N. Koçberber Kılıç and G. Dönmez / Hacettepe J. Biol. & Chem., 2021, 49 (3), 233-242

Research Article

Hacettepe Journal of Biology and Chemistry

journal homepage: www.hjbc.hacettepe.edu.tr

Bioactive Compound Activity Inducement of Thermophile *Cyanobacterium aponinum* Under Stress Conditions

Stres Koşulları Altında Termofil *Cyanobacterium aponinum* Biyoaktif Bileşik Aktivitesinin İndüklenmesi

Nur Koçberber Kılıç* and **Gönül Dönmez**

Department of Biology, Ankara University, Beşevler, Ankara, Turkey.

ABSTRACT

In this report, bioactive properties of 7 thermophile cyanobacteria isolated from thermal springs in Turkey were investigated. Of these, Strain H2 having the highest antimicrobial activity was identified as *Cyanobacterium aponium*. Bioactive character of cyanobacterial biomass was investigated with regards to different nitrogen concentrations (0.5 g/L, 1.0 g/L, 1.5 g/L, and 2.0 g/L), light intensities (1200lx, 2400 lx, 3600 lx, and 4800 lx), incubation periods (7 d, 14 d, 21 d, and 28 d), and temperatures (30°C, 40°C, 45°C, and 50°C). It was observed that the effectiveness of bioactive substances produced by cyanobacteria was induced by stress conditions. When *C. aponinum* was exposed to high light intensity or temperature, cyanobacteria produced more efficient bioactive compounds then other environmental conditions tested. The highest antimicrobial activity was found against *E. coli* 0157:H7 ATCC 35150 with biomass extracts obtained when cyanobacterium cultivated in media with 1.0 g/L nitrogen, at 45°C, under 3600 lx illumination after incubation for 14 days. For the first time with such an approach as in the current study, production of bioactive compounds by a thermophilic *C. aponinum* and optimization of the environmental conditions to obtain the most efficient biologically active compounds was investigated.

Key Words

Bioactive compounds; *Cyanobacterium aponinum*; thermophile; stress.

ÖZ

Bu çalışmada, Türkiye'de kaplıcalardan izole edilen 7 termofil siyanobakterinin biyoaktif özellikleri araştırılmıştır. Bunlardan en yüksek antimikrobiyel aktiviteye sahip olan Suş H2, *Cyanobacterium aponium* olarak tanılanmıştır. Siyanobakteriyel biyokütlenin biyoaktif karakteri, farklı azot konsantrasyonları (0.5 g/L, 1.0 g/L, 1.5 g/L ve 2.0 g/L), ışık yoğunlukları (1200lx, 2400 lx, 3600 lx ve 4800 lx), inkübasyon süreleri (7 gün, 14 gün, 21 gün ve 28 gün) ve sıcaklıklar (30°C, 40°C, 45°C ve 50°C) açısından araştırılmıştır. Siyanobakteriler tarafından üretilen biyoaktif maddelerin etkinliğinin stres koşulları tarafından tetiklendiği gözlenmiştir. *C. aponinum* yüksek ışık yoğunluğuna veya sıcaklığa maruz kaldığında, siyanobakteriler test edilen diğer çevresel koşullardan daha verimli biyoaktif bileşikler üretmiştir. En yüksek antimikrobiyel aktivite, siyanobakteri 1.0 g/L azot içeren bir ortamda, 3600 lx ışık şiddeti altında, 45°C'de 14 gün boyunca inkübasyondan sonra elde edilen biyokütleden alınan ekstraktlar ile *E. coli* 0157: H7 ATCC 35150'ye karşı bulunmuştur. Bu çalışmada ilk kez böyle bir yaklaşımla, termofilik *C. aponinum* tarafından biyoaktif bileşiklerin üretilmesi ve en etkin biyoaktif bileşikleri elde etmek için çevresel koşulların optimizasyonu araştırılmıştır.

Anahtar Kelimeler

Biyoaktif bileşikler; *Cyanobacterium aponinum*; termofil; stres.

Article History: Received: Mar 2, 2021; Revised: Feb 6, 2021; Accepted: Feb 6, 2021; Available Online: May 6, 2021.

DOI: https://doi.org/10.15671/hjbc.696762

Correspondence to: N.K. Kılıç, Department of Biology, Ankara University, Ankara, Turkey.

E-Mail: nrkilic@ankara.edu.tr

INTRODUCTION

Cyanobacteria and microalgae are rich source of unique and biologically active products known as bioactive compounds [1]. Among the microorganisms with potential for producing bioactive compounds, cyanobacteria appear as ideal candidates forming novel biologically active compounds [2]. They require only minimal nutrition using sunlight, carry out nitrogen and CO_2 fixation and grow fast by utilizing different carbon sources.

Several cyanobacteria can produce bioactive compounds with different biological activities like antibacterial, antifungal, anticancer and antiviral. Among cyanobacteria, extremophile ones coping with extreme conditions (high salt concentration, temperatures, etc.) have much greater potential in various biotechnological applications such as energy and production of useful substances. Such microorganisms maintain their vitality by producing more stable materials in adapting to changing environmental conditions [3-5]. Moreover, thermophilic cyanobacteria can produce biomass more quickly than mesophilic cyanobacteria by fixing CO_2 in the carbon cycle [6]. Properties like survival in different environments, rapid growth and plenty of biomass formation are required for effective biotechnological applications.

