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EFFECT OF 50 HZ EXTREMELY LOW MAGNETİC FİELD ON TOTAL CAROTENOİD PRODUCTİON OF SC8 ISOLATED FROM CAMALTİ SALTERN - IZMİR TURKEY

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ABSTRACT
This study aimed to investigate the influence of magnetic field as stress factor on production of carotenoids of halophilic bacterial strain SC8 isolated from Camalti saltern in western Turkey. Carotenoids are the most common pigments in nature which have a lot of benefits for human health. For instance, anti-oxidant and anti-aging effects. In this study, extraction of caretonids of SC8 isolate divided into two groups as control and experimental group. Experimental group was exposed to 50Hz, 1.5 mT magnetic field generated by a Helmholtz coil. Results indicated that time-varying magnetic fields have significant effect on caretonoid production as compared to control group. But no significant effect was seen on the bacterial viable number.

KEYWORDS: Magnetic field / carotenoid / Helmholtz coil / halophilic bacteria.

INTRODUCTION
Magnetic fields are created by man-made devices which humans are exposed constantly due to modern life technologies and its effects are inevitable. The use of bacteria which is simple unicellular organism thus very suitable and practical for metabolic impacts. Although many studies have been conducted about magnetic fields' effects on biological systems so far, our knowledge is still limited (Zhang et al., 2003).

Several effects caused by magnetic fields on biological systems have been reported for DNA synthesis, gene expression, ion transportation and antibiotic resistance mechanism (Robert,1992; King and Wai, 2000; Garip et al., 2011; Segatore et al., 2012). Magnetic fields also causes general stress response in many species (Pérez et al., 2010).

Some studies performed to analyze its effect of antimicrobial sensitivity, anti-cancer, enzymatic activity and bioremediation (Salmen et al., 2010; Cameron et al., 2007; Rochalska and Grabowska, 2007; Cebkowska, 1991) as well as working on antioxidant activities which drew a lot of attention because of its significant role in medicine and well-being of human. Carotenoids are one of which has a great impact as an antioxidant and anti-aging in human well-being (Lademann et al., 2011).

Carotenoids are common colorful pigments naturally occurring in a variety of organisms including plants, bacteria and algae. They play crucial roles as photoprotectant, light-harvesting, and also removing harmful radicals, having variable applications in medicine, nutritional sciences, dieting and cosmetics (Chidambara et al., 2005; Jie et al., 2014).

Effects of magnetic fields on carotenoids are poorly studied. In this study, we aim to study the effect of ELF-MF on carotenoid production by SC8 isolated from Camalti saltern.

MATERIALS AND METHODS
Helmholtz coil and time-varying magnetic fields
If an alternating current is passed through a coil, time-varying magnetic fields can be obtained. Therefore, an AC power source has been required for the experiment. To examine the interaction of the organism with a time-varying magnetic field, a pair of pure iron made Helmholtz coil has been used. The radius of coils is 12 cm each and distance between them is equal to their radius to adapt Helmholtz's concept. Number of the turn of each coil is 144. Both coils have a resistance of $13.5 \pm 0.2\ \Omega$. When a 15 V AC driver voltage with 50 Hz frequency is applied, the RMS value of the current read is 1.391 A. The AC peak value of time-varying magnetic field at the center of Helmholtz coil can be calculated by.

$$B_0 = \left(\frac{4}{5}\right)^{3/2} \frac{\mu_0 N I\sqrt{2}}{R}$$

where μ_0 is the permeability of free space, N is the number of turn of each coil, $I\sqrt{2}$ is the peak value of the measured AC current and R is the radius of each coil. Above equation has given the peak values of B which are ± 2.125 mT. Thus, the equation of time-varying magnetic field is

$$B = B_0 \sin(wt)$$

The image of our 50 Hz signal is shown in Figure 1. On the other hand, measurement of the magnetic field by a Gauss meter has given 1.5 mT which is approximately the RMS value of 2.125 mT.

First, to examine how the organism will react to the time-varying magnetic field in mains frequency (50 Hz) and between peak values of ± 2.125 mT, our target group has been placed at the center of Helmholtz coil. Then the coil is placed inside an incubator.

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Isolation and screening

Bacteria have been isolated randomly from different parts of Camalti saltern in August 2017 and carried in sterilized containers to the lab and immediately diluted and spread on Marin Agar media and incubated at 30°C for 7 days. Obtained colonies were screened for carotenoid content based on colony color and spectrophotometric absorbance (300-800nm) and thin layer chromatography.

