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Fatty acid profile of the edible filamentous cyanobacterium *Nostoc flagelliforme* at different temperatures and developmental stages in liquid suspension culture

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Abstract

The fatty acid profile of *Nostoc flagelliforme* (strains FACHB838 and CCAP1453/33), an edible terrestrial cyanobacterium, at different temperatures (i.e., 15, 20, 25 and 30 °C) and developmental stages (hormogonia, filaments, seriate and aseriate) in liquid suspension culture was investigated. The cyanobacterial species could be classified as Type IIB according to the existing taxonomic system based on fatty acid profiling, due to the presence of C18:3 and the absence of C16:2 and C16:3 fatty acids. Within the temperature range investigated, the content of C18:3n3 increased at the expense of C18:2n3 as temperature decreased, while the fatty acid suites remained unchanged. The degree of fatty acid unsaturation also increased with decreasing temperature, with the highest being 1.28 and 1.37 at 15 °C for the strains FACHB838 and CCAP1453/33, respectively. With respect to the effect of developmental stages, there was a slight variation in fatty acids composition and contents in the two strains without changing the fatty acid suite. At the aseriate stage at 25 °C, the highest C18 fatty acid proportion amounted to 63.17 and 65.08% and the degree of fatty acid unsaturation peaked at 1.32 and 1.31 in the strains FACHB838 and CCAP1453/33, respectively, indicating more long chain unsaturated fatty acids were accumulated at this stage.

Keywords: *Nostoc flagelliforme*; Filamentous cyanobacterium; Fatty acid; Temperature; Developmental stages

1. Introduction

Nostoc flagelliforme, formerly known as Nostoc commune var. flagelliforme, is an edible terrestrial cyanobacterium of great economic value. Field surveys show that it is distributed on arid or semiarid steppes in many countries around the world [1,2]. In China, wild *N. flagelliforme* is mainly found in eight western and north-western provinces [2]. It has been regarded as a food delicacy for more than 2000 years and its medicinal value has been recognised since ancient times [2,3]. As a result of the high profits associated with its commercial activities, picking and trading of *N. flagelliforme* has become a highly profitable enterprise. Unfortunately, the exploitation of this organism has led to environmental problems such as soil erosion and desertification and the species is now endangered to the point of extinction. The Chinese government has recently encouraged and funded a number of research projects to study the potential for its artificial mass cultivation. Because of its dual characteristics of bacteria and algae, *N. flagelliforme* may be grown well in liquid suspension culture, which may serve as an alternative for mass cultivation in order to meet the increasing market demand. Nevertheless, little is known about how this species behaves in liquid suspension culture especially in response to temperature changes. The study was made to complement a previous study on the possible exploitation of the biotechnological potential of *N. flagelliforme* [4].

As a mesothermal species, *N. flagelliforme* can survive within a temperature range of 94.8 °C, between −29.1 and 65.7 °C [5,6]. However, under artificial cultivation conditions coupled with an increase of humidity, its tolerance range decreases drastically. This indicates that it can tolerate either extreme temperature stress or high levels of humidity, but not a combination of both [6]. Although the

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fatty acid compositions of both natural desiccated colonies and liquid suspension cultures of *N. flagelliforme* have been reported recently [4,7], the fatty acid profile associated with temperature changes in liquid suspension culture has not been studied. Consequently, it is necessary to investigate how temperature changes will affect the organism and the resulting changes of fatty acid composition. Previous research also indicated that alternation of cell forms of the *Nostoc* species occurred in a regular manner during liquid suspension culture, leading to a very complex life cycle comprising four different colony morphologies: hormogonia; filaments; spiral aggregates (or seriate colonies) and aseriate (ball-like) colonies [8,9]. However, little information is available on the accumulation of fatty acids at different developmental stages.

It is known that n-3 polyunsaturated fatty acids are important for human health [10,11]. With α-linolenic acid (ALA, 18:3n3) as its dominant fatty acid and other essential nutrient ingredients, *N. flagelliforme* is already considered a health food. To improve the nutritional value of the cyanobacterial biomass, it is important to understand how its cellular fatty acid composition can be manipulated under a controllable growth conditions as in the cases of other cyanobacteria [12], green algae [13] and dinoflagellates [14].