Previous studies were mostly focused on mesophilic cyanobacteria having bioactive character. But, however, research on thermophilic cyanobacteria are very limited [1]. Fish and Codd reported that thermotolerant *Phormidium* species produced extracellular antimicrobial materials inhibiting growth of Gram-positive and Gram-negative bacteria, *Candida albicans*, and *Cladosporium resinae*, while not cyanobacteria [7]. Heidari et al. used *Oscillatoria subbrevis, O. tenius, O. limentica, O. angusta, O. articulate, Synechocystis aquatilis, and Synechoccous cerdrorum* that were isolated from Geno hot springs; the highest antimicrobial activity was found against *Bacillus subtilis* and *B. pumulis* with methanol-extracts of these thermophile cyanobacteria [8]. A thermophilic green algae *Cosmarium* sp. isolated from hot spring in north Tunisia was tested for its bioactive properties; extracts prepared by using different solvents showed significant antibacterial effects [9]. A recent report that was carried out with thermophile *C. aponinum* isolated from Polichnitos hot spring showed that extracts from *C. aponinum* had no antimicrobial activity against bacteria even the extracts were used at high dosage. In the same study, extracts of the cyanobacterium had significant insecticidal activity against *A. aegypti* larvae and anticancer activity against cancer cell line PC3 [10]. In the current study, a thermophile *C. aponinum* isolated from hot springs in Turkey was investigated for its bioactive compound production. For this purpose, different nitrogen concentrations, light intensities, incubation periods, and temperatures were tested to determine the most effective bioactive compound produced by *C. aponinum*. Our main goal was to investigate the optimal conditions for a highly efficient bioactive compound production by the tested cyanobacterium that could be used in various biotechnological applications like pharmaceutical industry. It is known that to work with an organism that can cope with extreme conditions is advantageous according to its more stable compounds production [11]. In addition, it has been known that extremophiles can resist microbial contaminations by growing well under hard environmental conditions like temperature, extreme pH, and high salt concentrations. Therefore, a thermophile cyanobacterium was used to obtain bioactive compounds, tested its possible antimicrobial activity, and conditions were optimized for the highest bioactive property in the current study. According to our knowledge, there is no work about investigating thermophile *C. aponinum* for biotechnological applications like in this work.

MATERIALS and METHODS

Cyanobacteria isolation, growth conditions

Thermal spring water samples from Haymana and Kızılcahamam, Turkey, were spread on Petri plates containing BG11 medium [12] with 12 g/L agar and incubated at 45°C under continuous illumination (2400 lux). The pH of the growth medium was adjusted to 7.5 with dilute (0.01 M) and concentrated (1 M) sulfuric acid or sodium hydroxide solutions. Cells from microcolonies on these plates were isolated by micromanipulation. The cyanobacterial cells were purified to aseptic conditions by streaking the cells repeatedly on the media with agar plate. The purified cells were transferred to liquid media. These liquid cultures were tested for bacterial contamination by plating on bacteriological media. Isolated and purified cyanobacterial cultures were identified according to morphological and genetic characteristic.

Cyanobacterial strains were cultivated in 250 ml Erlenmeyer flasks including 100 ml of BG11 medium (pH: 7.5) at 30°C, under continuous light intensity as 2400 lx at a growth chamber (BINDER, model: KBW 400 (E5.1), S.no: 15-13640) for 14 days.

Selection of Cyanobacteria

Cyanobacteria used in the study were tested to determine having the most effective bioactive compound. In these experiments, 7 different strains were inoculated in BG11 medium and after incubation period, the effectiveness of bioactive compounds were examined. Cyanobacterium producing the most effective bioactive compound was found and further trials were performed with this cyanobacterium.

Identification

DNA of the selected cyanobacteria (Strain H2) were amplificated using PCR amplification with 2 ml of genomic DNA, 0.4 mM deoxynucleotide triphosphate, 1.25 units of Taq DNA polymerase and use forward primer F5'AGAGTTTGATCMTGGCTCAG and reverse primer R5' TACGGYTACCTTGTTACGACTT. PCR program was adjusted to 95°C for 5 min followed by 30 cycles of 95°C for 30 sec, 50°C for 30 sec, and 72°C for 45 sec. DNA sequencing was performed with Bigdye Cycle sequencing kit v3.1 and ABI 3130XL Genetic Analyzer.

Production of bioactive compounds

After incubation period of 14 days, biomass was collected by centrifugation (MPW-351R) at 10.000 rpm for 5 min. Biomasses were freeze-dried (Millrock Technology, Inc., Kingston, NY 12401, USA) for overnight and 3 ml ethanol solvent (Purity: 96%) was added to 1 gram of dried biomass for 1 hour. After then, the mixture was centrifuged for 10.000 rpm for 5 min and supernatant was used as algal extract. These solutions were kept at 4°C and used within 2 days [13, 14].

Determination of effectiveness of bioactive compound

To find the effectiveness of bioactive compound, trials were designated with antimicrobial activity. Antimicrobial activity was determined by disc diffusion method [15]. Standard bacterial strains like *B. subtilis* ATCC 6633, *B. thermosphacta* ATCC 11509, *E. coli* 0157:H7 ATCC 35150, *E. coli* ATCC 25922, *Enterobacter cloacae* ATCC 700323, *S. aureus* ATCC BAA 976, *S. aureus* ATCC 25923, *S. aureus* ATCC 1026 were used to perform these experiments. Bacteria were cultivated in Nutrient Broth for 24 h, and were inoculated uniformly using sterile cotton swab onto Nutrient Agar to test the antibacterial activities of cyanobacterial extracts. Extracts were applied to sterile disks (40 µl/per disk) and impregnated disks were placed on the plates using sterile forceps properly spaced at equal distance. Plates were incubated 24 h for 30°C. Paper discs loaded with ethanol solvent were also checked for its effect against the standard bacterial strains. The plates were stored for 2h to allow of the extracts into the agar. Then, these plates were incubated for 24 h at 30°C for growth of bacteria. The zone of inhibition was measured and expressed in mm in diameter.

