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Inhibition of *Trichormus variabilis* and *Oscillatoriales cyanobacterium* Using Probiotic Lactic Acid Bacteria

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Abstract

Water blooms are a serious environmental problem affecting fresh and marine ecosystem. Application of probiotic bacteria has been introduced as an emerging and eco-friendly tool to regulate or inhibit cyanobacterial blooms. In the present study, we have isolated and identified two different cyanobacterial isolates from El Manzala Lake. Afterward, we examined the anticyanobacterial activity to evaluate their effect on cyanobacterial morphology. Furthermore, the effect on cyanobacterial behavior was evaluated through determination of biomass, reduction percentage of both chlorophyll, phycocyanin using the co-culture technique between the selected cyanobacterial isolates and probiotic bacteria (MT2, MT3 and MT4). In this study, the relative high occurrence of cyanobacteria was observed during spring and summer seasons. The isolated cyanobacteria were identified as *Trichormus variabilis* and *Oscillatoriales cyanobacterium*. Only, MT2 (*Lactobacillius sakei*) supernatant displayed anticyanobacterial activity with inhibition zone of 13.6±1.25 and 10±1.44 mm against *Trichormus variabilis* and *Oscillatoriales cyanobacterium*, isolated from El-Manzala Lake, respectively. Also, the same supernatant has caused in occurring great morphological changes represented by complete lysis of *Trichormus variabilis* filaments after 60 minutes. Strong decrease in some photosynthetic parameters in the co-culture between *Lb. sakei* and each of *Trichormus variabilis* and *Oscillatoriales cyanobacterium*, individually, was recorded. For *Trichormus variabilis*, after five days of incubation, the remarkable reduction percentage of chlorophyll and phycocyanin production was observed and reached 90.85±3.55% and 88.84±3.55%, respectively. In *Oscillatoriales cyanobacterium* co-culture, the reduction percentage reached 100±0.0 and 91.38±2.99% for chlorophyll and phycocyanin, individually.

Keywords: Cyanobacteria; *Trichormus variabilis; Oscillatoriales cyanobacterium*; *Lactobacillius sakei*, anticyanobacterial activity.

1. Introduction

Eutrophication, cyanobacterial blooming, and different environmental problems are the main factors which have many adverse effect on both natural environment and sustainable aquaculture [1]. Cyanobacteria are among the oldest organism in the world with extended evolutionary history where evidence of their presence started around 3500 million years ago [2]. Globally, many lakes and rivers are suffering from water blooms formed by cyanobacteria and algae. As they produce toxins which are harmful to all living organisms (fish, birds, animals and humans) [3, 4].

In general, cyanotoxins are originated from planktonic cyanobacteria, which occur in both fresh and brackish water. Cyanobacteria produce different types of cyanotoxins including microcystins are hepatoxin peptides that are destructive to liver tissue

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causing a severe public health problem. Microcystins are synthesized by different fresh water cyanobacterial species e.g *Microcystis* sp., *Anabaena* sp., *Nostoc* sp. and *Oscillatoria* sp., [5].

Probiotic bacteria have several advantages in comparison with previously reported cyanobactericidal bacteria, as the European Food Safety Authority, EFSA [19] classified them as safe bacteria. The mode of action associated with cyanobacteria or algae degradation by probiotic bacteria involves; (i) the direct attack to cyanobacterial/algal cells through cell-to-cell contact mechanism [20] and (ii) the indirect attack by lysis the cyanobacterial/algal cells through the extracellular metabolites produced by probiotic bacteria [21].

Thus, the aim of this study is to evaluate the effect of probiotic bacteria on the physiology of the dominant cyanobactria isolated from El Manzala Lake.

2. Experimental

2.1. Sampling location

El-Manzala Lake is the largest lake in the northeastern region of Egypt. It is located between three governorates; Dakahlia, Damietta and Port Said [22, 23]. It is bounded by the Suez Canal on the east and by Damietta branch of the Nile on the west. It is connected to the Mediterranean Sea through a narrow channel called Boughaz El-Gamil. Annually, it receives around 7500 million cubic meters of untreated effluent [domestic, agriculture and industry) from several drains and canals [24]. The most important drains are Faraskur, Al-Sarw, Baghous, Abu-Garida and Bahr El-Baqur [25]. Bahr El-Baqur is the most polluted drain because it is receiving untreated wastewater (domestic and industrial) from Cairo governorate. Thus, this drain contributes greatly to the deterioration of water quality in the lake.