Carotenoid Extraction

After satisfactory growth, both control and experimental samples centrifuged at 4200 rpm for 20 minutes. supernatant was discarded and pellets were harvested. Then Methanol/Hexane (1:2) containing butylhydroxytoluene (BHT) (0.1%; as antioxidant) was added to the pellet and cell disruption was achieved by sonication. Then centrifuged for 20 minutes and upper phase was analyzed at 300-800 nm.

UV-Visible Spectroscopy

Control and experimental extraction solutions UV spectra were recorded at 300-800 nm using spectrophotometer (Shimadzu UV-1800Series, Kyoto, Japan). The approximate content of total carotenoids was determined by measuring the optical density of the sample at 486nm.

Thin-Layer Chromatography

Thin-Layer chromatography (TLC) was used to confirm the existence of carotenoids in the extracts. After running of the extract on TLC silica gel in hexane: acetone (7:3) mobile phase, the antimony pentachloride (SbCl5): chloroform (1:10 v/v) solution sprayed on TLC silica gel to visualize the carotenoids as blue spots.

Time-varying magnetic field exposure

The bacterial sample was diluted and 0.5 absorption achieved at 600nm then 1ml was inoculated in 200 ml of Marin broth in 2 groups as control and test group. The test group was placed at the center of Helmholtz coil and subjected to 50Hz, 1.5mT. Both incubated for 72 hours at 30°C. Viable bacterial count was performed and extracts of carotenoids as control and experimental group were analyzed for total carotenoid content at 486 nm (λ_{max} of extraction).

DNA isolation and 16S rDNA amplification

DNA extraction was carried out by DNA extraction kit (Invitrogen) and 16S rDNA was amplified by fD1 (5' AGAGTTTGATCCTGGCTCAG 3') and rP1 (5'rPl GGTTACCTTGTTACGACTT3') universal primers. Initial denaturation was carried out for 4 min at 94°C. It was followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 1 min and extension at 72°C for 2 mins with 10 mins final extension at 72°C. The amplified DNA fragment was visualized using 1% agarose gel electrophoresis, then the DNA sample was sequenced at Izmir Yuksek Teknoloji Institute. The sequence was compared with reference 16S rRNA gene sequences available in NCBI GenBank database BLAST using blastn and a phylogenetic tree was constructed using Geneious 8 software.

RESULTS

Screening

The SC8 isolate was selected because of its orange colonial color and absorbance characteristics (Wang et al., 2011; De La Vega et al., 2011) of carotenoids (Figure 2). The characteristic absorption of SC8 sample in hexane solution indicated the existence of carotenoids. After running the extract on silica gel, blue spot appeared when antimony solution was sprayed which indicates that SC8 isolate contains carotenoids.

Magnetic field exposure and total carotenoids

After incubation period viable bacterial count is 1.6×10^8 CFU/ml for control and is 1.4×10^8 CFU/ml for experimental group. After measurements at 496 nm of both experimental and control groups, U-test was carried out and significant production in the experimental group was confirmed as it was compared to control group (Figure 3).

Mann&Whitney U-test was applied to examine whether the increase of carotenoid production of the organism under the time-varying magnetic field was statistically significant. First, the U-test values of the empirical

results of bacterial viable number without the effect of any magnetic field are calculated and the statistical value is found to be greater than the critical value ($U_{stat} = 12, U_{crit} = 5, \alpha = 0.05$) which indicates that there is no significant difference between the theoretical and empirical bacterial viable counts. Then, the U-test of the empirical results under the time-varying magnetic are calculated and this time, change in carotenoid production was found to be statistically significant. Statistical value is found to be less than the critical value ($U_{stat} = 0.5, U_{crit} = 5, \alpha = 0.05$), confirming that the effect of the time-varying magnetic field over the production of carotenoid exists.

16srDNA analysis

The sequence of bacterial strain isolated from camalti saltern designated as SC8 submitted to the genetic sequence database at the National Center for Biotechnology Information (NCBI), and identified *as Halobacillus locisalis* (100%) with accession number NR_025715.1, which was closely related to *Halobacillus yeomjeoni (*NR_043251.1), *Halobacillus salicampi (NR_146669.1), Halobacillus faecis (NR_114247.1)* and *Halobacillus trueperi* (NR_025459.1) with 98% identity. The phylogenetic tree of this 16S rRNA gene sequence (NR_025715.1) was constructed against 10 different *Halobacillus sp*. from GenBank by using Geneious 8 software (Figure 4).