Chlorophyll analysis

The chlorophyll (a) concentration in the media was determined in buffered aqueous 80% acetone solution. The concentration of chlorophyll was found with recording optical absorption at 646.6 and 663.6 nm by using Shimadzu UV 2001 model spectrophotometer (Japan) according to the equation given as below [16].

$$\text{Chlorophyll (a)}\ (\mu g/ml)=(12.25\times A_{663.6})+(2.55\times A_{646.6})$$

Bioactive compound production under different environmental conditions

Production of bioactive compounds by the selected cyanobacterium under different environmental conditions was investigated with regard to increasing nitrogen concentrations, light intensities, incubation periods, and temperatures. Unless other stated, cyanobacteria were cultivated in BG11 medium at 30°C, under continuous light intensity as 2400 lx at a growth chamber for 14 days.

To understand the effect of nitrogen concentration on production of bioactive compounds, experiments were performed in media with nitrogen concentrations as 0.5 g/L, 1.0 g/L, 1.5 g/L, and 2.0 g/L. Further trials were done with the condition that *C. aponinum* extracts had the highest antimicrobial activities.

Trials were carried out under different light intensities as 1200 lx, 2400 lx, 3600 lx, and 4800 lx to find the effect of light intensity on bioactive compound production by *C. aponinum*. Subsequent studies have been carried out with the condition in which the cyanobacterium produced the most efficient bioactive substance.

After optimization of nitrogen concentration and illumination for the highest antimicrobial activities of the extracts, the effect of incubation period was investigated. To determine the most effective bioactive compound produced by *C. aponinum* under different incubation periods, experiments were done with incubation periods as 7, 14, 21, and 28 days. Next experiments were

Table 1. Antimicrobial activity [zone of inhibition (mm)] of bioactive compounds extracted from cyanobacterial species isolated from Haymana and Kızılcahamam thermal springs (T: 45 °C; N concentration: 1 g/L; light intensity: 2400 lx; incubation period: 14 d).

Bacteria	Strain						
	H1	H2	H3	H4	H5	K1	K2
B. subtilis ATCC 6633	9±1	12±2	10±1	12±1	11±2	12±1	8±1
B. thermosphacta ATCC 11509	10±2	12±1	11±1	11.5±1	11±2	11.5±1	10±1
E.coli 0157:H7 ATCC 35150	11.5±1	13±1	12±1	13±1	11.5±1	13±1	11±1
E. coli ATCC 25922	8±1	14.5±1	12±1	12±1	12±2	13±1	10±1
E. cloacae 700323	8.5±1	11±1	11±1	12±2	11.5±2	12±1	10±2
S. aureus ATCC BAA 976	9±2	13±1	12±1	12±1	12±1	13±1	10±1
S. aureus ATCC 25923	7±1	12±1	12±2	12.5±1	11±1	12±2	11±1
S. aureus ATCC 1026	8±2	11±1	10±2	11±1	10.5±1	11±1	7±1

done with the incubation period due to the maximum antimicrobial activities of the samples. Effect of increasing temperature on bioactive compound production by the tested cyanobacterium were performed in another series of the experiments. To examine the effect of different temperatures, trials were performed with incubation temperatures as 30°C, 40°C, 45°C, and 50°C.

RESULTS and DISCUSSION

Selection of cyanobacterium producing the most effective bioactive compound and its identification

In these trials, 7 different cyanobacterial strains (Strain H1, Strain H2, Strain H3, Strain H4, Strain H5, Strain K1, and Strain K2) were used. The results obtained from these series of the experiments were summarized in Table 1. All cyanobacterial isolates had antimicrobial activity against all the tested standard bacterial strains. Ethanol-extracts of H1 affected *E. coli* 0157:H7 ATCC 35150 bacterium the most with an antimicrobial activity having 11.5 mm inhibition zone.

On the other hand, extracts from strain H2 had the highest bioactive properties against *E. coli* ATCC 25922 with an inhibition zone as 14.5 mm. Cyanobacterial strain H3 extracts had the maximum antimicrobial activity against four of the tested bacteria as *E. coli* 0157:H7 ATCC 35150, *E. coli* ATCC 25922, *S. aureus* ATCC BAA 976, and *S. aureus* ATCC 25923 (inhibition zones: 12 mm). Extracts from strain H4 affected *E. coli* 0157:H7 ATCC 35150 with the highest inhibition zone as 13 mm. Strain H5 had effective bioactive character against *E. coli* ATCC 25922 and *S. aureus* ATCC BAA 976 (inhibition zones: 12 mm); while strain K1 had the highest antimicrobial activity against *E. coli* 0157:H7 ATCC 35150, *E.coli* ATCC 25922, and *S. aureus* ATCC BAA 976 (inhibition zones: 13 mm). Among the cyanobacterial strains, strain K2 had the highest antimicrobial activity against *E. coli* 0157:H7 ATCC 35150 and *S. aureus* ATCC 25923 with inhibition zones as 11 mm. According to data obtained from these trials, further experiments were performed with Strain H2 related to its highest antimicrobial activity against the all tested bacteria.

The selected cyanobacterium (Strain H2) was identified by amplification and sequencing of its 16S rRNA gene. Phylogenetic analysis of the nearly complete sequence data was done by BLAST search. Alignment and further analysis in ARB database revealed that the cyanobacterium had a >99% similarity to *Cyanobacterium aponinum.* The isolated cyanobacteria submitted to NCBI Gen-Bank under accession number as MN 116003.

