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RESEARCH ARTICLE

Growth inhibition of *Microcystis aeruginosa* and adsorption of microcystin toxin by the yeast *Aureobasidium pullulans*, with no effect on microalgae

Zakaria A. Mohamed[1] · Saad Alamri[2,3] · Mohamed Hashem[2,4] · Yasser Mostafa[2]

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Abstract

This study evaluates the inhibitory effect of a yeast strain, *Aureobasidium pullulans* KKUY0701, isolated from decayed cyanobacterial bloom against harmful cyanobacterium *Microcystis aeruginosa* and determines the ability of this strain to remove microcystin (MC) toxin from the water. The antialgal activity of this yeast strain was assayed by co-cultivation with *M. aeruginosa*, diatom, and green algal species. The MC adsorption experiment was conducted in the presence of living and heat-inactivated yeast cells. Both yeast cells and filtrates caused a rapid reduction in the growth of *M. aeruginosa*, with complete death and cell lysis occurring after 3 days. The yeast strain did not exhibit any inhibitory effect on either green algae or diatoms. Both living and heat-inactivated yeast cells were capable of adsorption of MC on their surfaces. Inactivated yeast exhibited higher adsorption capacity and lower intensity than living yeast for the adsorption of MC toxin. The results of this study suggest that this yeast strain could be employed to selectively reduce cyanobacterial blooms in freshwaters. Moreover, the application of heat-inactivated yeast's biomass for toxin adsorption gives new possibilities in drinking water treatment plants.

Keywords Adsorption · Biological control · Lysis · Microcystis · Microcystin · Yeast

Introduction

Cyanobacterial blooms are a common phenomenon in freshwater environments worldwide. Due to progressive eutrophication of aquatic ecosystems and climate change, cyanobacterial blooms are expected to increase in the future (Paerl and Otten 2013). Cyanobacterial blooms are frequently constituted by toxin-producing species which exert negative impacts on aquatic food webs (Codd et al. 2005) and deteriorate the water quality for human use (Mohamed et al. 2015). Among blooming cyanobacteria, *Microcystis aeruginosa* is one of the most common and widespread species found in freshwater environments (Tanabe et al. 2018). In general, cyanobacterial blooms contain a diverse microbial community including viruses, bacteria, fungi, and some resistant zooplankters (Van Wichelen et al. 2016; Mohamed et al. 2018). Some of these microorganisms have a mutualistic relationship with *Microcystis*, while others display strong antagonistic effects (Li et al. 2011; Van Wichelen et al. 2016) that may be exploited in biocontrolling of the growth of harmful cyanobacteria in aquatic environments.

Numerous studies have been made on algicidal effects and feasibility of bacteria as control agents against harmful cyanobacteria (Ndlela et al. 2018; Weiss et al. 2019). Fungi were also found to exhibit algicidal activity against cyanobacteria including *M. aeruginosa*. These microorganisms attack cyanobacterial cells either directly via cell-to-cell contact (Jia et al. 2010a, 2010b) or indirectly via the release of algicidal compounds (Mohamed et al. 2014). Furthermore, some algicidal fungi such as *Trichaptum abietinum* and *Trichoderma citrinoviride* were able to degrade microcystin toxin in *M. aeruginosa* cultures (Jia et al. 2012; Mohamed

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✉ Zakaria A. Mohamed
mzakaria_99@yahoo.com

[1] Department of Botany and Microbiology, Faculty of Science, Sohag University, Sohag 82524, Egypt

[2] College of Science, Department of Biology, King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia

[3] Research Center for Advanced Materials Science (RCAMS), King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia

[4] Faculty of Science, Botany and Microbiology Department, Assiut University, Assiut 71516, Egypt