2.2. Water samples

Twenty different seasonal water samples were collected during the period from October, 2015 to September, 2016. The samples were collected from four different sites in El-Manzala Lake using bottles at a depth of 30 cm from water surface. These sites were at different distances (2, 4, 8 and 16 km) from Bahr El-Baqur drain as shown in (Fig. 1).

Fig. 1. Map of El – Manzala Lake showing sampling locations. Site 1, 2, 3 and 4 are at 2, 4, 8 and 16 km from Bahr El - Baqr drain, respectively.

All water samples were kept in ice box to transfer to the lab. Water samples were refrigerated at 4oC for not more than one day until examined in the in Biotechnology and Biodiversity Conservation lab., Centre of Excellence for Advanced Sciences, National Research Centre.

2.3. Isolation of cyanobacteria

The enrichment culture technique was applied to isolate cyanobacteria from the collected water samples. Twenty-five mL of each sample were aseptically added to 100 ml of BG-11 broth [26] in 250 conical flasks. Afterwards, the inoculated flasks were incubated at 30ºC under continuous illumination from Philips fluorescent white lamps at light intensity of 400 -500 lux.

2.4. Purification of isolated cyanobacteria

The techniques of several successive transfers and single filament isolation were applied to purify the obtained non axenic cultures of cyanobacteria:

a. Several successive transfers

The isolated cyanobacteria were successively subcultured several times in BG-11 broth and incubated for 3 - 4 weeks at 30ºC until the healthy and homogenous culture were obtained.

b. Single filament isolation

This technique depends on gliding movement and phototaxis of cyanobacteria. To examine the ability of cyanobacterial filaments to grow and swim toward a single source of light, the contaminated cultures of cyanobacteria were grown on BG-11 agar in Petri dishes. Once a single filament moved a sufficient distance on BG-11 agar, it was picked up under sterilized conditions and placed into a separate flask containing fresh liquid BG-11.

2.5. Identification of isolated cyanobacteria

a. Morphological characterization

Wet mount preparation was used to characterize the isolated cyanobacteria morphologically. According to Rippka, *et al*. [26]. the cyanobacteria morphotypes such as filamentous nature, size, shape of vegetative cells, presence of heterocyst and akinetes were identified and photographed using light microscope.

b. Molecular characterization

The molecular identification according to 16S rRNA gene sequencing was applied in Microbiology Research Lab., Cairo University Research Park (CURP), Faculty of Agriculture, Cairo University. The cyanobacterial cultures prepared in BG-11 broth were harvested by centrifugation at 12000g for 5 min. After washing the pellets for three times using 0.85% NaCl saline solution, the chromosomal DNA was extracted using GeneJET Genomic DNA purification Kit (Thermo scientific, Lithuania). DNA yield and purity were checked using both UV-Vis NanoDrop spectrophotometer (NanoDrop 2000, ThermoFisher Scientific, Germany) and agarose gel electrophoresis (Bio-rad, USA).

The 16S rRNA gene fragments were amplified using the universal primers (CYA106F), 5′-CGGACGGGT GAGTAA CGCGTGA-3′ and CYA781R, 5′-GACTACWGGGGT ATCTA ATCCCWTT-3′. The PCR products were purified using the gel extraction kit and sequenced in Macrogen (Seoul, Republic of Korea) using automatic ABI 370×1 DNA Sequencer (Applied Biosystem).

2.6. Culture of probiotic lactic acid bacteria

The probiotic isolates (MT2, MT3 and MT4) used in this study were previously isolated from Nile Tilapia fish intestines collected from El Manzala Lake, Port Saied Governaerate, Egypt. MT2, MT3 and MT4 were identified by 16S rRNA as *Lactobacillius sakei* (MK920968), *Staphylococcus hominis* (MK920968) and *Staphylococcus hominis* (MK920972). The probiotic isolates were grown individually in MRS broth at 37º C for 24hr, and the cells were removed by centrifugation at 4000 rpm for 15 min at 4ºC. The cell free supernatant (CFS) was filtered through a cellulose acetate membrane filter with a pore size of 0.22 µm (Chromatographic Specialties Inc., ON, Canada) to be used in the anticyanobacterial activity assessment.