There are only a few reports performing antimicrobial activity with extracts from thermophilic cyanobacteria [7-10]. Of the studies except one of them, thermophile *C. aponinum* was not used, while other thermophilic cyanobacteria as *Cosmarium* sp. [9], *Phormidium* sp. [7], *Oscillatoria spp., Synechocystis aquatilis, and Synechoccous cerdrorum* were used to obtain bioactive compounds. In these studies, authors found variable antimicrobial activities according to the cyanobacteria tested.

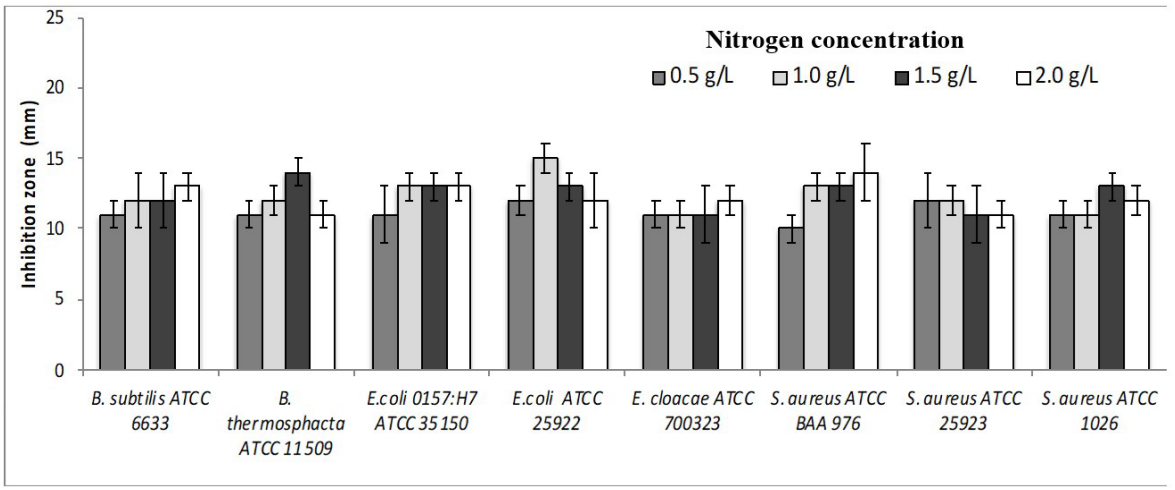

Figure 1. Antimicrobial activities [zone of inhibition (mm)] of bioactive compounds extracted from *C. aponinum* under different nitrogen concentrations (T: 30 °C; light intensity: 2400 lx; incubation period: 14 d).

The only work mentioned about thermophilic *C. aponinum* was performed by Mizerakis et al. [10]. In that study, there was no antimicrobial activity against bacteria, however, in the current study, ethanol-extracts of thermophilic *C. aponinum* showed high antimicrobial activity against several bacterial strains.

Bioactive compound production by *C. aponinum* cultivated in media with different environmental conditions

The effect of different nitrogen concentrations

To determinate the effect of different nitrogen concentrations onto production of bioactive compounds by *C. aponinum*, cyanobacterium was inoculated in to BG 11 media with 0.5 g/L, 1.0 g/L, 1.5 g/L, and 2.0 g/L nitrogen concentration. As it was shown in Figure 1, extracts from cyanobacterium grown in media with 0.5 g/L nitrogen, the highest antimicrobial activity was shown against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 with inhibition zones as 12 mm. Extracts obtained from *C. aponinum*, grown in media with 1.0 g/L nitrogen, the highest antimicrobial activity was shown against *E. coli* ATCC 25922 (inhibition zone: 15 mm). When the cyanobacterium was grown in medium containing 1.5 g/L of nitrogen, the extract prepared from that biomass showed the highest antimicrobial effect, producing 14 mm inhibition zone against *B. thermosphacta* ATCC 11509. The highest bioactive property was shown against *S. aureus* ATCC BAA 976 (inhibition zone: 14 mm) with the extracts obtained from biomasses from which *C. aponinum* cultivated in media with 2.0 g/L nitrogen concentration. According to these results, further experiments were done in media with 1.0 g/L nitrogen related to extracts having the highest bioactive property.

In these experiments, with an increase in nitrogen concentration, antimicrobial effect also increased or remained constant for the tested bacteria. Previous studies only focused on to determine growth, fatty acid production and accumulation due to the nitrogen stress in *C. aponinum* [17-19]. Such an attempt like in the current study has not been investigated yet.

Chlorophyll amounts were also determined under all the tested parameters. Chlorophyll contents of the *C. aponinum* were 2.2 µg/ml, 5.2 µg/ml, 2.6 µg/ml, and 2.4 when cyanobacteria were cultivated in media under 0.5 g/L, 1.0 g/L, 1.5 g/L, and 2.0 g/L nitrogen concentrations, respectively.