et al. 2014). However, little attention has been paid to algicidal activity of yeast on harmful cyanobacteria, though previous studies showed that strains of *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* possess antibacterial properties through the production of extracellular protease and/or secretion of inhibitory proteins (Chen et al. 2015). *Aureobasidium pullulans* is a yeast-like fungus, which can survive under different conditions including dry and wet environments, and a wide range of temperatures (DI Francesco et al. 2015). Some strains of *A. pullulans* have been found to produce lytic enzymes, such as chitinase and beta-1,3-glucanase, that inhibit the growth of pathogens (Vero et al. 2002). Isolates of *A. pullulans* have also exhibited antibacterial activity against Gram-negative and Gram-positive bacteria (Prasongsuk et al. 2018). Additionally, it has been reported that *A. pullulans* and its metabolites are safe for biotechnological and environmental applications (European Food Safety Authority 2011; Prasongsuk et al. 2018). *A. pullulans* has been used in agriculture as a suitable bioagent for the postharvest preservation of fruits and vegetables (Di Francesco et al. 2017). *A. pullulans* has also been reported to produce useful industrial enzymes, such as lipases, proteases, cellulases, amylases, mannanases, and xylanases, that have been applied in the textile, paper, detergent, food, and medical industries (Prasongsuk et al. 2018). The exopolysaccharide pullulan produced by *A. pullulans* has been used in medical applications for drug delivery (Gheorghe et al. 2008). Pullulan can also be used for coloring and food packaging, as it is able to form excellent films (Cheng et al. 2011).

Hence, this yeast could be a candidate for biocontrol of harmful organisms. This study aimed to determine the ability of *A. pullulans*, isolated from decayed cyanobacterial blooms, to inhibit the growth of the cyanobacterium *Microcystis aeruginosa* in order to control harmful cyanobacterial blooms in water sources. This study also investigates the ability of *A. pullulans* to detoxify and remove microcystin (MC) toxins produced by this cyanobacterium.

Materials and methods

Organisms

Aureobasidium pullulans strain KKUY0701 was isolated from decayed *Microcystis* bloom collected from a Saudi eutrophic lake (Wady Hali Dam Lake) on yeast extract-malt extract agar medium (YMA) containing 3 g L^{-1} yeast extract, 3 g L^{-1} malt extract, 5 g L^{-1} peptone, 10 g L^{-1} glucose at 25 °C, and pH 3.7 (Kurtzman and Fell 1998). The yeast was purified by repeated streaking on YMA medium. To obtain axenic culture, single yeast cells were transferred into 10-mL liquid YMA medium with 100 mg L^{-1} of chloramphenicol (Ravella et al. 2010). The axenicity of yeast cultures was confirmed by phase-contrast microscope (Carl Zeiss Axioskop 40, Germany) and inoculation on nutrient agar. The yeast strain was identified by sequencing of the D1/D2 domain of 26S rDNA region. Its DNA was extracted according the protocol described by Kurtzman and Robnett (1998) and primers NL1 (5′-GCATATCAA TAAGCGGAGGAAAAG-3′) and NL4 75 (5′-GGTC CGTGTTTCAAG ACGG-3′) were used to amplify the D1/D2 domain of the 26S rDNA region. The amplified fragments were purified and sequenced at Macrogen Company, Korea. The sequences were aligned with known 26S rDNA sequences in the GenBank database for the percentage homology. The phylogenetic tree was constructed using MEGA7 program to confirm the identity of the yeast strain. Cyanobacterial and microalgal strains used in this study were *M. aeruginosa* strain DRCK1, *Chlorella* sp. strain DRCK 6, and *Nitzschia* sp. strain DRCK18.

These strains were previously isolated using agar-plating method from Wady Hali Dam Lake by Al-Shehri (2010) and identified according to standard *morphological* features (John et al. 2003). Wady Hali Dam Lake is located in residential neighborhoods and surrounded by agricultural farms in Asir region, southwest Saudi Arabia (18° 76′ N and 41° 57′ E). Cyanobacterial and microalgal strains were grown axenically in BG11 medium at 25 ± 2 °C under continuous illumination at approximately 90 μmol photons m^{-2} s^{-1}. The purity of these strains was achieved by using a mixture antibiotics (rifampicin − 300 mg L^{-1}, streptomycin/penicillin − 25 mg L^{-1}) and antifungal nystatin (10 mg L^{-1}), which were incorporated into cultures for 2 days (Wilkens and Maas 2012). The axenicity of these cultures was assessed by phase-contrast microscope and inoculation of cells on nutrient agar. The cells of axenic cultures were then used for inoculation in the experiments of yeast algicidal activity. Exponentially growing cells of each strain were transferred separately into a 250-mL flask containing 100-mL BG-11 medium to give an initial cell density of 5×10^4 cells mL^{-1} for *M. aeruginosa*, 2×10^4 cells mL^{-1} for *Chlorella* sp., and 2×10^4 cells mL^{-1} for *Nitzschia* sp. *M. aeruginosa* strain used in our study has been proven to produce different microcystin variants including MC-RR, MC-YR, and MC-LR as determined by HPLC (Al-Shehri 2010).

