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Effect of Strain Storing and Reculturing Conditions on The *Cephalosporium* sp.Mycelium's Fatty Acid Composition

DAI Chuan-Chao YUAN Sheng SHI Yang
(The Biology Science College of Nanjing Normal University, Nanjing 210097, China)

Abstract: To get high content of docosahexaenoic acid (DHA, ω-3), the effects on the *Cephalosporium* sp. mycelium's fatty acid composition of strain storing and culturing conditions were studied. The strgins containing microorangism were stored at 20℃、4℃and −20℃ for different times respectively. The fatty acids were analyzed by GC. The results showed that the fatty acid composition was affected by the storing temperature、storing time no matter how the strain was activated on new agar before fermentation. If the strain was cultured on new agar before fermentation, the 10d period was the best storing time to get high

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作者简介：戴传超（1970-），男，副教授，博士，硕士生导师，从事微生物工程及微生物生态等方向研究。

DHA content. If not cultured, to the 20℃, the best time was 20d; to the 4℃, the best time was 25d and to −20℃, it was 5d. The polyunsaturated fatty acid (PUFA) changed the same tendency as the DHA's but at 4℃ not so. At 20℃, the index of unsaturated fatty acid (IUFA) and the 18:3 (α-)/18:2 changed with the same tendency as the DHA's. Storing the strain at −20℃, the PUFA、IUFA and DHA fluctuated at the lowest range. This study could give a theoretical reference for producing high yield of DHA by microorganisms.

Key words: *Cephalosporium* sp.; strains storing and culturing; docosahexaenoic acid (DHA); fatty acid composition; polyunsaturated fatty acid (PUFA)

菌种储藏温度及菌种活化对头孢霉脂肪酸组分的影响

戴传超，袁 生，史 央
（南京师范大学生命科学学院，江苏 南京，210097）

摘 要：为了获得高含量的二十二碳六烯酸（DHA），对不同储藏温度对头孢霉脂肪酸组分的影响进行研究。将菌种储藏在20℃，4℃和－20℃三种温度下，比较不同储藏时间及菌种活化对菌丝脂肪酸组分的影响。结果表明菌种储藏温度、时间及发酵前菌种是否活化都影响脂肪酸组分。发酵前活化菌种，DHA含量均在10d达到最大；不活化菌种，20℃储藏菌种，DHA在20d可以达到最大；4℃，在25d可以达到最大；－20℃，在5d可以达到最大。除4℃外，其它两种温度下多不饱和脂肪酸变化和DHA变化趋势均一致。20℃，脂肪酸不饱和指数和18∶3/18∶2的变化与DHA变化一致。－20℃储藏菌种，如发酵前不活化菌种，其DHA，脂肪酸不饱和指数和多不饱和脂肪酸变化幅度均为六组最小，可能在这种温度下，菌丝调节脂肪酸变化的能力受到损伤。本研究可以为发酵生产多不饱和脂肪酸提供理论参考。

关键词：头孢霉；菌种储藏温度；菌种活化；二十二碳六烯酸；脂肪酸
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The ω-3 fatty acids such as docosahexaenoic acid (22:6, DHA) had very important physiological functions. It was reported that DHA exhibited beneficial effects in the cardiovascular disease, hypertension and cancer. DHA was also reported beneficial to development of the baby's brain and the eyes [1~5]. For the limited resources, a lot of studies were done on finding new sources. A lot of microorganisms contained polyunsaturated fatty acid (PUFA) and the culture was not restricted by the season. So it became one source of PUFA. Some kinds of microorganism had been researched for producing DHA [6~8]. Although a lot of research had been done on the DHA produced by microorganism, it still would not be the main source for the DHA content and its yield was low. A strain of *Cephalosporium* sp. contained DHA and α-linolenic acid was screened [9]. It was found that in different batches ferment, the DHA's yield showed greaty discrepancy. If the strain was stored in refrigerator, its DHA yield was lower than that stored at 20℃ sometimes. So we inferred that the storing temperature and time might be one of the factors that would affect output. In this paper, we discussed the effect to fatty acid composition when the strain was stored at different temperature, different time no matter how culturing. Four kinds of index including the content of polyunsaturated fatty acid (PUFA), index of unsaturated fatty acid (IUFA), 18:3 (α-)/18:2 and ΣC18/ΣC16 could often be used in the fatty acid researches to estimate the fatty acid change [10~12]. In this paper we used these indexes to study the storing temperature, storing time and reculturing's effects on the fatty acid composition. We hope to find some measures to improve the DHA yield.

