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Screening on DHA-producing Antarctic bacteria N – 6 and its cultural conditions

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Abstract In Antarctic, the geography and climate differs from those in other places, and the bacteria there have adapted well to the environment there. Two hundred strains of bacteria were isolated from the sea ice in Antarctica. The bacteria were screened for DHA by means of GC, with fish oil as the standard. Seven strains containing DHA or EPA were obtained, among which the strain of No. N-6 was outstanding. And the component of DHA was identified by GC-MS. The relative content of DHA in N-6 was 8.72%, and total lipids in dry bacteria was 22.54%. The effects of some factors, including temperature, salinity and pH value, on the growth and DHA-content of strain N-6 were studied. The results show that the DHA-content is relatively high when in low temperature and high pH, and the bacterium is psychrophilic, alkalophilic.

Key words Antarctic, bacteria, GC-MS, unsaturated fatty acids

1 Introduction

Polyunsaturated fatty acids (PUFAs) have remained as a natural product due to the synthetic difficulty of reproducing the methylene interrupted double bond sequence by industrial chemistry. Their significance to animals and humans lies in their biological activity, as precursors for groups of nutritionally important compounds and as essential cellular components(Meyer *et al.* 1999). Interest in the production of PUFAs, especially DHA and EPA, from alternative sources for use in aquaculture feeds and human health supplements has fuelled recent research into the molecular biology of PUFA production in sea ice microorganisms(Mock and Kroon 2002a, b).

In order to colonize habitats of extremely low temperatures, Antarctic microorganisms have developed outstanding molecular adaptations at the ultrastructural and metabolic levels (Russel 1990; Russel and Fukunaga 1990; Gounot 1991; Arpigny *et al.* 1994; Feller *et al.* 1996). PUFA in membrane lipids is concerning acclamatory of adaptational changes of psychrophiles in response to low ambient temperatures(Hazel 1989; Hazel 1995; Cossins and Macdonald 1989; Tasaka *et al.* 1996; Suutari *et al.* 1997). An increased proportion of DHA helps to maintain the static order and dynamic properties of membranes which is vi-

tal for the function of cells and whole organisms. In the study, several strains of bacteria containing DHA were obtained, and N-6 was the prominent. It is a well-known fact that fatty acids composition by microorganisms is greatly influenced by physical factors such as pH, temperature and by others factors such as media composition (Suzuki *et al*. 1991; Nichols DS, McMeekin *et al*. 1994). The effects of some factors, including salinity, temperature and pH value, on the growth and DHA-content of strain N-6 were studied. The results show that the DHA-content is relatively high when in high salinity, low temperature and high pH. With the merit of fast growth and high production of DHA at low temperatures, N-6 and its DHA might be potentially used in food industries in future.

2 Materials and Methods

2.1 Culture conditions and harvest

Two hundred strains of bacteria were isolated from the sea ice in Antarctica (68°30′E, 65°00′S) during the 19th Antarctic Scientific Expedition of China (2001–2002), all of which were conserved in the Key Laboratory of Marine Bio-active Substances, State Oceanic Administration, China.

The bacteria were cultured in 2216E sea water medium (ZoBell 1946) at 8 ℃ with shaking at 100 rpm. The cultured cell mass was collected by centrifugation at 8000 rpm for 10 min, then washed with distilled water and centrifuged for two times and lyophilizated finally.

2.2 Preparation of fatty acid methyl esters and lipid extraction

Fatty acid methyl esters (FAME) were produced by direct transesterification of cell biomass (Dionisi *et al*. 1999). The weighed dry cell mass (40 mg) were added to sealed test tubes containing 5 ml $CHCl_3$/MeOH/HCl (1∶10∶1, v/v/v), filled with pure N_2 and heated at 75 ℃ for 1 h. After cooling, 5 ml distilled water was added and FAME were extracted with 1 ml hexane.

Total solvent extract of complex lipids were produced by the modified Bligh and Dyer extraction method (Bligh and Dyer 1959; White DC *et al*. 1979).

2.3 Instrument analysis

Analyses of FAME were performed using 6890N GC and 5973N MS equipped with HP-5MS, 30 m × 0.25 mm ID × 0.25 μm capillary column, flame ionization detector (FID) and NIST. Nitrogen was used as the carrier gas. The retention time of the components from all samples were compared with that of methyl esters of DHA in fish oil, the known standards. Identification of DHA was achieved by comparison to that in NIST. Operating conditions were listed as following. The samples were injected at 250 ℃, and the oven was temperature-programmed from 50 ℃ to 250 ℃ with the speed of 25 ℃/min, then maintained for 7 min.