2.7. Evaluating the interaction between probiotic bacterial isolates and cyanobacteria

The probiotic LAB with the greatest anticyanobacterial activity, were studied to evaluate their effect on cyanobacterial morphology. Furthermore, the effect on behavior of cyanobacteria was evaluated through determination of dry weight and the reduction percentage of both total chlorophyll and phycocyanin applying the co-culture technique.

2.7.1. Antagonistic activity of probiotics against cyanobacteria

To apply the disc diffusion assay, the cultures of isolated cyanobacteria were cultivated in BG-11 broth for 14 days and harvested by centrifugation at 3000 rpm for 20 min. The pellet was washed twice with PBS and mixed with molten BG-11 medium. The inoculated medium was poured into a Petri plate and the sterilized paper disc (7mm in diameter) saturated with 40 µL of the crude cell-free supernatant (C - CFS), prepared from probiotics as mentioned before, was placed on it. The plates were incubated at 30 ºC under continuous illumination for 4 days [27]. Appearance of a detectable clear zone around the disc was considered as a growth inhibition of cyanobacteria by probiotics.

2.7.2. Effect of probiotics on cyanobacterial morphology

The C-CFS of probiotics was added to cyanobacterial culture (14 days) in a percentage of 20%. At ten minutes interval for one hour, changes in the cyanobacterial morphology were examined microscopically. These changes were compared with untreated cyanobacterial cultures.

2.7.3. Effect of probiotics on cyanobacterial physiology

Effect of probiotics on cyanobacterial physiology was assessed applying the co-culture technique to determine the variations of dry weight and cyanobacterial ability to produce chlorophyll and phycocyanin. In this technique, 225 mL of cyanobacterial broth culture were mixed with 25 mL of probiotic LAB broth culture (105cfu/mL) [28]. The BG-11 broth culture containing cyanobacteria only was considered as a control. Every 24hr intervals for 5 days of incubation at 30°C, the cyanobacterial behavior was evaluated through determining the reduction percentage of both total chlorophyll and phycocyanin, and estimation of the dry weight.

2.7.3.1. Chlorophyll content determination

The method of Seely *et al*. [29] was applied to estimate the total chlorophyll concentration. In this method, 5 mL of the co-culture were filtered through 0.45 µm membrane filter using millipore filtration system. Then, 3 mL of 95% DMSO were added to the filtered cells in a 10 mL tube, and the mixture was sonicated and incubated at 70°C for 5 minutes in an ultrasonic water bath, and centrifuged at 8000 rpm for 5 minutes. The optical density (OD) of the supernatant was measured at a wavelength of 666 nm using a spectrophotometer (Jasco, V -630). The total chlorophyll concentration (mg/L) was calculated with the following equation:

Total chlorophyll (mg/L) = E666 × D × F

where, E666 represents OD666, D is a ratio of volume of extract/volume of sample and F is a value of 11.3 (factor to equal the reduction in absorbance).

2.7.3.2. Phycocyanin content determination

To determine the total phycocyanin, the biomass was sonicated at 37 kHz using (Elma Sonic 30H) for 40 seconds to break up the filaments and release the water phycobiliproteins pigments, followed by centrifugation at 8000 rpm/5 min to remove filament debris [30]. The optical density (OD) of the supernatant was measured at a wavelength of 620 and 652nm. Phycocyanin concentration (µg/mL) was calculated according to the following equation [31]:

Phycocyanin concentration (µg /mL) = [OD620-(0.474OD652)] / 5.34.

2.7.3.3. Dry weight determination

To estimate the dry weight of cyanobacteria, 20 mL of homogeneous suspensions was centrifuged at 10000 rpm for 15 min., washed and dried overnight at 60-70 ºC [32].

2.8. Statistical analysis

All experiments were performed in triplicates. The experiments were analyzed using Paired Samples T-Test. The differences were considered significant for p values < 0.05.