Algae-yeast co-cultivation

A. pullulans cells were collected in sterile distilled water and co-cultivated at a final concentration of 1.2×10^6 cells mL^{-1} with each microalga or cyanobacterium in BG11 medium under the same conditions outlined above for 5 days. We did not add YMA medium in the experiment to test the ability of *A. pullulans* to lyse algal or cyanobacterial cells and use their constituents as a carbon source. Microalgal cultures without yeast were used as a control. Yeast cells cultivated in BG11 medium served as negative procedural control. The growth of *M. aeruginosa* and microalgae was estimated as a cell density

and determined daily by a hemocytometer under a microscope. At the same time, yeast cells were stained with methylene blue (0.1%) and counted by a hemocytometer using the same method used by Thomson et al. (2015). At the end of the experiment, yeast cells in completely lysed algal cultures were harvested by polycarbonate filters. The filtrate of this lysed culture was filtered again through 0.2-μm Millipore membrane filter and added at different dilutions (1, 10, 50%, V/V) to each 250-mL flask containing 100-mL BG-11 medium and algal inoculum at the same initial density mentioned above. Algal culture without fungal filtrate was used as a negative control. Both control and treated cultures were incubated under the same growth conditions outlined above. The cell density of cyanobacterial and microalgal cultures was monitored daily by a hemocytometer. All the experiments were conducted in triplicate.

Microcystin removal

To investigate the capability of the yeast *A. pullulans* for removing MC released in fungal-cyanobacterial cultures, an aliquot was taken daily from yeast-treated and filtrate-treated *Microcystis* cultures. *Microcystis* cultures without yeast were used as control. The samples were centrifuged at 6000×*g* for 15 min, and MC concentration in the supernatant was determined by enzyme-linked immunosorbent assay (ELISA) according to the method of Carmichael and An (1999) using commercial kits purchased from Abraxis (54 Steamwhistle Drive Warminster, PA 18974).

Microcystin adsorption by yeast

To investigate whether the disappearance of MC in the medium of yeast-*Microcystis* cultures over the incubation period is due to biodegradation by viable yeast or its potential adsorption on the fungal cell wall, the adsorption experiment was conducted using heat-inactivated yeast. Yeast cells were grown in several conical flasks containing 100-mL YMA medium for 24 h at 37 °C. After incubation, cells were collected by centrifugation (6000×*g*, 10 min) and washed with 0.9% NaCl. The yeast pellet concentration resulting from each flask was 2.3×10^7 cells mL^{-1} as determined by hemocytometer. The yeast pellets (viable cells) of some flasks were suspended in 0.9% NaCl and inoculated in 100-mL conical flask containing 50-mL YMA medium, pH 7.2. Yeasts pellets of other flasks were inactivated by heating at 80 °C for 12 h in an oven. The inactivity of yeast was confirmed by attempting to culture on YMA agar, with no yeast colonies growing on the plates. This procedure allowed to exclude the involvement of a potential metabolic conversion of toxin by viable cells (Petruzzi et al. 2015). The weight of each yeast pellet used in adsorption experiments was about 75 mg dry weight. All conical flasks either inoculated with viable or inactivated yeast cells were dosed with crude toxin extract containing MC at different concentrations (0.5, 1, 2, 4, 6, 8, and 10 μg L^{-1}). The flasks were placed in a thermostatically controlled shaker (200 rpm) at 30 °C for 1 h. Thereafter, the contents of flasks were centrifuged at 6000×*g* for 10 min at 30 °C. The supernatants were collected and used for the analysis of microcystin remaining in the solutions (i.e., not bound or degraded) by ELISA. YMA medium containing only microcystin but not-containing viable or inactivated yeast cells was used as control. All experiments were performed in triplicate. The MC adsorption efficiency (*R*%) by yeast was calculated using the following equation:

$$R\% = \frac{(C_0 - C_f)}{C_0} \times 100$$

where C_0 and C_f are the initial and final concentrations of microcystin (μg L^{-1}), respectively.

