GC-MS Determination of Fatty Acids in Arachidonic Acid High-Yield Strain Induced by Low-Energy Ion Implantation

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With a novel mutation technique, low-energy ion beam implantation, which was originated in our laboratory, an arachidonic acid high-yield strain (*Mortierella alpina*) was obtained. The mycelium of this strain was dried and treated with boron fluoride—methanol solution. The fatty acids were determined by gas chromatography—mass spectrometry. The result showed that there were saturated and unsaturated fatty acids in the dried mycelium. The relative content of arachidonic acid was 70.2 % among all fatty acids. In addition, there were linoleic acid (3.985 %) and γ-linolenic acid (1.576 %) in the dried mycelium.

Due to their specific biological activity, polyunsaturated fatty acids have aroused great concerns in recent years [1—3]. Arachidonic acid (AA), which belongs to n-6 series of polyunsaturated fatty acids, is an important intermediate product and a precursor of many eicosanoic acid derivatives, such as prostaglandin, prostacyclin, thromboxane, and leukotriene. As a precursor, AA can be catalyzed to prostaglandin by enzyme, which sharply reduces the cost of the production. On the other hand, due to its particular function in brain and eye growth, AA was first and mostly applied in infant nutrition field, such as the addition in liquid milk and in infant compound food. Until now over 80 countries have approved AA application as food additive. Besides infant compound food, the application of AA has expanded to the fields of healthy food, health care products, cosmetics, and biopharmacy [4—6]. This is consistent with the importance and novel functions of AA [7—9].

There have been some reports on AA in some kinds of mycelial fungi and microalgae since 1980. Due to the great advantages of microorganism fermentation, such as growing at a high speed, abundance of fatty acid and AA, giving a stable yield, many researchers show interests in such projects. Although there are several reports about AA fermented by microorganism in the world, the concentration of AA is very low [10—12].

In 1980's *Yu* [13] discovered the genetic effect in rice mediated by low-energy ions. Since then, ion beam implantation has been used widely to improve crops and microorganism character. Accumulating evidences have shown that three factors including energy absorption, mass deposition, and charged ions exchange, may play an essential role in low-energy ion bio-effects. So we carried out a mutation experiment with ion beam implantation and an AA high-yield strain was screened. The fatty acids in dried mycelium were analyzed by gas chromatography—mass spectrometry, which provided convincing evidence for potential applications of this strain.

EXPERIMENTAL

Mortierella alpina was used as original strain in this research. First, 0.2 cm^3 spore suspension of original strain was put in dish and dried by sterile wind, then it was irradiated by N^+ ions. After ion irradiation, the samples were washed by sterile water. Then they were inoculated in shaking flask containing 100 cm^3 liquid medium with 6.0 % glucose, 1.2 % peptone, 0.8 % yeast extract, 1.0 % peanut cake, pH = 8.5, and shaken at 200 rpm. All cultures grew for about seven days at 28 ± 1 ℃. At the end, fungal mycelia were harvested by suction filtration and washed with 50 cm^3 of water. After being dried and treated, they were analyzed with GC-MS.

The N^+ ion was produced by the CASIPP (Chinese Academy of Sciences, Institute of Plasma Physics) ion beam bioengineering instrument and its energy was 25 keV [14, 15]. The experiment apparatus is shown in Fig. 1 as a schematic diagram of an ion implanter. The samples were spread on the film over glass substrates and placed in the target chamber. For ion irradiation, the pulse technique was used and the pulse time was

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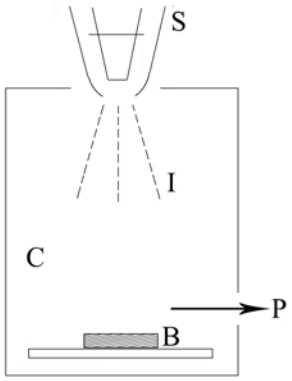

Fig. 1. Schematic diagram of an ion implanter. S – ion source, I – ion beam, C – chamber, P – pump, B – sample.