3. Results

3.1. Relative occurrence of cyanobacteria in El Manzala Lake

The relative occurrence of cyanobacteria in El-Manzala Lake was analyzed during the period from October, 2015 to September, 2016 (Table 1). In each site, the high prevalence of cyanobacteria was perceived in summer season (July, 2016 – September, 2016). The weak occurrence was observed during the period from October, 2015 to January, 2016.

Table 1

Relative occurrence of cyanobacteria in El Manzala Lake

Season	Relative occurrence of cyanobacteria in the selected sites			
	Site 1	Site 2	Site 3	Site4
Autumn (Oct., 2015)	+	+	+	+
Winter (Jan., 2016)	+	+	+	+
Spring (Mar., 2016)	+ +	+ +	+ +	+
Summer (July, 2016)	+ + +	+ + +	+ + +	+ +
Late Summer (Sept., 2016)	+ + +	+ + +	+ + +	+ + +

+++; good occurrence of cyanobacteria. ++; moderate occurrence. +; weak occurrence.

3.2. Isolation, purification and identification of cyanobacteria

A total of 15 cyanobacterial isolates was collected and only 6 isolates could be completely purified and

identified through characterization of their morphological attributes and molecular profile. Morphologically, according to Rippka *et al*. [26], the microscopic examination revealed that the isolates could be categorized into two groups. Isolates MN2, MN5 and MN6 of the first group were filamentous non heterocystous cyanobacteria (Fig. 2a), whereas the isolates MN1, MN3 and MN4 were filamentous heterocystous cyanobacteria (Fig. 2b).

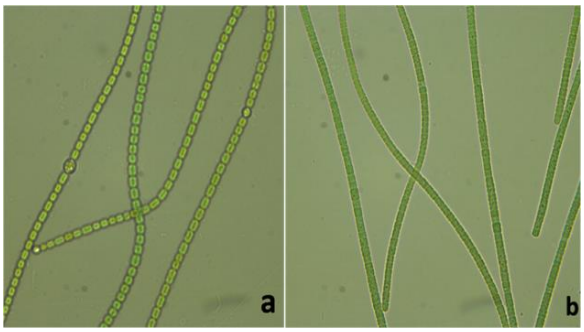

Fig. 2. Light micrograph (630x) of morphology of representative cyanobacteria isolated from El- Manzala Lake.

From each group, one isolate (MN2 from 1st group and MN1 from 2nd group) was selected randomly for the molecular identification using 16s rRNA sequencing. The results showed that MN1 isolate (584bp) had 99.51% nucleotide base homology with *Trichormus variabilis* MACC-57 (MH702203.1). The MN2 isolate (583bp) had 100% nucleotide base homology with *Oscillatoriales cyanobacterium* YACCYB534 (MH683781.1). The two sequences were submitted to the gene bank data base and the accession number assigned for MN1 and MN2 were MW024901 and MW024902, respectively. The isolates identified genetically were from site 1 in the lake.

3.3. Evaluating the effect of probiotic bacteria on cyanobacteria

The antagonistic activity of cell-free supernatant prepared from probiotic *Lb. sakei* and *Staph. hominis* was assessed against isolated *Trichormus variabilis* and *Oscillatoriales cyanobacterium*. After 4 days, only *Lb. sakei* supernatant displayed anticyanobacterial activity with inhibition zone of 13.6±1.25 and 10±1.44 mm against *Trichormus variabilis* and *Oscillatoriales cyanobacterium*, respectively.

Figures (3 and 4) illustrate the morphological changes occurred in both *Trichormus variabilis* and *Oscillatoriales cyanobacterium* in the presence of cell-free extract of *Lb. sakei*. For *Trichormus variabilis*, after 10 minutes, the changes appeared and represented by cells and heterocysts swelling, fragmentation and discoloring of filaments (Fig. 3.2). These changes were observed for another 20 minutes (Fig. 3.3 and 3.4). After 40 minutes, the shape of heterocysts changed from globular to oval, and lysis of some filament parts was obvious (Fig. 3.5). After 60 minutes, lysis of most filaments was clear (Fig. 3.6). Figure (4) shows the changes occurred in *Oscillatoriales cyanobacterium*. For 30 minutes, there were no obvious morphological alterations to be observed (Fig. 4.1 – 4.4). After 40 min., the filaments were partially segmented (Fig. 4.5). Weak swelling of the cells, and deformation of some filament ends were observed after 60 minutes (Fig. 4.6).

