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THE PRESENCE OF EICOSAPENTAENOIC ACID (20:5 n-3) IN LIPIDS FROM FRESH WATER BACTERIA ISOLATED FROM INTESTINAL LIQUID FROM ARCTIC CHARR, *SALVELINUS ALPINUS* (L.).

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ABSTRACT

The Arctic charr, *Salvelinus alpinus* (L.), was stripped of faecal pellet and intestinal liquid. Microorganisms were isolated from the intestinal liquid at 12°C and 4°C. Fatty acid analyses of microorganisms grown in a lipid free Tryptic Soy Broth medium added glucose (TSBg) showed the presence of 5-8% eicosapentaenoic acid (20:5 n-3). Differences in fatty acid composition was found in total fatty acids of bacteria isolated from intestinal liquid at 12°C and 4°C and thereafter grown in a TSBg medium at 4°C.

INTRODUCTION

Previous investigations concerning the fatty acid composition in microorganisms have often showed that polyunsaturated fatty acids (PUFA) do not occur (see Ratledge and Wilkinson, 1988). However, it has been reported that deep-sea bacteria, *Vibrio marinus* (De Long and Yayanos, 1986) a marine *Alteromonas* sp. (Wirsen et al., 1987) and *Shewanella Alteromonas putrefaciens* contain eicosapentaenoic acid (20:5 n-3) (Yazawa et al., 1988 a;b).

No investigations so far have shown that freshwater bacteria contain PUFA. The aim of this study was to investigate whether intestinal bacteria isolated from freshwater fish contain long chain PUFA.

MATERIAL AND METHODS

The feed consisted of a moist pellet based on casein, dextrin and coconut oil (Table 1). A detailed description of the different premixes added to the feed is given by Olsen et al. (1990). The feed components were thoroughly mixed and then pelleted, and stored at -80°C prior to use.

Alevins of Arctic charr, *Salvelinus alpinus* (L.), were fed a commercial feed (Tess Elite Pluss, Skretting LTD) from the initial feeding stage until a mean weight of approximately 10 g was attained. Thereafter the fish were given the experimental diet for 180 days.

The feeding experiment was set up with 30 Artic charr. Experimental design is given by Ringø and Nilsen (1987). This study was undertaken under the natural photoperiod at 70°N from mid October to mid April at an average water temperature of 6°C ± 1.0°C.

Ten fish, weight about 40 g, were anaesthetized in 0.3% benzocaine, thereafter stripped by pressing the belly of the fish until the intestinal liquid flowed the faecal pellet. The intestinal liquid was separated by sedimentation from the faecal pellet after a respite of 2 min.

Total viable counts were performed on TSAg plates containing TSA (Tryptic Soy Agar), 40 g/l and glucose, 5 g/l. Samples of intestinal liquid were diluted in sterile 0.9% saline and 0.1 ml volumes of appropriate dilutions were spread on the surface of the TSAg plates. The plates were incubated at 12°C and 4°C, and inspected daily for up to 4 weeks. After enumeration, a representative selection of colonies were subcultured on TSAg plates.

After confirmation of culture purity bacteria were cultured in TSBg medium, containing TSB (Tryptic Soy Broth) 40 g/l and glucose, 5 g/l. Thereafter, the bacteria culture (0.8 ml) was added glycerol (0.2 ml) and stored at -80°C for further identification.

Seventeen and ten bacterial isolates, isolated from intestinal liquid at 12°C and 4°C, respectively, were grown in lipid free TSBg medium at 4°C to an

Table 1. Ingredients (gram per kg dry weight) of the diet.

Casein	706.5
Dextrin	170.0
Gelatin	17.0
Premix I	30.0
II	1.7
III	1.8
IV	20.0
Coconut oil	53.0

A detailed description of the added premixes is given by Olsen et al. (1990).

optical density (OD 600) of approximately 0.7. Thereafter, sterile culture medium (TSBg) was inoculated with 0.25% (vol/vol) of each bacterial isolate and cultivated at 4°C. Microorganisms were harvested at three different growth phases (exponential, stationary and death). The bacteria were centrifugated at 5.000 rpm for 15 min in a Sorvall centrifuge (model RC2-B), and then bacteria were dissolved in 5 ml methanol. The bacteria cells were sonicated for 60 sec and extracted with chloroform: methanol (2:1, v/v). The extract was left at 12°C for several hours, and then evaporated to remove the solvent (Kates, 1972). Methyl esters of fatty acids were prepared by H_2SO_4 catalysed transesterification of total lipid in methanol (Christie, 1982), and were analysed by gas chromatography (Hewlett Packard, Model 5890 A) using a SP 2330 capillary column (30 m x 0.25 mm i.d) and helium as carrier gas. The temperature program employed was 60°C for 1 min, followed by an increase of 30°C/min to 180°C for 7 min and thereafter 5°C/min to 240°C. Individual fatty acids were identified by comparison with known standards (Supelco 4-7019, 4-7042, 47033, 4-5589) and quantified using a Hewlett-Packard 3393A integrator.