The Freundlich isotherm equation was used to fit the adsorption isotherm data.

$$q_e = KC_e{}^{1\backslash n}$$

where q_e = adsorption concentration in solid sorbents (mg g^{-1}), C_e = equilibrium concentration in bulk solution (μg L^{-1}), and K and n = empirical constants of Freundlich equation related to the capacity of the adsorbent (inactivated or viable yeast) for microcystin and the affinity of the adsorbent, respectively. All the adsorption data were plotted on logarithmic scale, and the regression isotherm coefficients were calculated.

Bound microcystin release assay

This assay was performed to evaluate the firmness of MC adsorption by living and inactivated yeast cells (i.e., is the binding of yeast-MC reversible or irreversible?). The potential release of MC adsorbed on the yeast surface was assessed according to the method of Petruzzi et al. (2015) with some modifications. Briefly, pellets of living and inactivated yeast with adsorbed MC collected from adsorption experiment were suspended in BG-11 medium (pH 7.2), mixed for 1 min, and incubated at room temperature (25 °C) for 1 h with shaking. Subsequently, the yeast cells were centrifuged (6000×*g*, 10 min) and the supernatant was collected to determine the amount of MC potentially released back into the washing solution. MC in the washing solution was determined by ELISA as described in the "Microcystin removal" section.

Statistical analysis

Data were tested to ensure they met the assumptions of ANOVA (independence, homogeneity of variance, and normality). All data used for the present study met the

assumptions. Differences in the cell density and MC concentrations between control and treated cultures were evaluated by one-way repeated-measures analysis of variance (ANOVA) followed by Tukey's post hoc test ($P < 0.05$).

Results

A yeast strain isolated from decayed *Microcystis* bloom was found to inhibit the growth of *M. aeruginosa*. The strain was designated as KKUY0701and identified using the molecular technique to determine its correct phylogenetic position. The genomic DNA was extracted from the isolated yeast strains, and primers NL1 and NL4 were used for the amplification and sequencing. The sequence data obtained from the amplification of the D1/D2 region of the 26S rRNA gene was compared with the sequences of 26S rDNA regions in the GenBank by means of BLAST search of the National Center for Biotechnology Information (NCBI) databases. Alignment of the 26S rRNA gene sequences of the yeast isolate with sequences obtained by doing a BLAST search revealed up to 99% similarity with *Aureobasidium pullulans*, and it was given GenBank accession number as MK141707. The phylogenetic tree indicated that strain KKUY0701 shared one clade cluster with *Aureobasidium pullulans*; therefore, it was identified as *Aureobasidium pullulans* KKUY0701 (Fig. 1).

The yeast strain KKUY0701 showed algicidal activity towards the cyanobacterium *M. aeruginosa* rather than green algae and diatom species. During the first day of co-cultivation with living yeast, the cell density of *M. aeruginosa* exhibited sharp reduction (84%) compared with control ($F = 27.4$, df = 1, $P = 0.0004$), and their cells were almost completely lysed after 3 days (Fig. 2A). The yeast filtrate also showed an inhibitory effect on *M. aeruginosa*, and this inhibition increased with the increase in the proportion of filtrate in the cultures ($F = 18.3$, df = 2, $P = 0.0002$) (Fig. 2B). The 50% extract proportion caused complete growth inhibition of *M. aeruginosa* within the first day of incubation, while the lowest extract proportions (1, 10%) reduced the growth but without complete lysis of the cells (Fig. 2B). Moreover, the growth inhibition of *Microcystis* cells by yeast filtrate varied with the incubation time. The longer the incubation time is, the stronger the growth inhibition. On the other hand, neither living yeast cells nor their culture filtrate exhibited a remarkable antialgal activity towards the green alga *Chlorella vulgaris* ($F = 0.01$, df = 3, $P = 0.9$) and the diatom *Nitzschia* sp. ($F = 0.02$, df = 3, $P = 0.8$) compared with control cultures (Figs. 3 and 4).

After co-cultivation for 5 days with algal cultures, the cell number of yeast co-incubated with *Microcystis* culture increased significantly ($F = 1497$, df = 1, $P < 0.001$) compared with its initial concentration (Fig. 5). Conversely, the cell number of yeast did not significantly change when co-incubated with *Chlorella* ($F = 4.9$, df = 1, $P = 0.1$) and *Nitzschia* compared with their initial cell numbers ($F = 2.5$, df = 1, $P = 0.2$) (Fig. 5).