The effect of different light intensities

In these series of the experiments the increment in light intensities varied the antimicrobial character of the bioactive compounds. Figure 2 summarized that with an increase in light intensities, antimicrobial activity of the extracts increased also. When *C. aponinum* cultivated at 1200 lx light intensity, extracts obtained under these conditions showed the highest antimicrobial activity against *E. coli* 0157:H7 ATCC 35150 and *S. aureus* ATCC BAA 976 (inhibition zones: 14 mm). Under the same conditions, the lowest antimicrobial activity was found

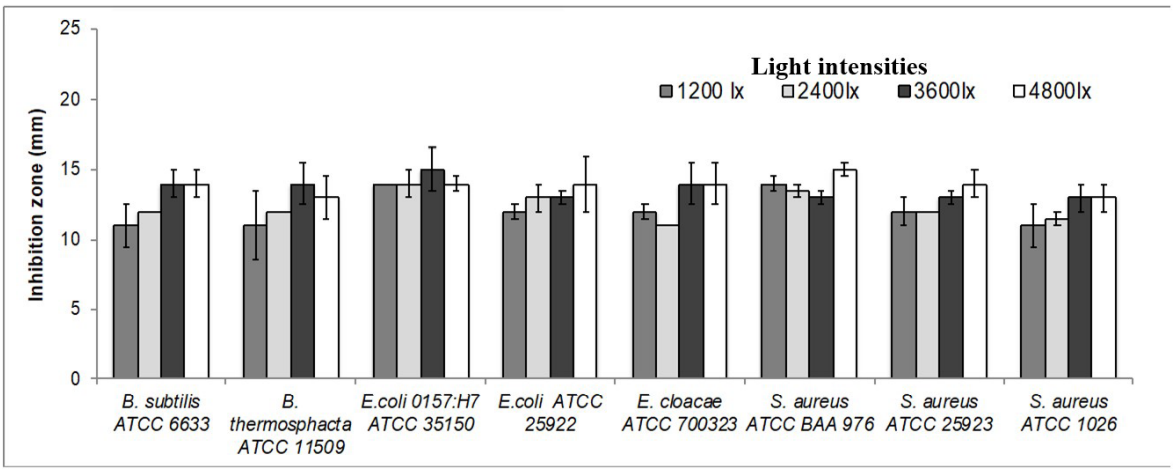

Figure 2. Antimicrobial activities [zone of inhibition (mm)] of bioactive compounds extracted from *C. aponinum* under different light intensities (T: 30°C; N concentration: 1 g/L; incubation period: 14 d).

against *B. subtilis* ATCC 6633, *B. thermosphacta* ATCC 11509 and *S. aureus* ATCC 1026 bacteria with inhibition zones as 11 mm. When cyanobacterium was cultivated at 2400 lx light intensity, the highest antimicrobial activity was shown against E. coli 0157:H7 ATCC 35150 with an inhibition zone as 14 mm, while the lowest inhibition zone was 11 mm against *E. cloacae* ATCC 700323. With bioactive compounds obtained from *C. aponinum* grown at light intensity as 3600 lx, the highest antimicrobial activity was found as 15 mm against *E. coli* 0157:H7 ATCC 35150. Under the same light intensity, bioactive compounds showed high antimicrobial activity against the all bacteria tested with inhibition zones ranged from 13 or 14 mm. When light intensity was increased to 4800 lx, cyanobacterium still produced effective bioactive compounds. Under these conditions, the most effective compound was found against *S. aureus* ATCC BAA 976 (inhibition zone: 15 mm), while antimicrobial activities for other bacteria varied from 13 or 14 mm. At the end of these experiments, further experiments were done under 3600 lx light intensity.

Gris et al. showed that thermophilic *C. aponinum* produced extracellular compounds under different environmental conditions [20]. In that study, the formation of extracellular polymer by *C. aponinum* was induced with an increase in light intensity and it was constant above the light intensity as 70 µmol photons m^2/s. Another previous study about thermophile *C. aponinum* mentioned the effect of stress conditions as temperature, pH, CO_2 and light quality on *C. aponinum* growth [21]. In the same study, cyanobacterium was exposed to four light qualities; of these, under white light with 60 µmol photons m^2/s intensity, *C. aponinum* achieved a high growth rate. In the current study, when the increment of the illumination from 16 µmol photons m^2/s to 32, 48, or 64 µmol photons m^2/s, *C. aponinum* produced more efficient bioactive compounds.

According to the results, with an increase in light intensity, chlorophyll amount was also increased by the cyanobacterium. Under 1200 lx, chlorophyll content was 3.1 µg/ml; while it was 4.4 µg/ml under 2400 lx. When light intensity was increased to 3600 lx and 4800 lx, chlorophyll amounts were found as 6.1 µg/ml, 4.8 µg/ml, respectively.

The effect of different incubation periods

Antimicrobial activities of bioactive compounds extracted from *C. aponinum* cultivated with different incubation periods were given in Figure 3. When cyanobacterium was grown with an incubation period as 7 days, obtained extracts showed lower antimicrobial activity than extracts taken from biomasses cultivated other incubation periods. Extracts obtained from biomasses after incubation for 7 days showed the highest bioactive properties against *E. coli* 0157:H7 ATCC 35150 and *E. cloacae* ATCC 700323 (inhibition zones: 12 mm). It was found that the most effective bioactive compound was produced after incubation for 14 days. Under these incubation period, *E. coli* 0157:H7 ATCC 35150 was affected the most by the extracts of *C. aponinum* with an incubation zone as 17.5 mm. After incubation for 21 days, bioactive compounds obtained from cyanobacterium