Eicosapentaenoic acid (20:5 n-3) was further identified by GC-MS using a 5970 MSD (Hewlett-Packard) connected to a Hewlett-Packard work station 9000-300.

RESULTS AND DISCUSSION

Colony Forming Units (CFU) of aerobic microorganisms in the intestinal liquid were $7.3x10^4$ at 12°C incubation, and $8.2x10^4$ at 4°C incubation on TSAg plates.

It is generally accepted that bacteria do not contain polyunsaturated fatty acids (PUFA) and that such fatty acids can only be formed *de novo* by photosyntethic organisms. However, microorganisms isolated from intestinal liquid contained eicosapentaenoic acid (EPA) (20:5 n-3) when grown in a lipid free TSBg medium (Table 2). The proportion of EPA in microorganisms isolated at 12°C and thereafter grown at 4°C (cultivation study I) accounted for 8.5% of total fatty acids when harvested in the exponential phase. However, the EPA content decreased during growth and accounted for 6.1% of total fatty acids in microorganisms harvested in the death phase. A somewhat lower proportion of EPA during the growth phases was found in cultivation study II, bacteria isolated and grown at 4°C. Neither EPA nor other PUFA were detected in sterile TSBg medium (results not shown).

The presence of EPA has been reported in the following marine bacterial species: the deep-sea bacterial *Vibrio marinus* (De Long and Yayanos, 1986),

Table 2. Composition of the major fatty acids (%) in the microorganisms isolated from intestinal liquid at 12°C (cultivation study I) and 4°C (cultivation study II) and thereafter grown in TSBg medium at 4°C. Samples were analysed in microorganisms taken in (A) exponential, (B) stationary phase and (C) death phase.

Fatty acids	Cultivation study I			Cultivation study II		
	A	B	C	A	B	C
12:0	3.1	2.9	3.3	1.1	1.1	0.9
14:0	2.2	1.2	1.2	1.5	1.1	0.8
15:0	1.9	2.3	2.2	1.6	1.8	1.4
15:1	1.6	1.7	1.3	1.0	1.0	0.8
16:0	9.3	8.2	7.4	9.4	10.9	11.8
16:1 [a]	37.2	35.0	32.6	40.4	36.6	23.9
17:1	5.8	6.4	5.1	4.7	7.2	12.8
18:1 [b]	7.3	8.1	9.5	14.1	14.7	17.8
20:5 (n-3)*	8.5	6.8	6.1	6.3	6.4	4.6

16:1 [a]; 16:1 (n-9) + 16:1 (n-7)

18:1 [b]; 18:1 (n-9) + 18:1 (n-7)

*; identified by GC-MS

Alteromonas sp. (Wirsen et al., 1987) and *Shewanella Alteromonas putrefaciens* isolated from intestinal contents of horse mackerel *(Trachurus japonicus)* and Pacific mackerel *(Pneumatophorus japanicus)* (Yazawa et al., 1988 a;b). These findings indicate that microorganisms do contain PUFA more frequently than earlier suggested.

The major fatty acid in the lipids from all growth experiments was palmitoleic acid (16:1). However, the proportion of this fatty acid remained constant, and accounted for approximately 35% of total fatty acids in cultivation study I, while a decrease from c. 40% to 24% was found during growth in cultivation study II.

The proportion of 17:1 in the three growth phases in cultivation study I accounted for approximately 5.5% of total fatty acids. However, an increase in the proportion of 17:1, from c. 5% to c. 13% of total fatty acids was found during growth of bacteria isolated at 4°C and cultivated at 4°C (cultivation study II). The amount of 18:1 increased from 14 to 18% during cultivation study II, while this fatty acid remained constant (c. 9%) during the three growth phases of cultivation study I.

Based on the fatty acid composition in the three growth phases of the two cultivation studies (Table 2) we suggest that these differences are due to different bacterial species isolated from the intestinal liquid at 12° and 4 °C. Identification of the microorganisms is in progress.

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