1 Material and Methods

1.1 Microorganisms

Cephalosporium sp. was screened and identified by the microbiological lab of the biology science college of Nanjing Normal University, China [9].

1.2 Storing and culturing condition, analysis method

The strains were transformed to new PDA slope culture 3d at 20℃ then stored at 20℃、4℃ and -20℃ for different time. Every time, two slopes strain were taken out in each temperature. One

slope strain was transformed to new PDA agar and cultured at 20℃ for 3d,the other one not transformed; then the sterilized water was added to the agar. The mycelium was scraped and the water with the mycelium was put into the liquid potato dextrose medium for 6d at 20℃, agitating at a reciprocal speed of 150rpm.There was 100ml liquid in the 500ml flask. Mycelia were harvested by suction filtration in a Buchner funnel washed twice by distilled water. Fatty acid analysis: Lipid was extracted and fatty acid was methylated according to [9,13,14] procedure. The fatty acid composition values were given in mol%. The gas chromatography was equipped with a 30m ×0.25mm ×0.25 μm fused silica capillary column coated with HP-innowax (crosslinked polyethylene glycol). Three period temperature programmings were used (170℃2min,10℃/min to 190℃2min,5℃/min to 210℃2min,10℃/min to 220℃ 8min).The injector and detector temperature was 240℃. Fatty acid esters were identified by comparison of their retention times with standard fatty acid esters. The standard fatty acid esters were bought from SIGMA Co.(St. louis, MO). The standard fatty acid esters included 14:0,16:0,16:1,18:0,18:1,18:2,18:3(ω-3),20:4(ω-6),20:5(ω-3),22:6(ω-3)and were eluted in that order. The index of unsaturated fatty acid (or degree of lipid unsaturation) was calculated according to the method of Choi and coworkers[12].ie: IUFA=(1×%monoene + 2×%diene + 3×triene + ….) sum of all know fatty acids. The unrecognizable peaks in the gas chromatogram of each sample were calculated as monoene in this calculation.

2 Results

2.1 Storing the strain at 20℃

If the strain was cultured on new agar before the fermentation, the results showed that the 10d was the best storing time to get high content of DHA. The PUFA、IUFA、18:3 and 18:3/18:2 changed with the same tendency as the DHA's. The 18:1 changed reversed tendency with the DHA's. The changing of the other fatty acids did not coincide with the changing tendency of DHA's(table1).No matterhow cultured the strain on the new agar before fermentation, the tendency of fatty acid variation was different with that of the cultured. DHA content yielded highest at 20d. At 10d,it yielded the minimum. The DHA、PUFA and IUFA fluctuated at lower range than that of the cultured. The PUFA、18:3 and 18:3/18:2 showed the same tendency as the DHA's. When the value of 18:3/18:2 and Σ C18/ Σ C16 got higher at the same time, the content of DHA could get high. If the two values were low at the same time, the DHA content was low too (table 2).

2.2 Storing the strain at 4℃.

To the 4℃, the fatty acid composition changed with the

Table 1 The effect of storing time on fatty acid composition when the strain was stored at 20℃ and cultured on new agar before fermentation (mol%)

Fatty acid	Storing time (d)					
	0	5	10	15	20	25
16:0	16.38	21.04	18.36	14.06	14.46	12.80
16:1	6.13	7.51	9.17	8.34	3.56	6.51
18:0	6.11	1.57	3.26	1.85	6.54	1.31
18:1	19.91	11.96	3.36	3.55	18.34	3.80
18:2	17.55	20.70	16.84	23.21	22.09	14.17
18:3 α -	5.71	2.93	21.56	14.83	6.79	6.44
20:5	0	0	0	0.34	0.22	0
22:2	19.87	20.31	7.18	11.43	9.47	21.02
22:6	2.57	2.81	9.76	7.80	2.83	3.54
其它	5.76	11.17	10.51	14.40	15.70	30.41
Σ C18/ Σ C16	2.19	1.30	1.64	1.94	2.98	1.33
18:3/18:2	0.33	0.14	1.28	0.64	0.31	0.45
Σ PUFA	45.70	46.75	55.34	57.81	41.40	45.18
IUFA	139.18	138.31	194.31	189.73	139.14	151.66