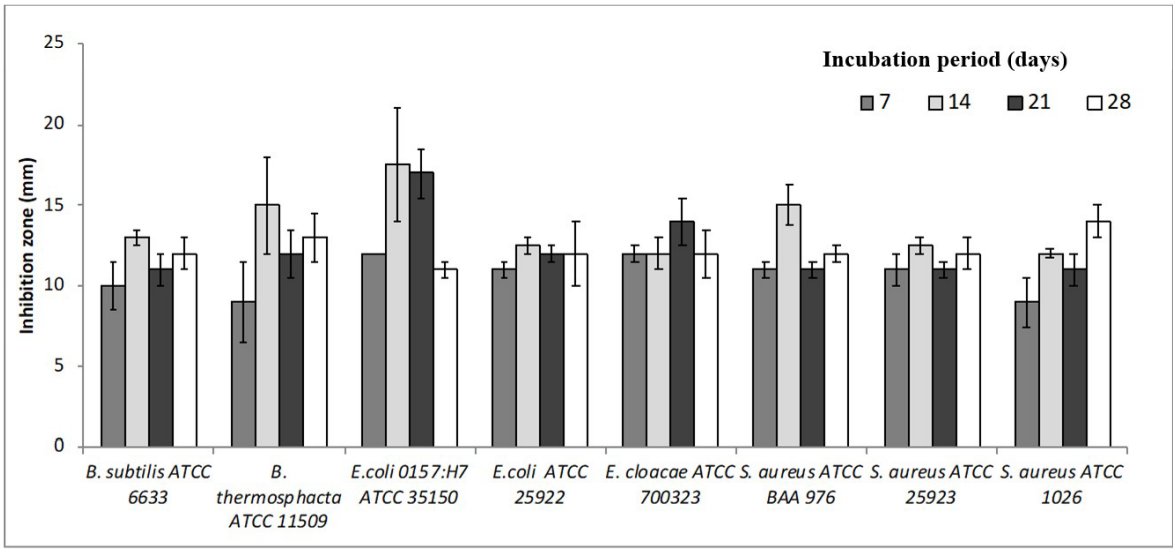

Figure 3. Antimicrobial activities [zone of inhibition (mm)] of bioactive compounds extracted from *C. aponinum* under incubation periods (T: 30°C; N concentration: 1 g/L; light intensity: 3600 lx).

had the maximum antimicrobial activity against *E. coli* 0157:H7 ATCC 35150 (incubation zone: 17 mm). Trials in which the tested cyanobacterium was cultivated for 28 days, extracts had their highest antimicrobial activity towards *S. aureus* ATCC 1026 (inhibition zone: 14 mm).

Incubation period is a substantial factor for cyanobacterial bioactive compound formation. These unique biologically active compounds can be produced via primary metabolism, like proteins, fatty acids, vitamins, and pigments, or can be formed from secondary metabolism [22]. It was previously determined by Noaman et al. that *Synecoccus leopoliensis* showed the maximum antimicrobial properties with extracts obtained when cyanobacteria were cultivated with an incubation period for 14 or 15 days [23]. In the current study, it was also found that *C. aponinum* had the most efficient bioactive compound when the incubation period was 14 days.

The highest chlorophyll amount was determined after incubation for 14 and 21 days (6.2 µg/ml). Chlorophyll content of the cyanobacterium was 1.9 µg/ml and 5.1 µg/ml when *C. aponinum* was cultivated in media under different incubation periods as 7 d and 28 d, respectively. The most effective compounds were produced after incubation for 14 days. Thus, incubation period was optimized as 14 d.

The effect of temperatures

To understand the effect of increasing temperatures onto production of bioactive compounds, cyanobacterium was cultivated at 30°C, 40°C, 45°C and 50°C. As it was shown in Figure 4, extracts from cyanobacterium grown at 30°C, the highest antimicrobial activity was shown against *B. thermosphacta* ATCC 11509 with an inhibition zone as 13 mm. Extracts obtained from *C. aponinum* grown in media at 40°C, the highest antimicrobial activity was shown against *B. subtilis* ATCC 6633 and *S. aureus* ATCC 1026 (inhibition zones: 13 mm). When the cyanobacterium was grown in medium at 45 °C, the extract prepared from that biomass showed the highest antimicrobial effect, producing 18 mm inhibition zone against *E. coli* 0157:H7 ATCC 35150. The highest bioactive property was shown against *E. coli* ATCC 25922 (inhibition zone: 14 mm) with the extracts obtained from biomasses from which *C. aponinum* cultivated at 50 °C. In these experiments, it was determined that the bioactive effectiveness of the extracts increased which were obtained from the biomass when the temperature was increased from 30°C to 45°C. Increasing the temperature by more than 45°C caused a decrease of cyanobacterial growth, and later the cells dead. This result was easily visible because of color change of the cells from blue-green to brown.