The results of toxin analysis in the medium of treated and control cultures of *Microcystis* revealed an increase in MC concentrations released into the medium of cultures treated with either living yeast or its filtrate compared with control cultures ($F = 4.9$, df = 2, $P = 0.03$) (Fig. 6). The highest MC concentrations released into in the medium of *M. aeruginosa* cultures treated with living yeast or yeast filtrate were obtained after 3 days incubation, coinciding with the complete lysis of *Microcystis* cells (Figs. 2 and 6). After that, the released microcystins decreased sharply in the medium of *M. aeruginosa* cultures treated with living yeast and became undetectable at day 6 ($F = 518$, df = 5, $P < 0.001$). Conversely, MC quantities released after 3 days into the medium of *M. aeruginosa* cultures treated with yeast filtrate showed non-significant variation along the incubation period ($F = 1.8$, df = 5, $P < 0.2$) (Fig. 6).

The results of adsorption isotherm experiments showed that both living yeast and heat-inactivated yeast were able to bind MC toxins. The adsorption efficiencies of MC by inactivated yeast (31–56%) differed significantly ($F = 39$, df = 1, $P = 0.003$) from those of living yeast (19–43%) (Fig. 7). Furthermore, the adsorption percentages of MC by either living yeast or inactivated yeast differed ($F = 268.4$, df = 6, $P < 0.0001$) with the initial concentrations of MC used in the experiment (Fig. 7). The highest adsorption percentages of MC by inactivated (56%) and living yeast (43%) were obtained at initial MC concentration of 2 μg L^{-1} and then adsorption percentages decreased with increasing toxin concentration (Fig. 7).

Additionally, sorption isotherms of MC onto the living and inactivated yeast were well fitted to the Freundlich model, and the fitting parameters are listed in Table 1. Inactivated yeast exhibited higher K values (i.e., high adsorption capacity) and lower $1/n$ values (i.e., high adsorption intensity) than living yeast for the adsorption of MC toxin. The results of the toxin release assay revealed that the firmness of MC adsorption by yeast differed between living and inactivated cells ($F = 9361.5$, df = 1, $P < 0.06$). The binding of MC onto living yeast was reversible with about 80% of the initially binding MC releasing back into the washing solution (Table 2). Conversely, the complex of inactivated yeast-MC was much firmer, with no detectable levels of initially binding MC releasing back into the washing solution (Table 2).

Discussion

The present study clearly demonstrates the ability of the yeast *A. pullulans*, isolated from decayed *Microcystis* bloom, to inhibit the growth of the cyanobacterium *M. aeruginosa* and