2.4 *Effect on growth rate and DHA content of temperatures, pH and salinity*

In the experiment of temperatures, N-6 was cultured in 2216E seawater medium (inoculated with 5% of inoculum) at 0 ℃, 5 ℃, 10 ℃, 15 ℃, 20 ℃ and 25 ℃ with shaking at 100 rpm for 96 h.

In the experiment of pH, N-6 was cultured in 2216E seawater medium (inoculated with 5% of inoculum) with different pH, 5.0, 6.0, 7.0, 7.5, and 8.0, with shaking at 100 rpm for 96 h.

In the experiment of salinity, N-6 was cultured in 2216E seawater medium (inoculated with 5% of inoculum) with different salinity, 1/2 folds salinity of seawater, 3/4 folds salinity of seawater, 1 folds salinity of seawater, 3/2 folds salinity of seawater and 2 folds salinity of seawater, with shaking at 100 rpm for 96 h.

All media components used were of highest purity grade available commercially in China.

3 Results

3.1 *Analysis on fatty acids and Identification of DHA*

Seven strains containing DHA or EPA were obtained, among which the outstanding containing DHA, was recorded as N-6 (Tab. 1). In Fig. 1, the peak at 12.615 min ought to be methyl ester of DHA compared with the retaining time of the known DHA methylester in Fig. 2, and its relative content in the total lipids was 8.72%. The result of total solvent extract showed that the total lipids in dry bacteria of n-6 was 22.54%.

Table 1 Relative contents of DHA and EPA in 7 strains of Antarctic bacteria/%

Lipid acid	Strain						
	N-4	N-6	12-3	262	18	82	17-1
DHA content	—	8.72	—	1.55	—	—	0.54
EPA content	0.24	—	1.68	—	0.25	0.68	0.56

Note "—" meas the content of EPA or DHA is extremely low (or without EPA and DHA).

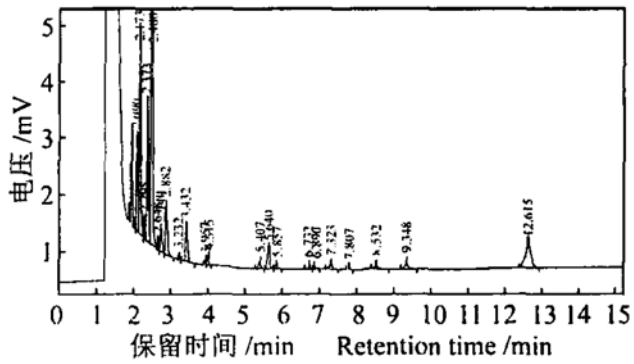

Fig. 1 GC chat of fatty acid methyl ester of No. N-6 bacterium

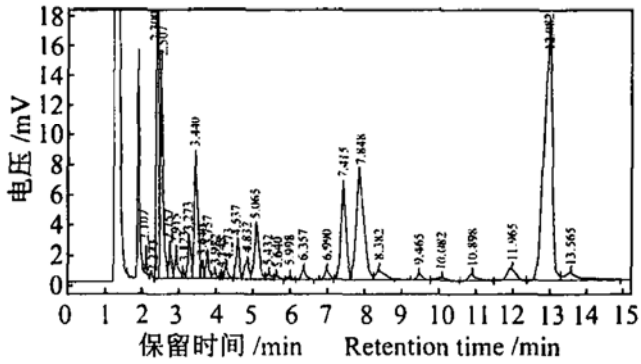

Fig. 2 GC chat of fish oil fatty acids methyl ester

The component of DHA was identified with 5973N MS by comparison with known standards using NIST. The partial MS spectrum is shown in Fig. 3.

3.2 *Effect on growth rate and DHA content of temperatures, pH and salinity*

The growth curve were protracted with DHA percentage and OD_{560} as y-axis, with temperature, pH and salinity as *x*-axis, respectively (Fig 4–6).