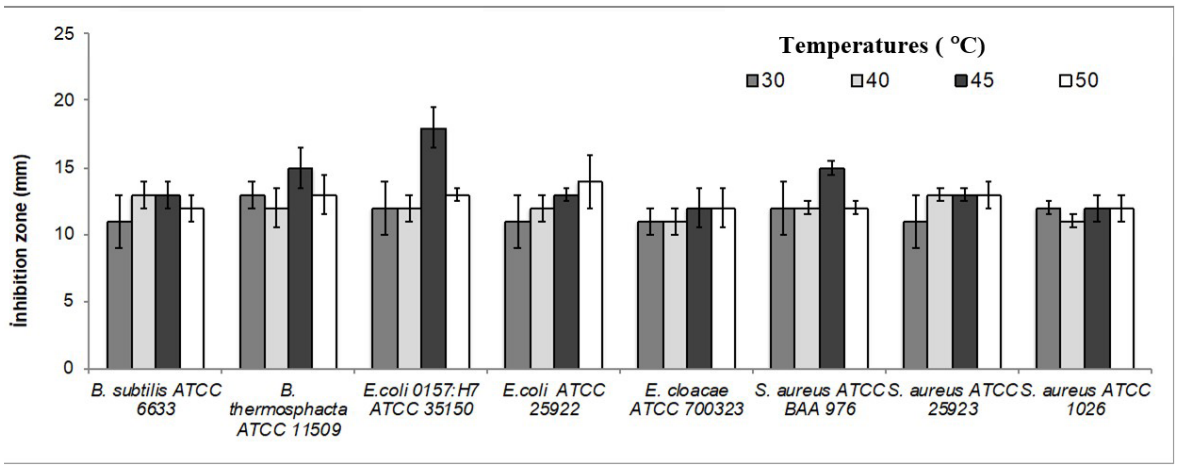

Figure 4. Antimicrobial activities [zone of inhibition (mm)] of bioactive compounds extracted from *C. aponinum* under different temperatures (N concentration: 1 g/L; light intensity: 3600 lx; incubation period: 14 d).

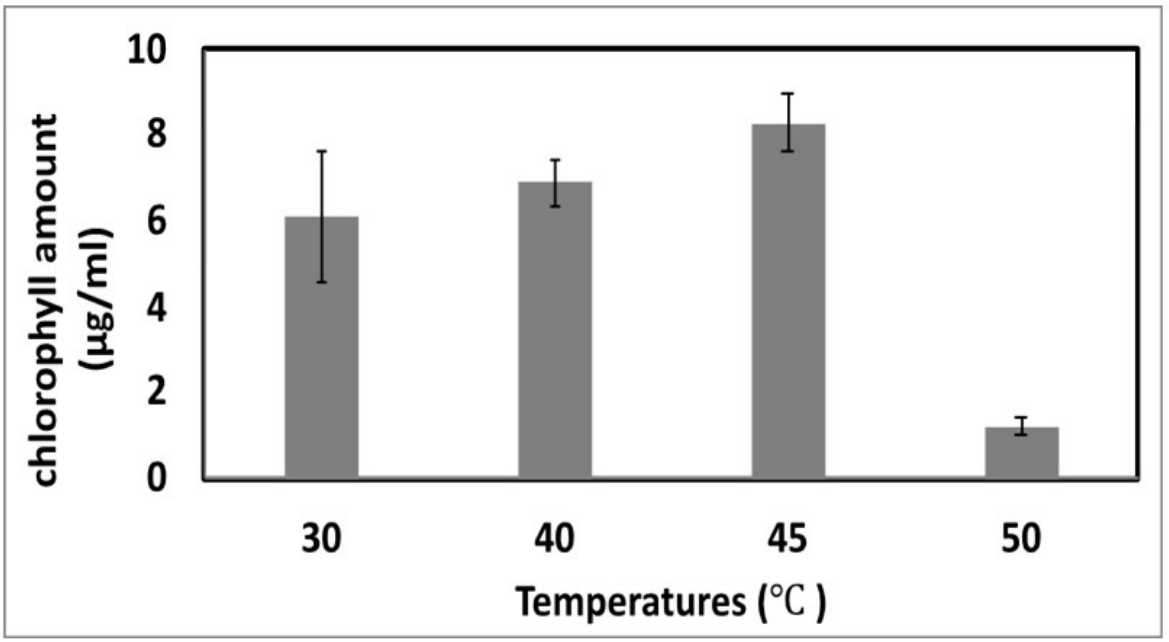

Figure 5. Chlorophyll amounts of *C. aponinum* under different temperatures (N concentration: 1 g/L; light intensity: 3600 lx; incubation period: 14 d).

The most effective parameter which affected the cyanobacterium was temperature. It was clearly understood from Figure 5 that the tested cyanobacterium produced the highest amount of chlorophyll under 45°C as 8.3 µg/ml. Under 30°C and 35°C, *C. aponinum* had chlorophyll amounts as 6.1 µg/ml and 6.9 µg/ml, respectively, while the content was 1.2 µg/ml when cultivated at 50°C.

It was previously reported that thermophilic *C. aponinum* can be survived up to 50°C [20, 21]. Gris et al. found the maximum growth rate at 40°C, extracellular polymer production at 35°C [20], while Meng et al. determined the highest biomass production at 35°C [21]. In the current study, the most efficient bioactive compound was observed when cyanobacterium was cultivated under 45°C. The tested cyanobacterium was affected mostly temperature parameter and antimicrobial activity had its highest rate when temperature was increased.

CONCLUSIONS

Formation of bioactive compounds by *C. aPoninum* isolated from thermal springs of Turkey was investigated in the current study. The study was concluded that extracts of this thermophilic cyanobacterium had antimicrobial activity against several standard bacterial strains. In addition, it was found that efficiencies of the bioactive compounds could be induced via exposure to stress conditions like high light intensities and temperatures. According to our knowledge, this issue will be firstly presented by the current work. The highest efficiency was found in extracts of *C. aponinum* when the extremophile cyanobacterium was cultivated in media with 1 g/L nitrogen, at 45°C, under 3600 lx light intensity after incubation for 14 days. These findings obtained from the current study has indicated that thermophile *C. aponinum* might be a good promising candidate in several biotechnological applications such as designing of new antibacterial drugs.