16:0 Palmitic acid; 16 1:Palmitoleic acid; 18:0: Stearic acid; 18:1: Oleic acid; 18: 2: Linoleic acid;18:3 α -: α -Linolenic acid; 20:5: Eicosapentaenoic acid; 22:2:Docosadienoic acid ; 22:6: Docosahexaenoic acid Σ C16=16: 0+16: 1; Σ C18=18: 0+18: 1+18: 2+18: 3; PUFA: Polyunsaturated fatty acid. IUFA=16:1+18: 1+18: 2*2+18: 3*3+22:6*6+others.

storing time too (table 3, 4). The DHA、PUFA and IUFA fluctuated at far low range than that at 20℃.It showed that the strain stored at 4℃ was more stable than that at 20℃. All the consistency to the DHA disappeared. If cultured before fermentation, the best storing time to get high content of DHA was 10d. If not cultured before fermentation, 25d was the best time.

2.3 Storing the strain at -20℃.

To the -20℃, the fatty acid composition varied with the storing time too. To the cultured, 10d of storing was the best time to get higher content of DHA. PUFA and IUFA changed with the same

Table 2 The effect of storing time on fatty acid composition when the strain was stored at 20℃ and not cultured on new agar before fermentation (mol%)

Fatty acid	Storing time (d)					
	0	5	10	15	20	25
16:0	16.36	13.02	23.92	18.48	14.08	17.00
16:1	6.13	5.26	9.57	4.80	12.57	7.59
18:0	6.11	8.01	1.95	6.82	8.39	5.64
18:1	19.88	19.03	17.03	22.18	16.05	8.49
18:2	17.53	17.71	14.09	24.40	25.33	30.43
18:3 α -	5.70	4.81	3.49	5.77	16.07	13.24
20:5	0	0.10	0.55	0.55	0.01	1.29
22:2	19.87	9.86	19.62	13.02	0.84	2.81
22:6	1.71	4.21	1.24	1.55	6.10	4.57
其它	6.73	17.98	8.54	2.42	0.54	8.58
ΣC18/ΣC16	2.19	2.71	1.09	2.54	2.47	2.32
18:3/18:2	0.33	0.27	0.25	0.24	0.63	0.44
ΣPUFA	44.79	36.69	38.98	45.30	48.37	52.33
IUFA	134.85	137.59	123.19	133.61	166.44	165.03

Table 3 The effect of storing time on fatty acid composition when the strain was stored at 4℃ and cultured on new agar before fermentation (mol%)

Fatty acid	Storing time (d)					
	0	5	10	15	20	25
16:0	16.38	8.68	12.18	14.89	14.02	16.85
16:1	6.13	7.47	5.38	3.70	3.68	1.85
18:0	6.11	2.20	3.77	7.62	6.69	7.89
18:1	19.91	18.08	20.40	23.99	23.82	32.07
18:2	17.55	18.44	18.49	20.98	18.11	14.87
18:3 α -	5.71	4.51	2.91	5.35	4.75	5.07
20:5	0	0.04	0	0.34	0.33	0.09
22:2	19.87	21.44	31.27	19.54	19.16	15.70
22:6	2.57	0.73	2.84	2.83	3.06	1.13
其它	5.76	18.41	2.77	0.76	6.39	4.48
ΣC18/ΣC16	2.19	2.68	2.59	3.11	3.01	3.20
18:3/18:2	0.33	0.24	0.16	0.26	0.26	0.34
ΣPUFA	45.70	45.16	55.51	49.03	45.40	36.87
IUFA	139.18	141.82	153.82	144.19	142.64	122.01

trend as the DHA's (table 5). If the strain was not cultured before fermentation, the PUFA、IUFA and DHA fluctuated at lowest range in the six groups. All the consistency to DHA had disappeared except PUFA(table 6). This suggested that the low temperature damaged the strain's ability of regulating fatty acid composition.