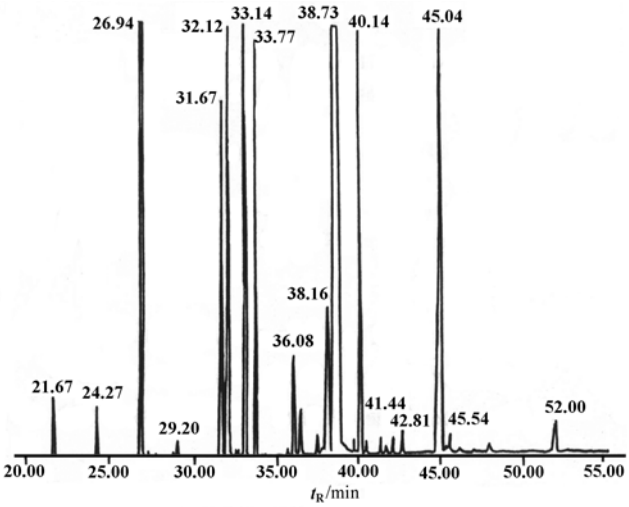

Fig. 2. The total ion current chromatogram of fatty acid methyl esters.

Table 1. Contents of Fatty Acids in Dried Mycelium

Fatty acid	Retention time/min	Content/%
Myristic acid (C14:0)	21.67	0.221
Pentadecanoic acid (C15:0)	24.27	0.174
Palmitic acid (C16:0)	26.94	6.315
Stearic acid (C18:0)	31.67	2.236
Oleic acid (C18:1)	32.12	3.676
Linoleic acid (C18:2)	33.14	3.985
γ-Linolenic acid (C18:3)	33.77	1.576
Arachidic acid (C20:0)	36.08	0.049
Arachidonic acid (C20:4)	38.73	70.201
Behenic acid (C22:0)	40.14	2.657
Lignoceric acid (C24:0)	45.04	5.302
Others		3.806

10 s. For each pulse, the irradiation ion dose was $D_0 = 10^{15}$ cm^{-2}.

All reagents (boron fluoride—methanol, potassium hydroxide, petroleum ether) were anal. grade.

The GC-MS analyses were performed on an Auto-System (Perkin—Elmer, USA) connected to a silex capillary column of DB-5 (30 m × 0.25 mm × 0.25 m) made by SULPECOS.

At chromatography temperature parameters were set as follows: injector at 250 ℃, ion source 200 ℃. Oven program: initial temperature: 80 ℃ for 20 s, 80 ℃ to 240 ℃ at 4 ℃ min^{-1}, 240 ℃ for 10 min. Carrier gas was helium; column pressure 100 kPa, split rate 1:30. 1.0 mm^3 sample was injected. The energy of the EI source of the Perkin—Elmer mass spectrometer was 70 eV.

Sample Preparation

The dried mycelium was weighed (0.3 g) and added to 0.5 cm^3 of potassium hydroxide—methanol solution (c = 0.5 mol dm^{-3}). This mixture was heated for 30 min at 60 ℃. Then it was added to 1.0 cm^3 boron fluoride—methanol solution (φ_r = 1:2).

The solution was heated for 30 min at 60 ℃, then it was added to 1 cm^3 of petroleum ether and 1 cm^3 of saturated salt solution. Finally, supernatant fluid was injected into the GC-MS system.

RESULTS AND DISCUSSION

The total ion current chromatogram of fatty acid methyl esters is shown in Fig. 2.

The content of all fatty acids compared with the standard sample was calculated (see Table 1).

C_{12}—C_{24} saturated and unsaturated fatty acids were separated by the above chromatogram. The oil of the strain largely belongs to unsaturated fatty acids (79.438 %). However, it should be noticed that arachidonic acid (retention time 38.73 min, content 70.201 %) is topmost among all fatty acids in the mycelial oil. The contents of γ-linolenic acid (retention time 33.77 min) and linoleic acid (retention time 33.14 min) among those fatty acids were 1.576 % and 3.985 %, respectively.

The methyl linoleate, methyl γ-linolenate, and methyl arachidonate mass spectra are shown in Fig. 3.