References

1. A. Patel, L. Matsakas, U. Rova, P. Christakopoulos, A perspective on biotechnological applications of thermophilic microalgae and cyanobacteria, Biores. Technol., 278 (2019) 424-434.
2. S. Dobretsov, R.M.M. Abed, S.M.S. Al Maskari, J.N. Al Sabahi, R. Victor, Cyanobacterial mats from hot springs produce antimicrobial compounds and quorum-sensing ınhibitors under natural conditions, J. Appl. Phycol., 23, (2011) 983-993.
3. A. Drobac-Čik, T.I. Dulic, D.B. Stojanovic, Z.B. Svircev, The ımportance of extremophile cyanobacteria in the production of biologically active compounds, Matica Srpska J. Nat. Sci., 112, (2007) 57-66.
4. C. Pumas, P. Vacharapiyasophon, Y. Peerapornpisal, P. Leelapornpisid, W. Boonchum, M. Ishii, C. Khanongnuch, Thermostablility of phycobiliproteins and antioxidant activity from four thermotolerant cyanobacteria, Phycol. Res., 59, (2011) 166-174.
5. N. Mezhoud, F. Zili,, N. Bouzidi, F. Helaoui, J. Ammar, H.B. Ouada, The effects of temperature and light intensity on growth, reproduction and eps synthesis of a thermophilic strain related to the genus *Grasiella*, Bioproc. Biosyst. Eng., 37 (2014) 2271-2280.
6. J-L. Leu, T-H. Lin, M.J.P. Selvamani, H-C. Chen, J-Z. Liang, K-M. Pan, Characterization of a novel thermophilic cyanobacterial strain from taian hot springs in Taiwan for high CO_2 mitigation and c-phycocyanin extraction, Process Biochem., 48 (2013) 41-48.
7. S.A. Fish, G.A. Codd, Bioactive compound production by thermophilic and thermotolerant cyanobacteria (blue-green algae), W. J. Microbiol. Biotechnol., 10 (1994) 338-341.
8. F. Heidari, H. Riahi, M. Yousefzadi, M. Asadi, Antimicrobial activity of cyanobacteria ısolated from hot spring of geno, Middle-East J. Sci. Res., 12 (2012) 336-339.
9. R. Challouf, R. Ben Dhieb, H. Omrane, K. Ghozzi, H. Ben Ouada, Antibacterial, antioxidant and cytotoxic activities of extracts from the thermophilic green alga, *Cosmarium* sp., Afr. J. Biotechnol., 11 (2012) 14844-14849.
10. P. Mizerakis, P. Stathopoulou, G. Tsiamis, M.N. Baeshen, J.A. Mahyoub, A.M. Elazzazy, S. Bellou, E. Sakoulogeorga, I-E. Triantaphyllidou, T. Mazioti, P. Katsoris, G. Aggelis, Bacterial diversity of the outflows of a polichnitos (lesvos, greece) hot spring, laboratory studies of a *Cyanobacterium* sp. strain and potential medical applications, Ann. Microbiol., 67 (2017) 643-654.
11. M.S., Urbieta, E.R., Donati, K-G., Chan, S., Shahar, L.L., Sin, K.M. Goh, Thermophiles in the genomic era: biodiversity, science, and applications, Biotechnol. Adv., 33 (2015) 633-647.
12. R. Rippka, Recognition and identification of cyanobacteria, Method Enzymol., 167 (1988) 28-67.
13. A.R. Rao, A.H. Reddy, S.M. Aradhya, Antibacterial Properties of *Spirulina platensis, Haematococcus pluvialis, Botryococcus braunii* micro algal extracts, Curr. Trend Biotechnol. Pharm., 4 (2010) 809-819.
14. J. Pradhan, B.K. Das, S. Sahu, N.P. Marhual, A.K. Swain, B.K. Mishra, A.E. Eknath, Traditional antibacterial activity of freshwater microalga *Spirulina platensis* to aquatic pathogens, Aquac. Res., 43 (2012) 1287-1295.

15. P.R. Murray, E.J. Baron, M.A. Pfalle, F.C. Tenover, R.H. Yolke, Manual of Clinical Microbiology (6th ed.) Washington, DC, United States, ASM Press., (1995) 1482 pp.
16. R.J. Porra, W.A. Thompson, P.E. Kreidemann, Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy, BBA-Bioenergetics, 975 (1989) 84-394.
17. T.C. Hopkins, E.J.S. Graham, J. Schwilling, S. Ingram, S.M. Gómez, A.J. Schuler, Effects of salinity and nitrogen source on growth and lipid production for a wild algal polyculture in produced water media, Algal Res., 38 (2019) 101436
18. K.C. Wu, K.C. Ho, C.C. Tang, Y.H. Yau, The Potential of foodwaste leachate as a phycoremediation substrate for microalgal CO_2 fixation and biodiesel production, Environ. Sci. Poll. R., (2018). https://doi.org/10.1007/s11356-018-1242-9
19. S.E. Karatay, G. Dönmez, Microbial oil production from thermophile cyanobacteria for biodiesel production, Appl. Energy, 88 (2011) 3632-3635.
20. B. Gris, E. Sforza, T. Morosinotto, A. Bertucco, N. La Rocca, ınfluence of light and temperature on growth and high-value molecules productivity from *Cyanobacterium aponinum*, J. Appl. Phycol., 29 (2017) 1781-1790.
21. F. Meng, H., Cui, Y., Wang, X. Li, Responses of a new isolated *Cyanobacterium aponinum* strain to temperature, pH, CO_2 and light quality, J. Appl. Phycol., 30 (2018) 1525-1532.
22. M.G. de Morais, B. da Silva Vaz., E.G. de Morais, J.A. Costa, Biologically active metabolites synthesized by microalgae, Biomed Res. Int., Article ID 835761 (2015) 1-15.
23. N.H. Noaman, A. Fattah, M. Khaleafa, S.H. Zaky, Factors Affecting antimicrobial activity of *Synechococcus leopoliensis,* Microbiol. Res., 159 (2004) 395-402.