FIGURE LEGENDS

Fig1. *Graph of the time-varying magnetic field applied to the target group.*

Fig2. *Carotenoid absrorbance spectra of SC8 between 300-800 nm. As* λ_{max} *of 486, 455 and 428 nm.*

Fig3. *Total carotenoid absorption in control and experimental group (MF: Time-varying magnetic field). Viable bacterial count for control is 1.6 x* 10^8 CFU/ml *and* $1.4x10^8$ *CFU/ ml for MF exposure group. There is no significant effect on viable bacterial count whereas MF significantly increased the total carotenoid content.*

Fig4. *Comparative phylogenetic analysis of the SC8 isolate with, Halobacillus sp. (NR_025715.1), (NR_146669.1), (NR_041360.1), (NR_114247.1), (NR_043251.1), (NR_025459.1), (NR_029304.1), (NR_042860.1), (NR_025545.1) and (NR_041246.1) strains from GenBank.*

FIGURES

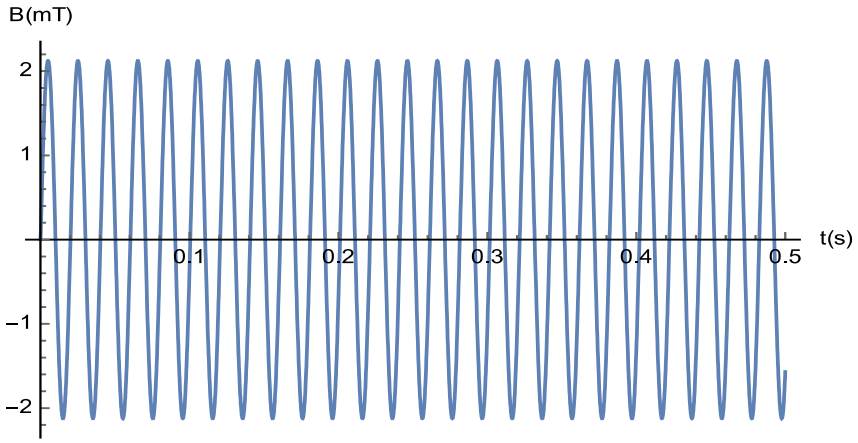

Figure 1.

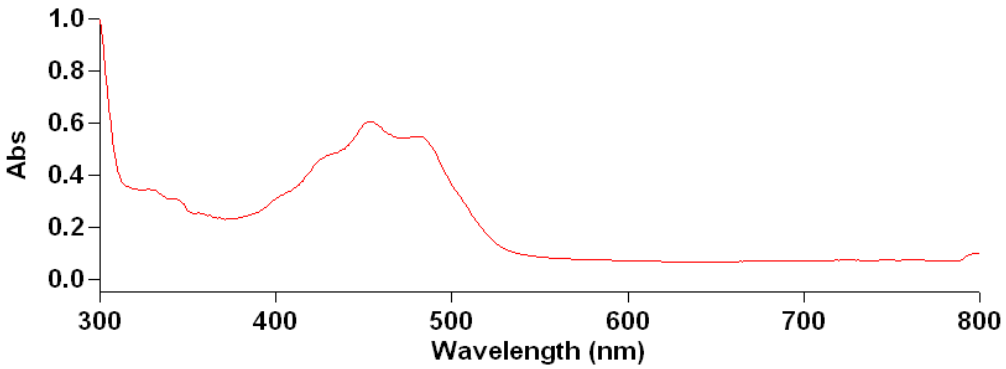

Figure 2.

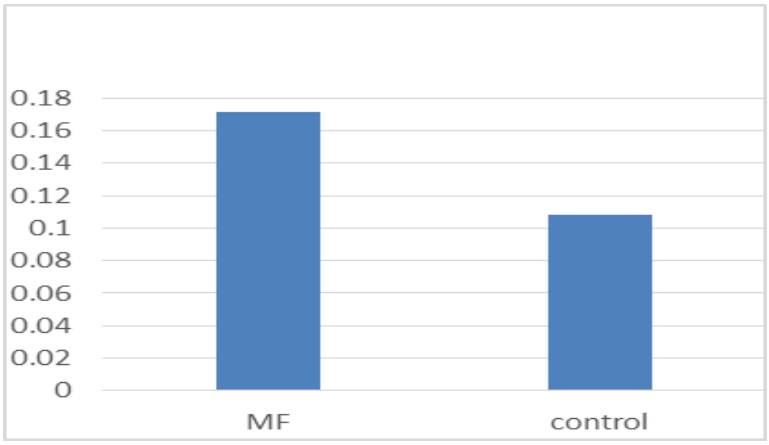

Figure 3.