3 Discussion

Temperature was the main factor affecting the fatty acid composition. The low temperature was beneficial to form the PUFA[10]. Although a lot of studies about the effect of culture temperature on fatty acid composition had been done, but the effects of strains storing time on different temperature no matter how cultured on new agar before ferment had been hardly studied. The

Table 4 The effect of storing time on fatty acid composition when the strain was stored at 4℃ and not cultured on new agar before fermentation (mol%)

Fatty acid	Storing time (d)					
	0	5	10	15	20	25
16:0	16.36	18.36	16.66	7.40	15.13	8.06
16:1	6.13	1.46	3.42	5.99	2.89	3.05
18:0	6.11	6.70	9.05	5.36	5.26	6.55
18:1	19.88	11.57	31.48	16.19	15.16	16.58
18:2	17.53	16.75	14.67	25.88	20.67	11.05
18:3 α -	5.70	7.21	5.62	7.34	5.59	2.83
20:5	0	0	0	0.05	0.05	0.05
22:2	19.87	5.69	12.12	17.35	12.68	14.64
22:6	1.71	1.13	2.01	2.80	1.85	3.56
其它	6.73	31.12	4.96	11.64	20.72	14.61
ΣC18/ΣC16	2.19	2.13	3.03	4.09	2.59	3.33
18:3/18:2	0.33	0.43	0.38	0.28	0.27	0.26
ΣPUFA	44.79	30.78	34.43	53.42	40.83	32.13
IUFA	134.85	117.43	122.39	159.36	133.08	115.74

Table 5 The effect of storing time on fatty acid composition the strain was stored at -20℃ and cultured on new agar before fermentation (mol%)

Fatty acid	Storing time (d)					
	0	5	10	15	20	25
16:0	16.38	15.45	16.11	15.74	15.58	20.19
16:1	6.13	6.37	5.36	2.11	3.47	4.84
18:0	6.11	2.84	5.50	5.08	4.13	4.30
18:1	19.91	15.43	17.19	14.32	15.26	18.61
18:2	17.55	19.83	26.30	21.06	15.37	18.74
18:3 α -	5.71	4.63	9.49	8.62	4.44	5.75
20:5	0	0	0	0.26	0.07	0
22:2	19.87	17.25	9.98	10.72	14.54	13.11
22:6	2.57	3.04	4.04	1.0	1.45	2.0
其它	5.76	15.16	6.04	21.10	25.69	12.45
ΣC18/ΣC16	2.19	1.96	2.72	2.75	2.06	1.89
18:3/18:2	0.33	0.24	0.36	0.41	0.29	0.31
ΣPUFA	45.70	44.76	49.80	41.66	35.87	39.60
IUFA	139.18	143.28	153.83	134.25	126.62	128.85

Table 6 The effect of storing time on fatty acid composition the strain was stored at -20℃ and not cultured on new agar before fermentation (mol%)

Fatty acid	Storing time (d)					
	0	5	10	15	20	25
16:0	16.36	14.58	17.50	16.61	18.01	14.46
16:1	6.13	4.91	6.52	1.72	2.32	4.02
18:0	6.11	5.72	5.22	7.13	5.42	5.48
18:1	19.88	25.45	23.41	35.46	27.77	21.67
18:2	17.53	21.79	17.62	19.23	19.62	15.67
18:3 α -	5.70	5.14	5.76	6.88	5.87	3.45
20:5	0	0	0	0.09	0.05	0.07
22:2	19.87	13.49	16.24	7.79	8.73	17.95
22:6	1.71	3.03	4.08	0.99	1.44	2.03
其它	6.73	5.88	3.63	4.08	10.78	15.21
ΣC18/ΣC16	2.19	2.98	2.17	3.75	2.89	2.50
18:3/18:2	0.33	0.24	0.33	0.38	0.30	0.22
ΣPUFA	44.79	43.45	43.71	34.99	35.70	39.17
IUFA	134.85	140.40	143.05	122.38	124.02	131.0