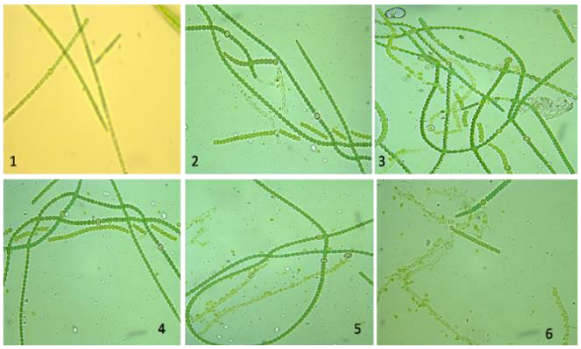

Fig. 3. Microscopic examination (630x) of *Trichormus variabilis* in the presence of *Lb. sakei* extract (1: after 0 min., 2: after 10 min., 3: after 20 min., 4: after 30 min., 5: after 40min. and 6: after 60 min.).

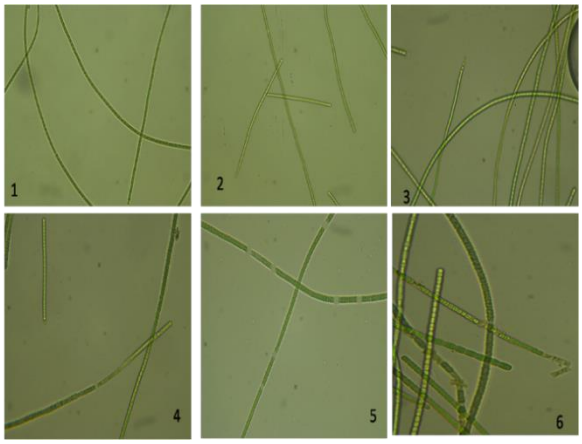

Fig. 4. Microscopic examination (630x) of *Oscillatoriales cyanobacterium* in the presence of *Lb. sakei* extract (1: after 0 min., 2: after 10 min., 3: after 20 min., 4: after 30 min., 5: after 40min. and 6: after 60 min.).

Tables (2 and 3) showed a strong decrease in some photosynthetic parameters in the co-culture between *Lb. sakei* and each of *Trichormus variabilis* and *Oscillatoriales cyanobacterium*, individually. Generally, there was no significant difference in the chlorophyll and phycocyanin production during the last 4 days.

For *Trichormus variabilis*, the production of chlorophyll and phycocyanin declined after 24hr of incubation with *Lb. sakei* with percentages of 17.4±1.37 and 11.62±12.49%, respectively. After five days of incubation, the remarkable reduction percentage of chlorophyll and phycocyanin contents were observed and reached 90.85±3.55% and 88.84±3.55%, respectively (Table, 2).

In *Oscillatoriales cyanobacterium* co-culture, the reduction percentage of chlorophyll and phycocyanin, after 24hr, were 20.81±2.4 and 20.49±3.58%, respectively. The reduction percentage increased drastically after five days of incubation and reached 100±0.0 and 91.38±2.99% for chlorophyll and phycocyanin, respectively (Table, 3).

Also, the effect of co-culture between *Lb. sakei* and selected cyanobacterial isolates on biomass production was evaluated through determination of cyanobacterial dry weight. The dry weight of *Trichormus variabilis* biomass decreased by 11.96±6.3 after 24hr. The extreme decreasing was recorded after 5 days as the reduction percentage reached 92.44±1.23%. Conversely, the biomass production of *Oscillatoriales cyanobacterium* was increased by 4.93±8.30% after 24hr. The biomass production decreased by 81.58±4.94% after 5 days.

4. Discussion

Cyanobacterial blooms are considered as a serious environmental hazard to the water ecosystem. These blooms produce toxins, which are harmful to fish, birds, wild animals, livestock, and potentially humans. Also, they cause water quality deterioration. Therefore, there are several strategies, as physical sedimentation, application of chemical and biological algaecides, were applied for controlling the blooms – forming cyanobacteria.