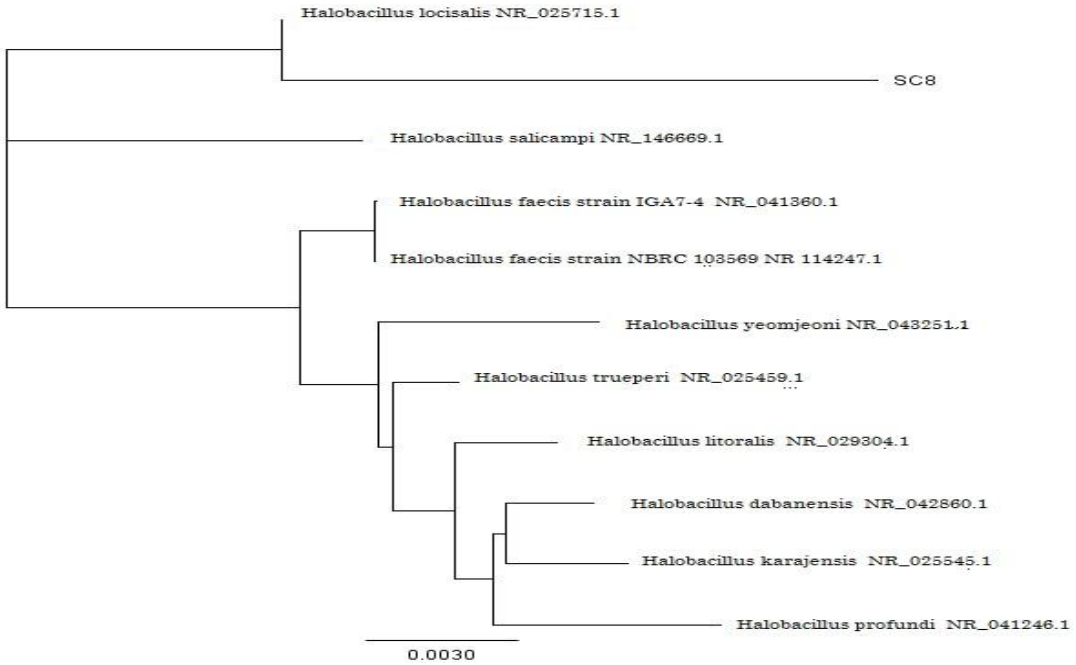

Figure 4.

DISCUSSION

Results showed that 50Hz 1.5mT magnetic field had no significant effect on the bacterial viable number but significantly increased the carotenoid production. Although there have been many studies of magnetic field effect on pigment production on plants and algae the effect on bacterial carotenoids is poorly studied.

In one study on *C. vulgaris* magnetic field had no effect on carotenoid content (Hai-Ying et al., 2008). Whereas study performed on tomato seeds showed an increased amount of lycopene significantly (Aspasia et al., 2014). In another experiment of 10mT SMF conducted on *D. salina* showed an increase of B-carotenoid content (Yamaoka et al., 1992).

Another study showed 25% increase in total carotenoid when Chlorella kesleri subjected to 30-60mT (Lenon et al., 2017).

And also Darcy et al. reported similar findings in chlorella kesleri when subjected to 10mT (Small et al., 2012). In a similar study with *C. vulgaris*, Benavente-Valdés et al. reported that under photoheterotrophic conditions production of total carotenoids increased (Benavente et al., 2016).

Based on different studies so far, the mechanism behind magnetic field stress is not clear yet. Hirano et al. implies that SMF increased free radical concentration and hence this could help to explain amplified pigment production (Morio et al., 2014). Furthermore, Timmel C. R. et al. reported that low magnetic field results in an increased free radical concentration due to oscillation of zero-quantum coherences which leads to interconversion of singlet and triplet radicals and changes in yield of recombination of products (Timmel et al., 1998). Sahebjamei et al. also reported that magnetic fields can cause oxidative stress by changing electrons spin orientation which leads to enhancing concentration and lifetime of free radicals (Hassan et al., 2007).

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