results showed that if the strain was stored at low temperature (-20℃) for a short time (e.g. 5～10d), its ability to synthesize DHA would increase. While for longer time, there was a reverse effect. Whether the strains were cultured before fermentation was an important factor that would affect the fatty acid composition. In fact, storing the strain at 20℃ for successive culture.the results suggested the strain cultured only for 3d before fermentation could not get high content of DHA. If the strain was cultured at 20℃ for 20d before fermentation or cultured for 10～15d and then cultured for the second time on new agar for 3d, it could get high content of DHA. Storing the strain at -20℃ for more than 10d, the PUFA ,IUFA and the ability to synthesize DHA decreased gradually. This suggested -20℃ was harmful to the strain. Storing the strain at this temperature and not cultured before fermentation, the range from the maximums to the minimum of the PUFA was only 8.97%. It was the lowest of the six conditions. If cultured in new slope agar, the range would amount to 13.94%. This suggested that the strain cultured on new slope agar could decrease the hurt from the low temperature. To the 4℃, the hurt had not showed. The studies suggested the PUFA changed with the same trend as the DHA's except at 4℃. Maybe it could be an index to examine the ability to synthesize the DHA. The consistency was in accordance with our previous study that the desaturation from 18:1 to 18:2 was the key step for synthesizing DHA [9]. Storing at 20℃, the 18:3/18:2 and IUFA changed with the same tendency as the DHA's, which suggested under the suitable condition, the desaturation from 18:2 to 18:3 was critical too. In most cases, the value of ΣC18/ΣC16 and the DHA's synthesis had no consitency, which agreed with our previous studies that the optimum conditions to desaturation enzyme and chain elongation enzyme were different[10].

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壳聚糖-Zn（Ⅱ）配合物对尿素的吸附行为的研究

郎惠云，蔡 健，魏永锋
（西北大学化学系，陕西 西安 710069）

摘 要：本文提出了利用高分子聚合物壳聚糖-Zn（Ⅱ）配合物吸附尿素的方法。实验结果表明，最佳吸附条件为：室温下，溶液pH在6～7之间，尿素的起始浓度为2.0mg/ml，反应时间为3.0h，最大吸附量可达83.97mg/g。

关键词：壳聚糖；锌；配合物；吸附；尿素

Urea Adsorption by Chitosan-Zn(Ⅱ) Complex

LANG Hui-yun,CAI Jian,WEI Yong-feng
(Department of Chemistry,Norhtwest University, Xian 710069,China)

Abstract: A method for urea adsorption by chitosan-Zn(Ⅱ) complex was studied.The optimal conditions were obtained from experiments.The adsorption capacity of the complex for urea was 83.97mg/g under room temperatur and pH value between 6～7,while the original concentration of urea was 2.0mg/ml.The adsorption time was 3.0h.

Key words: chtiosan; zinc; complex; adsorption; urea

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尿素是肾功能衰竭和尿毒症患者血液中存积的主要毒性成分，高效地清除尿素一直是生物医药领域中人工肾和口服尿素吸附剂研究的重要课题。由于常规的吸附剂存在吸附容量低、选择吸附性差、生物和血液相容性不好等缺点，因此，尿素吸附剂的研究一直未能取得突破性进展。设计合成新型尿素吸附剂，将其用于吸附性人工肾或透析人工肾中透析液的同步再生是人们关注的热点。

目前，常见的除去尿素的方法有：①活性炭吸附法，该方法的缺点是吸附容量很低，仅为9.0mg/g[1]，而且用作人工肾材料时，微碳粒易脱落，有造成人体内栓塞的危险；②利用脲酶降解尿素分子形成NH_4^+和CO_3^{2-}，然后再通过阳离子交换树脂除去NH_4^+的方法降低尿素含量[2]，该方法的缺点是在除去NH_4^+的同时却影响了人体内正常的离子平衡；③用合成的高分子聚合物与金属离子形成的配合物来分解尿素[3]，

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作者简介：郎惠云（1942-），教授，主要从事药物分析和医用敷料的研究。