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Influence of static magnetic field exposure on fatty acid composition in *Salmonella* Hadar

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ABSTRACT

We have been interested, in this work, to investigate the effect of the exposure to static magnetic field at 200 mT (SMF) on the fatty acid (FA) composition of *Salmonella enterica* subsp *Enterica* serovar Hadar isolate 287: effects on the proportion of saturated and unsaturated fatty acids (SFAs, UFAs), cyclopropane fatty acids (CFAs) and hydroxy fatty acids after exposure to the static magnetic field at 200 mT (SMF). Analysis with Gas Chromatography-Mass Spectrometry (GC-MS) of total lipid showed that the proportion of the most fatty acids was clearly affected. The comparison of UFAs/SFAs ratio in exposed bacteria and controls showed a diminution after 3 and 6 h of exposure. This ration reached a balance after 9 h of treatment with SMF. So we can conclude that *S.* Hadar tries to adapt to magnetic stress by changing the proportions of SFAs and UFAs over time to maintain an equilibrium after 9 h of exposure, thus to maintain the inner membranes fluidity. Also, a decrease in the proportion of hydroxy FAs was observed after 6 h but an increase of this proportion after 9 h of exposure. Concerning CFAs, its proportion raised after 6 h of exposure to the SMF but it decreased after 9 h of exposure. These results are strongly correlated with those of *cfa* (cyclopropane fatty acid synthase) gene expression which showed a decrease of its expression after 9 h of exposure.

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1. Introduction

Recently, the sources of electromagnetic field had prodigiously increased. Consequently, exposure level of different biological systems to electromagnetic fields is increasingly important. Many studies have been carried out to evaluate the potential harmful effects of magnetic field [3,15,33]. It has been shown that the effect of the magnetic fields on the growth and bacterial viability depend on many factors such as magnetic field intensity, time exposure and the type of organism. Many researchers have shown that the exposure of bacteria to magnetic fields, can significantly affect the viability. Indeed, viability of *E. coli* under extremely low-frequency electromagnetic fields (ELF-EMF) 0.1 T was 100 times higher than controls [38]. Also, the exposure of *Staphylococcus aureus* and *E. coli* to 4 mT-20 Hz ELF-EMF for 6 h induced an inhibition of CFUs (colony forming units) compared to their controls [15]. At the same context, the exposure of *S.* Hadar to a SMF shows a significant decrease in bacterial viability after 6 h followed by a recovery after 9 h [3]. In the same way, it was proved that *Salmonella typhi* growth was inhibited when exposed to an extremely low frequency electromagnetic waves (ELF-EMW-ELF-EM) "at resonance frequency" [33]. Magnetic fields may affect DNA and gene expression of living organisms. In this regards, it was shown that magnetic field induced DNA degradation in case of *E. coli* [45]. Moreover, in case of *S.* Hadar, SMF exposure induces a significant increase in mRNA expression level of *katN* gene encoding for a nonhemic catalase, *DnaK*, gene encoding for a heat chock protein and *rpoA* and gene encoding for the α-subunit of the RNA polymerase [3]. However, the exposure of *E. coli* to 60 Hz magnetic field of 3 mT hasn't induced any qualitative changes in protein synthesis [6]. In another context, a decrease in biofilm cell mass was also detected after exposure of *Helicobacter pylori* to ELF-EMF [16]. Besides, tests in *Salmonella* Hadar and *Pseudomonas aeruginosa* were carried out to show a possible improvement in the effectiveness of antibiotics in combination with magnetic field. In this case, magnetic field did not have undesirable effect. In contrast, it decreased their antibiotic sensitivity [13,25].

On the other hand, bacterial membranes, which consist principally of lipids, have a very important role into the adaptation

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process of bacteria to different environmental stress. In fact, membranes of Gram negative bacteria represent a powerful barrier against external toxic agents (chemical, physical or biological assault) [26]. Changing membrane integrity and permeability, which are essential for bacterial defense [21,35], is often caused by a change in the lipid compositions [27,35]. pH and temperature may significantly affect lipid membrane composition. These changes in the composition of cell membrane may affect the membrane fluidity in order to maintain the functionality and the membrane integrity face to stress conditions [32,36]. In fact, a decline in membrane unsaturated fatty acid and an augmentation in SFAs and CFAs were observed in growth temperatures in *Salmonella enterica* serovar Typhimurium [44]. An increase of long-chain and saturated fatty acids were detected in *E. coli* with the increasing of growth temperature [30]. Furthermore, an augmentation in the unsaturated/saturated fatty acids ratio was observed after sufficient exposure time of wild-type and *dam* mutant in *S. typhimurium*. From which the membrane integrity as well as its fluidity were increased [29].

As a first study which highlights the effect of such type of stress: static magnetic stress (200 mT) on fatty acid composition in a prokaryote model: *Salmonella* Hadar, we are interested in this work to follow its influence on the proportion of saturated fatty acids, unsaturated fatty acids, cyclopropane fatty acids and hydroxy fatty acids in *Salmonella* Hadar. This in order to examine the ability of prokaryote microorganisms to develop an adaptation strategy to the environmental stress induced by exposure to SMF.