In view of the uniqueness of *N. flagelliforme* and its biotechnological potential in liquid suspension culture, the present study was aimed to unveil the fatty acid composition of *N. flagelliforme* at different growth temperatures and developmental stages.

2. Materials and methods

2.1. Axenic suspension cultures

N. flagelliforme FACHB838 and *N. flagelliforme* CCAP 1453/33 were obtained from the Institute of Hydrobiology, the Chinese Academy of Sciences, Wuhan, PR China and the culture collection of algae and protozoa (CCAP), UK, respectively. Prior to inoculation, the culture was microscopically observed to confirm that hormogonia of the *Nostoc* species were the dominant cell form (this was achieved by sub-culturing several times), accounting for above 98% of total number of cells. Inocula (8%, v/v), acclimated to the appropriate temperature for 1 week, were added to each 250 ml Erlenmeyer flask containing 100 ml of BG-11 medium [15]. Temperature was adjusted to four different regimes: 15, 20, 25 and 30 °C. All the cultures were then incubated under axenic conditions in an orbital incubator (Gallenkamp, UK) at 120 rpm. Continuous illumination was provided by an array of cool white fluorescent tubes with a mean photon flux density of 60 $\mu E\,m^{-2}\,s^{-1}$) at the flask surface. The cell pellets were harvested and freeze-dried for further analyses after being alternately centrifuged at 4100 × *g* and washed with DI water several times. Each experiment was performed with three replicates.

To avoid contamination in the inoculum, a modified approach based on the procedures of Caudales and Wells [16] and Rippka [17] was used. Briefly, the cultures were homogenised with an ultrasonic microtip for ca. 2 min. After the ultrasound procedure, the colonies were observed under a light microscope of 40× magnification to ensure that most of them were in strings of 3–5 cells. They were then washed three times with the BG-11 medium by centrifugation at 1000 × *g* for 5 min. The supernatant was discarded and the algal pellets re-suspended. Finally, a 0.1 ml suspension culture was evenly distributed in the medium-based plates solidified by Difco Bacto agar. After 2–3 days, the confirmed algal colonies with agar cores were expelled into liquid media for suspension culture. These procedures were repeated 2–4 times and both the macroscopic and microscopic colonies were microscopically observed immediately afterwards, to confirm complete purification.

2.2. Fatty acid analyses

The method described by Vazhappilly and Chen [18] was employed for the analysis of fatty acids. Briefly, lyophilised cell pellets were weighed (ca. 20 mg) in Teflon-lined, screwcapped centrifuge tubes and the lipids of the cells were extracted according to the procedure described by Bligh and Dyer [19]. In order to trans-esterify the fatty acids into fatty acid methyl esters (FAMEs), 1 ml butylated hydroxytoluene (50 $mg\,l^{-1}$ in methanol) (BHT, Sigma, St. Louis, USA) as antioxidant and 0.8 ml internal standard was added. The mixture was incubated at 70 °C for 15 min. Three milliliter of 5% methanolic-HCl and 0.8 ml of boron trifluoride (Sigma, St. Louis, USA) were added to facilitate the extraction process and the mixture was further incubated in a water bath at 80 °C for 1 h. The FAMEs were extracted by the addition of 1 ml of DI water and 2 ml hexane. The upper hexane phase was decanted and the extraction procedure was repeated twice with hexane. The pooled hexane solution containing FAMEs was evaporated to dryness under a nitrogen stream. The FAME residue was then dissolved in 1.5 ml hexane and centrifuged at 14,000 rpm for 10 min. The supernatant was analysed with an HP6890 gas chromatograph (Hewlett Packard, USA) equipped with an HP7673 automatic injector, a flame ionization detector (FID) and an HP-INNOWAX^{TM} capillary column (HP 19091N-133, 30 m long, 0.25 mm diameter and 0.25 μm film thickness). Two microlitre of the sample was injected in a splitless injection mode. The inlet and detection temperature were maintained at 250 and 260 °C, respectively and the oven temperature was programmed from 170 to 220 °C at increments of 1 $°C\,min^{-1}$. High purity nitrogen was used as the carrier gas. FAMEs were identified by a comparison of their retention times against those of the authentic standards (Sigma, St. Louis, USA). Different fatty acids were quantified by comparing the peak areas of their corresponding FAMEs with that of the internal standard (heptadecanoic acid, C17:0, Sigma, St. Louis, USA).