エイコサペンタエン酸(EPA)産生細菌(SCRC-2738)のEPA生産に及ぼすアルギニンの影響

誌名	日本水産學會誌
ISSN	00215392
著者	鈴木, 信雄 井上, 玲 鹿野, 真弓
巻/号	57巻7号
掲載ページ	p. 1407-1407
発行年月	1991年7月

Short Paper

Effect of Arginine on the Production of Eicosapentaenoic Acid (EPA) in EPA-elaborating Bacterium SCRC-*2738*

Nobuo Suzuki,*1 Akira Inoue,*2 Mayumi Shikano,*2 Kazunaga Yazawa,*2 and Kiyosi Kondo*2

(Received October 2, 1990)

Eicosapentaenoic acid (EPA) is effective for the protection and the curing of thrombosis, atherosclerosis, and subsequent blood-circulating diseases.[1] A *Shewanella*-like ("*Alteromonas*-like" according to the former classification[2]) bacterium SCRC-2738, isolated from the intestine of a Pacific mackerel, was shown to contain EPA as a major fatty acid component of the cell,[3] and is expected to be utilized for the industrial production of EPA. It is necessary to establish the optimal culture condition for such purpose. We observed that SCRC-2738 grow well in the PY medium without additional carbohydrate such as glucose (unpublished data), and this suggests that SCRC-2738 might require certain amino acid(s) for its growth. In this study, we searched out the amino acid(s) to be most required and examined the effect of the amino acid(s) on the growth and EPA content of SCRC-2738.

SCRC-2738 was cultured in 100 m*l* of PY broth, which was composed of 1% peptone (Kyokuto) and 0.5% yeast extract (Merck) in 50% artificial sea water (ASW, "Jamarin S"; Jamarin Laboratory), in a 500-m*l* flask with triple baffle at 15°C for 18 h with a rotary shaker (180 rpm). Before and after the culture, the medium was collected and deproteinized by the addition of 10% sulfosalicylic acid, and the amino acid composition of filtrate was quantitated by an amino acid analyzer (Model 835, Hitachi). One of the most consumed amino acids was arginine, which decreased from 75.5 μg/m*l* to the undetectable amount during the culture. Other amino acids favored by SCRC-2738 were leucine (from 134.2 μg/m*l* to 30.6 μg/m*l*) and phenylalanine (from 82.7 μg/m*l* to 21.1 μg/m*l*).

The complete cosumption of arginine led us to examine the promoting effect of arginine on the growth and EPA content of SCRC-2738. One loop of SCRC-2738 on a PY slant was inoculated into 5 m*l* of PY broth and incubated at 15°C with shaking until the optical density (OD) at 610 nm reached 8.0. Then 1 m*l* of the culture was inoculated into 100 m*l* of PY broth without or with arginine (0.01% or 0.1%) in duplicate cultures. Bacterial growth was monitored by OD at 610 nm with a spectrophotometer (Spectronic 20D, Milton Roy Company). EPA content of whole culture or dry cells was measured by the method described previously.[4] Figure 1 shows the time-course of the growth (A) and the EPA content (B) of SCRC-2738. The growth was slightly enhanced by the addition of arginine. The EPA content in the arginine-supplemented medium was considerably greater than that of control. At 24-h incubation, arginine (0.1%)-supplemented culture contained 75.1 mg/*l* of EPA, whereas the control 64.5 mg/*l*. In a separate experiment, arginine (0.1%)-supplemented culture contained 87.8 mg/*l* of EPA and the control contained 61.5 mg/*l*. Thus, arginine increased the EPA content of culture with little affection on the growth. These data suggest the possibility of increase of EPA content in individual cells by the addition of arginine. Then, we measured the EPA content of dry cells. At 18-h culture, cells grown in arginine-supplemented media contained larger amount of EPA (11.6 or 11.95 mg/g dry cells in 0.01% or 0.1% arginine-medium, respectively) than that of control (10.45 mg/g dry cells). Thus, EPA content increased 11.0% or 14.4%, respectively.

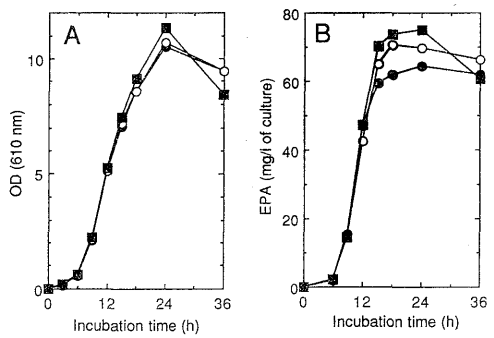

Fig. 1. Effect of arginine on the growth (A) and the EPA content (B) of SCRC-2738. Data are expressed as averages of duplicate cultures. Control, ●; 0.01% arginine, ○; 0.1% arginine, ■.

These results indicated that the increased concentration of arginine in the culture medium might alter the composition of fatty acids of this bacterium. The fatty acid composition of marine bacteria[3,4] as well as enteric bacteria[5] has been reported to be altered in response to its culture condition, *e.g.* growth temperature. Particularly, the relative amount of EPA among total fatty acids increases at the lower culture temperature in marine bacteria.[3,4] Our results showed the possibility that certain amino acid(s) in the culture medium may alter the fatty acid composition of bacteria. Further studies are required to reveal the detail of this phenomenon and to clarify its mechanism.

The effect of arginine is expected to be applied for a simple and easy method for the production of EPA using strain SCRC-2738 without the use of low-temperature cultivation, which may cause a high cost.

Acknowledgment

We are very grateful to K. Takada for his continuous support during the course of this study.

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*1 Hoko Fishing Co., Ltd., Kamiwada, Yamato, Kanagawa 242, Japan (鈴木信雄：宝幸水産(株)食品総合研究所).
*2 Sagami Chemical Research Center, Sagamihara, Kanagawa 229, Japan (井上 玲，鹿野真弓，矢澤一良，近藤 聖：(財) 相模中央化学研究所).