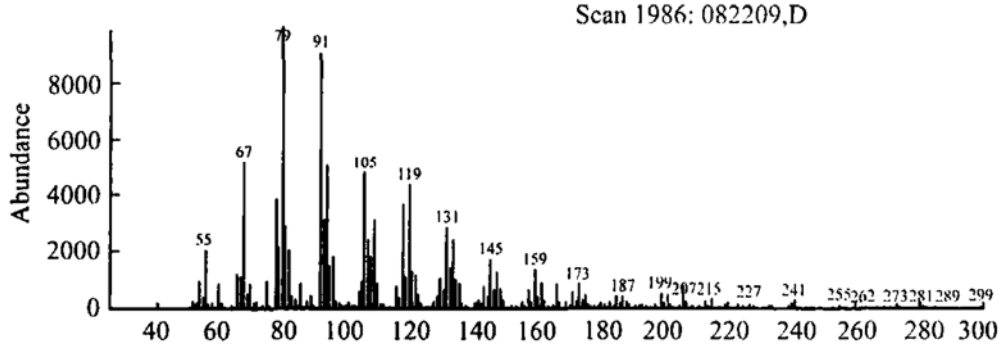

Fig 3 GC-MS chat of DHA methyl ester in No. N-6 bacterium

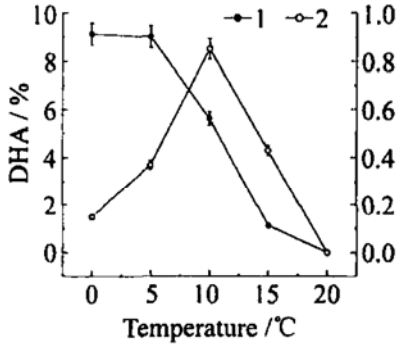

Fig 4 Effect of temperature on growth and DHA content of N-6
1: DHA content; 2: OD_{560}

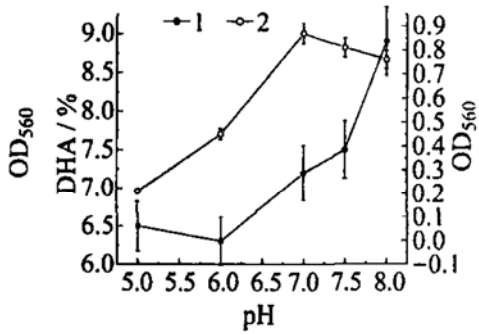

Fig 5 Effect of pH on growth and DHA content of N-6
1: DHA content; 2: OD_{560}

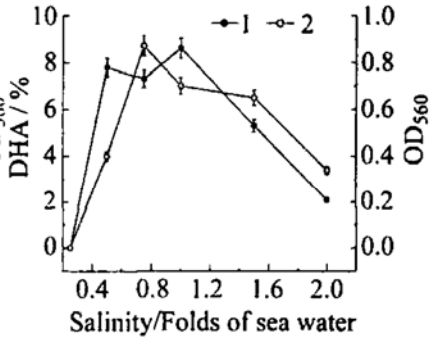

Fig 6 Effect of salinity on growth and DHA content of N-6
1: DHA content; 2: OD_{560}

4 Discussion

Cold-adapted microorganisms are generally understood to achieve their physiological and ecological successes in cold environments as a result of unique features in their proteins and membranes and their genetic responses to thermal shifts (Jody 2002). PUFA-producing taxa are generally characterised by combined psychrophilic and halophilic growth and contain further fatty acid components of sufficient novelty to act as qualitative biomarkers, and specific fatty acids may be used as indicators of PUFA-producing bacteria in environmental samples (Nichols *et al.* 2001). This ability even was proposed as functional genes for the phylogeny of marine bacteria (Russell and Nichols 1999).

The optimal temperature for N-6 is around 10 ℃, and N-6 can grow well in the appreciably alkalimedia and the DHA-content is relatively high, suggesting that N-6 is the psychrophilic and alkalophilic. The percentage of DHA decreased markedly at growth temperatures above the optimal region, indicating that DHA may play a critical role in the modulation of membrane fluidity and lipid phase at lower growth temperatures than the optimal region. Other bacteria and yeasts had been reported to contain an increasing proportion of unsaturated fatty acids as the growth temperature decreases (Russell *et al.* 1995; Berry and

Foegeding 1997).

As fish oil fails to meet the increasing demand for purified DHA, alternative sources are being sought, and some microalgae containing DHA were screened (Wen and Chen 2003; Xu *et al.* 2004; Xu *et al.* 2001). But there are some limitations in cultivation on a large scale, including sunlight, growth rate, etc., all of which can be easily solved with the psychrophilic N-6 if cultured in chemostat at a low temperature, and the advantage of running the bioreaction at low temperature is avoiding the risk of contamination by othermicroorganisms. The purpose of this study on Screening DHA-producing Antarctic bacteria is very cheerful, and much more of their talents will be exploited in future.

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