2. Materials and methods

2.1. Magnetic field exposure

The SMF was induced as described before by a pair of cylindrical coils (diameter 20 cm, length 13 cm each), powered by a transformer [3]. The 2 bobbins were separated by 6 cm. The coils were water-cooled and the temperature inside the coils was regulated at the value of the laboratory temperature (25 °C). An Erlenmeyer-shaped glass double phial (external diameter, 6 cm; external height, 8 cm; internal diameter, 4 cm; internal height, 7 cm) was used as sample holder. The exposures were carried out with a magnetic induction (B) of 200 mT in the middle of the double phial. The temperature was maintained at 37 °C inside the glass double phial by water circulation, using an incubator system composed of pump and resistance. The samples were positioned in the center between the 2 coils. Bacterial cultures were exposed to the SMF for 3, 6 and 9 h; subsequently, the culture was centrifuged at 2000 g for 10 min. For the control experiments, the bacterial cultures were similarly positioned, except that the magnetic field was turned off. A digital Teslameter was used to check regularly the induction of the SMF.

2.2. Bacteria

Salmonella enterica subsp *Enterica* serovar Hadar isolate 287 (6.8: Z10: e, n, X) was made available to us by the Laboratory of Water and Food Surveillance of the Pasteur Institute of Tunis. It was isolated from turkey meat. Bacteria stored at −20 °C in 15% sterile glycerol were re-cultured for the purposes of this work. Pre-cultures were performed overnight at 37 °C in 10 ml of culture medium (culture medium composition: Tryptone 10 g/l, Meat extract 5 g/ml and Sodium chloride 5 g/ml) in tubes and then diluted to the same initial concentrations corresponding to an OD600 equal to 0.1 in the double phial.

2.3. Lipid analysis

2.3.1. Lipid extraction

Bacterial cultures with an $OD_{600nm} = 0.1$ obtained from the pre-cultures that have been diluted in sterile medium, are exposed to 200 mT SMF at 37 °C. The bacterial pellet is recovered from approximately 1 L of bacterial culture by centrifugation (4500 rpm, 15 min, 4 °C) and stored at −20 °C. The bacterial lysate was then washed with 0.9% NaCl and then centrifuged at 9000 rpm for 2 min. To extract the cell lipids the method produced by Ref. [41] was followed with little modification. The pellets were re-suspended in 2 ml of sterile water and 7.5 ml methanol/chloroform (2:1 v/v) was added. The mixture was shaken few minutes and allowed to stand for 1 h. Then, pellets were recuperated by centrifugation (5 min at 3000 g) and a second extraction was effectuated by addition of methanol/chloroform/water (2:1:0.8; v/v/v). 4 ml of chloroform and 4 ml of water was mixed to the too supernatants and the lower phase was recuperated. The lower phase (chloroform) containing lipid is recovered by centrifugation (4000 rpm, 10 min), dried at 40 °C in Rota vapor then mixed with 1 ml of hexane and dried under a stream of nitrogen gas in order to avoid lipid oxidation. These extracts are stored in a freezer at −20 °C to avoid the risk of degradation.

2.3.2. GC-MS analysis

The analysis was carried out by gas chromatography and gas chromatography coupled with mass spectrometry (GC and GC-MS).

2.3.3. Analysis by gas chromatography CPG-FID

Analysis and separation of the total lipid extracts is carried out by a HP5890-series gas chromatograph which is equipped with a flame ionization detector (FID), a Split-splitess injector, an HP INNOWAX capillary column 30 m long, 0.25 mm internal diameter and 0.25 μm film thickness, and the stationary phase of which consists of polyethylene glycol. The operating chromatographic conditions are: the temperature of the injector: 250 °C, the temperature of the detector: 280 °C. And the temperature of the column is programmed from 50 °C to 250 °C at a rate of 5 °C/min with two bearings, one at 50 °C and lasts 1min and the other at 250 °C and lasts 42 min. The carrier gas is nitrogen with a flow rate of 1.2 ml/min 1 μl is the injected volume (diluted 1% in hexane).

The determination of all the constituents was carried out by co-injection of pure controls analyzed under the same conditions and carried out using the HP Chemstation software which makes it possible to assimilate the percentages of the areas and peaks of the chromatogram to the percentages of the various constituents. A Bacterial Acid Methyl Ester (BAME) Mix standard (Sigma Aldrich) was injected just before sample injection.

2.3.4. GC-MS coupling analysis

The identification of the fatty acid chemical components (diluted 1% in hexane) was carried out by GC-MS coupling, composed of a gas chromatograph (HP 5890 - SERIE II) coupled to a quadrupole mass spectrometer (HP-MSD 5972 A) in electronic impact mode. The column used is an HP INNOWAX polar column 30 m long, 0.25 mm internal diameter and 0.52 μm film thickness, the stationary phase of which is polyethylene glycol, diphenyl and 95% dimethyl polysiloxane. The temperatures of the injector, the source and the interface are respectively 250, 175 and 280 °C. The carrier gas is helium (flow rate of 1.2 ml/min), the pressure is 9 psi. The temperature of the polar column is programmed from 50 °C to 250 °C at a rate of 5 °C/min with two stages, one at 50 °C and lasts 1 min and the other at 250 °C and lasts 20 min. The temperature of the apolar column is programmed from 50 °C to 280 °C at a rate of 5 °C/min. With two stages, one at 50 °C and lasts 1min and another

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