Some species of bacteria, viruses, protozoans, and fungi were reported as promising potential inhibitors of cyanobacterial blooms in freshwater and marine ecosystems [33, 9, 34,27]. Specifically**,** bacteria are considered as one of the crucial biological agents to control the phytoplankton blooms in water through inhibiting or degrading harmful algal growth [35, 36, 34, 37].

Table 2

Effect of co-culture between *Trichormus variabilis* and *Lactobacillus sakei* on production of chlorophyll and phycocyanin pigments, and cyanobacterial dry weight.

Time (Days)	Chlorophyll		Reduction %	Phycocyanin		Reduction %	Dry weight		Reduction %
	Control	Treated		Control	Treated		Control	Treated	
0	2.23 ± 0.06	2.30 ± 0.17	-	1.60 ± 0.26	1.60 ± 0.10	-	3.42 ± 0.37	3.42 ± 0.28	-
1	2.30 ± 0.56	1.90 ± 0.17	17.4±1.37	1.75 ± 0.35	1.40 ± 0.26	11.62±12.49	3.65 ± 0.57	3.00 ± 0.10	11.96±6.3
2	2.73 ± 0.15	1.42 ± 0.18 **	38.37±4.36	1.95 ± 0.13	0.90 ± 0.18 **	43.75±10.82	4.67 ± 0.21	2.80 ± 0.26 **	17.84±9.33
3	3.27 ± 0.15	0.94 ± 0.06 **	59.18±2.57	2.21 ± 0.10	0.65 ± 0.15 **	59.45±8.26	4.90 ± 0.66	1.87 ± 0.23 **	45.09±8.24
4	3.57 ± 0.21	0.62 ± 0.11 **	73.17±2.74	2.40 ± 0.10	0.40 ± 0.05 **	74.80±4.75	5.10 ± 0.56	0.99 ± 0.02 **	70.92±2.44
5	4.26 ± 0.36	0.21 ± 0.09 **	90.85±3.55	2.96 ± 0.24	0.18 ± 0.06 **	88.84±3.55	5.60 ± 0.54	0.26 ± 0.06 **	92.44±1.23

The results of co-culture were analyzed statistically using independent samples T-Test for each parameter in each day

Table 3

Effect of co-culture between *Oscillatoriales cyanobacterium* and *Lactobacillus sakei* on production of chlorophyll and phycocyanin pigments, and cyanobacterial dry weight.

Time (Days)	Cholorphyll		Reduction %	Phycocyanin		Reduction %	Dry weight		Reduction %
	Control	Treated		Control	Treated		Control	Treated	
0	3.41 ± 0.11	3.41 ± 0.09		2.85 ± 0.28	2.85 ± 0.28		2.32 ± 0.09	2.32 ± 0.09	
1	3.85 ± 0.49	2.70 ± 0.10 *	20.81±2.40	3.01 ± 0.01	2.26 ± 0.14 *	20.49±3.58	2.83 ± 0.08	2.48 ± 0.17 *	12.3
2	4.25 ± 0.30	1.88 ± 0.30 **	44.70±10.0	3.56 ± 0.05	1.93 ± 0.06 **	31.85±6.8	3.25 ± 0.13	2.36 ± 0.20 **	27.3
3	4.58 ± 0.14	1.31 ± 0.20 **	61.60±5.30	4.02 ± 0.07	1.32 ± 0.11 **	53.28±7.19	3.88 ± 0.11	2.00 ± 0.10 **	48.4
4	4.93 ± 0.11	0.74 ± 0.12 **	89.69±2.58	4.35 ± 0.28	0.55 ± 0.04 **	80.6±1.99	4.22 ± 0.12	1.91 ± 0.10 **	54.7
5	5.28 ± 0.13	0.35 ± 0.08 **	100±0.0	4.68 ± 0.20	0.24 ± 0.06 **	91.38±2.99	4.84 ± 0.12	0.42 ± 0.13 **	91.3

The results of co-culture were analyzed statistically using independent samples T-Test for each parameter in each day

The present study assessed whether isolated probiotic lactic acid bacteria (LAB); *Lb. sakei* and *Staph. hominis* could be used to effectively control isolated cyanobacteria represented by *Trichormus variabilis* and *Oscillatorials cyanobacterium*.