CONCLUSION

With our novel low-energy ion beam implantation mutation technique, an AA high-yield strain was screened, which, together with previous researches [16—18] showed that this technique is very effective. This study laid a foundation for industrialized production of AA. Owing to the low-cost property of microbiological fermentation, we believe that AA will have a potential market place. As one of the most important polyunsaturated fatty acids, AA shows varied applications in infant growth, nutrition of pregnant and lying-in women and prevention of cardiovascu-

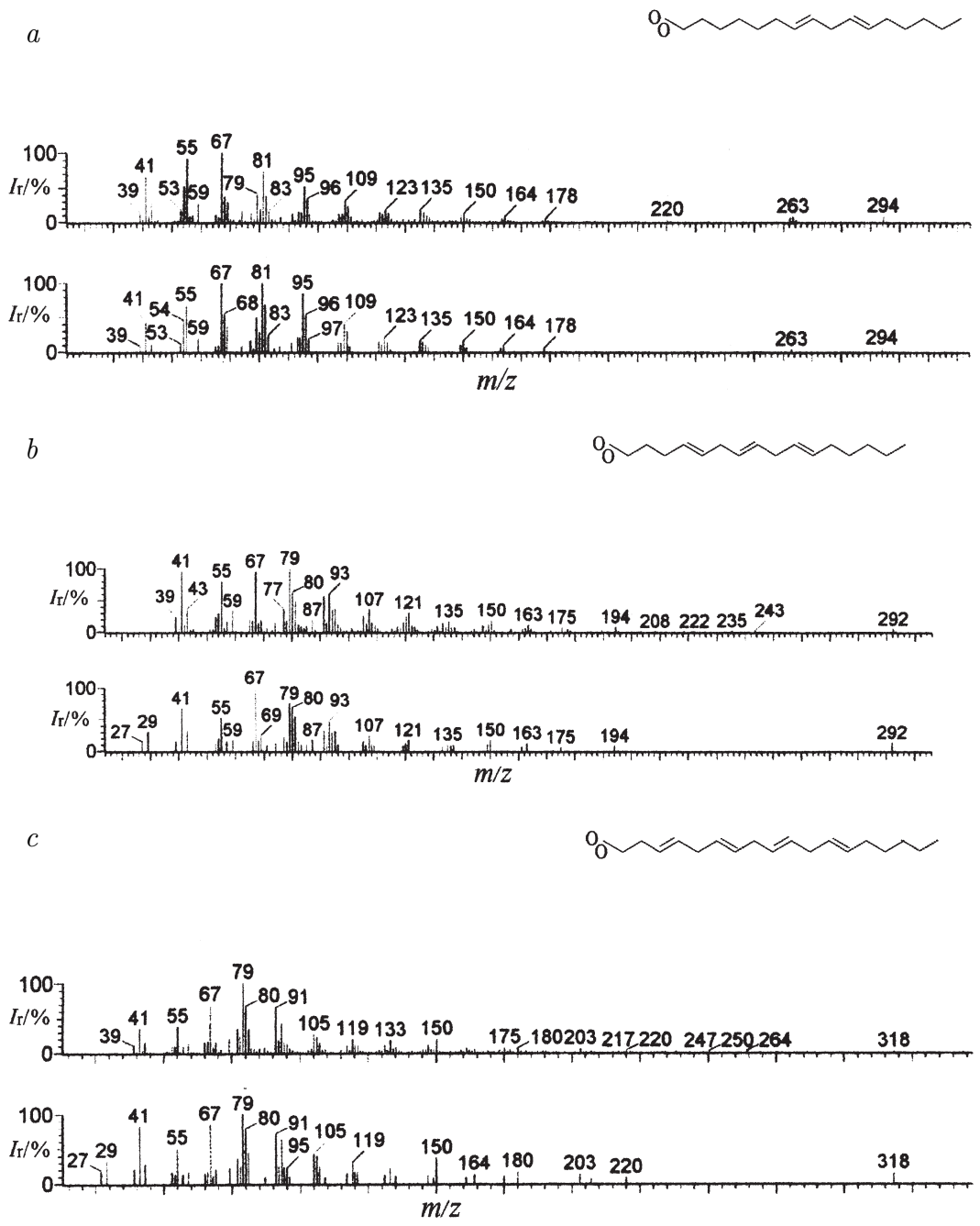

Fig. 3. Mass spectrum of methyl linoleate sample (upper) and standard (lower) (*a*), of methyl γ-linolenate (*b*), of methyl arachidonate (*c*).

lar diseases. AA plays a decisive role in the course of human intelligence development. With the popularization of AA concept and the constant breakthrough of the follow-up researches and developments, the applications of AA products will be expanded further.

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