/N 비가 *Mucor* sp. FA-007의 균체 생육 및 지질생성에 미치는 영향을 조사하였다. 탄소원으로는 포도당이, 질소원으로는 황산암모늄과 질산나트륨이 균체내 유지함량 및 감마-리놀렌산 수율 측면에서 양호한 것으로 나타났다. C/N 비의 변화에 따른 영향에서는 포도당과 황산암모늄을 사용할 경우 C/N 비 40일때에 최고의 지질생산량을 나타냈으며, 지질생산성 측면에서 효과적인 초기 pH는 4.6이었다. 또한 본 실험에 사용한 곰팡이는 35℃에서 생육하지 못하였고, 15℃에서 최대의 지질생성 및 균체생육을 보였다.