The results showed that, only *Lb. sakei* had the ability to inhibit the growth of both cyanobacterial isolates. *Trichormus variabilis* was more susceptible, than *Oscillatorials cyanobacterium*, to the anticyanobacterial activity of *Lb. sakei* as the largest inhibition zone of 13.6±1.25 mm was appeared against it. Also, Morphologically, *Trichormus variabilis* was more sensitive as the complete lysis of filaments was observed after 60 minutes. This action was not observed in *Oscillatorials cyanobacterium* culture. Shunyu *et al*. [38] found that *Bacillus cereus* has the ability for lysis of *Aphanizomenon flosaquae*. The morphological changes including discoloring, expansion and rupture were observed in *Anabaena* inoculated with *Lactobacillus paraplantarum* [4].

Evaluating the biomass production of cyanobacteria in the presence of *Lb. sakei* cells revealed that, the growth of *Oscillatorials cyanobacterium* was stimulated for 2 days comparing with *Trichormus variabilis*. In the co-culture, the growth of *Oscillatorials cyanobacterium* increased by 4.93±8.30% after 24hrs, whereas the growth of *Trichormus variabilis* decreased by 11.96±6.3%.

Generally, the modes of bacteria as anticyanobacterial agents could be summarized into direct attack and release of extracellular compounds with antibacterial activity [17, 39, 11]. Ren *et al.* [27], Kim *et al.* [40] and Kang *et al.* [4] (stated that proteins, heat resistant non proteinaceous compounds, peptides, amino acids, fatty acids, organic acids, hydrogen peroxide, diethyl ether, ethyl acetate, bacteriocins, antibiotics, biosurfactants, hydroxylamine and lipid peroxidants are responsible for cyanobacteriocidal activity in different probiotic bacteria.

The results of co-culture experiments confirmed that the probiotic *Lb. sakei* was effective in diminishing the photosynthetic activity through monitoring the phycocyanin and chlorophyll production for five days. After five days, the chlorophyll and phycocyanin produced by *Trichormus variabilis* decreased by 90.85 and 88.84%, in average. The highest decreasing in pigments production was observed with *Oscillatorials cyanobacterium* as the production reduced by 100 and 91.38% for chlorophyll and phycocyanin, respectively. Shun-Yu *et al*. [3] found that after 7 days incubation of *Bacillus cereus* with each of *Anabaena flosaquae* and *Oscillatoria tennis*, individually, chlorophyll production decreased by 77.3 and 77.2%, respectively. Also, they found a decrease in phycocyanin content in *Anabaena flosaquae* after 6 days of incubation by 72.4 %.

Chlorophyll and phycocyanin play an important role in photosynthesis process. Phycocyanin is a pigment-protein complex from the light-harvesting phycobiliprotein family. It is an accessory pigment to chlorophyll [41]. Chlorophyll not only has a chemical role in the photosynthesis process, but also it acts as a light absorber [42]. Also, in the

fluorescence resonance energy transfer, FRET, phycocyanin is an intermediate from phycoerythrin to chlorophyll [42]. Consequently, the impairment chlorophyll and phycocyanin contents in *Trichormus variabilis* and *Oscillatorials cyanobacterium* in the presence of *Lactobacillus sakei* cells will influence the photosynthetic activity of them.

Finally, it was found that the probiotic *Lactobacillus sakei* could be applied as a good biological agent to control the growth and activity of *Trichormus variabilis* and *Oscillatorials cyanobacterium*. Generally, LAB are considered as the best candidate to be used as biological control for cyanobacteria comparing with other bacteria characterized as cyanobacteriocidal agents as *Pseudomans*, *Bacillus*, *Cytophaga*, *Arthrobacter* and *Vibrio* in different studies [43, 44, 34, 4].

Conclusions

Here we introduce the use of probiotic lactic acid bacteria (LAB) as a biocontrol agent for elimination of cyanobacteria in surface water. We presented *Lactobacillus sakei* as probiotic bacteria which have the ability to preclude the growth of *Trichormus variabilis* and *Oscillatorials cyanobacterium* in the in-vitro experiments. Correspondingly, more studies are required to identify the anticyanobacterial substances produced by *Lb. sakei* against *Trichormus variabilis* and *Oscillatoriales cyanobacterium*. Also, the potential of *Lb. sakei* to regulate the abundance of cyanobacteria in water should be assessed.

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