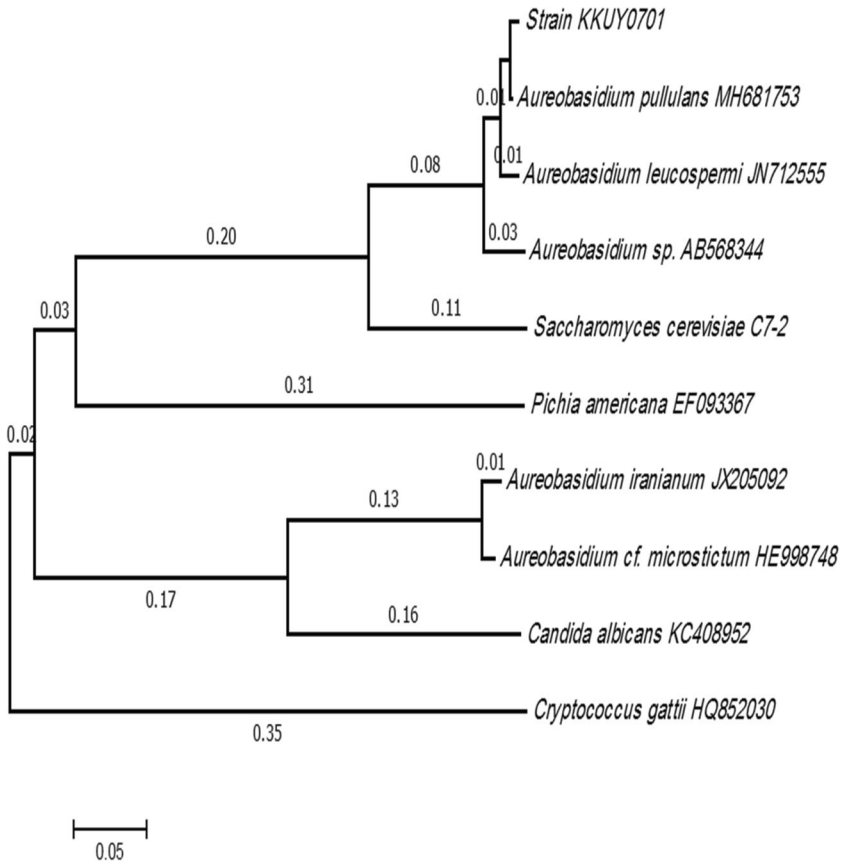

Fig. 1 Phylogenetic tree of the evolutionary relationships of yeast strain KKUY0701with different species using the neighbor-joining method. The optimal tree with the sum of branch length is 1.64922120. The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Branch length shorter than 0.01 was omitted

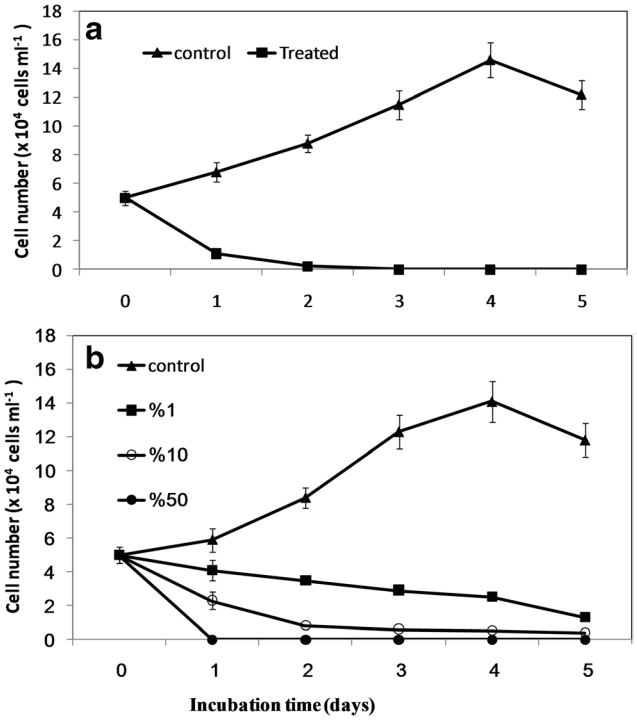

Fig. 2 Changes in the growth of *Microcystis aeruginosa* co-cultivated with living cells (**A**) and filtrate (**B**) of *A. pullulans* at different amounts (1%, 10%, 50%, v/v)

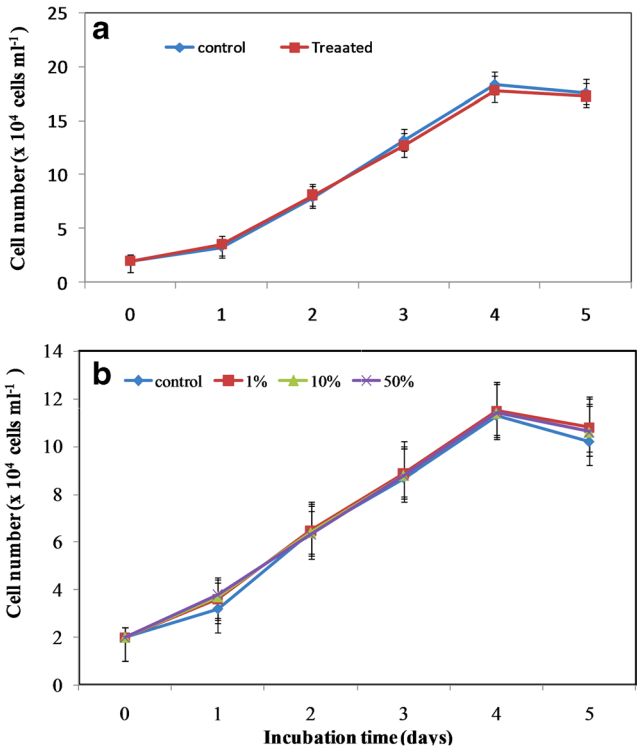

Fig. 3 Changes in the growth of *Chlorella* sp. co-cultivated with living cells (**A**) and filtrate (**B**) of *A. pullulans* at different amounts (1%, 10%, 50%, v/v)

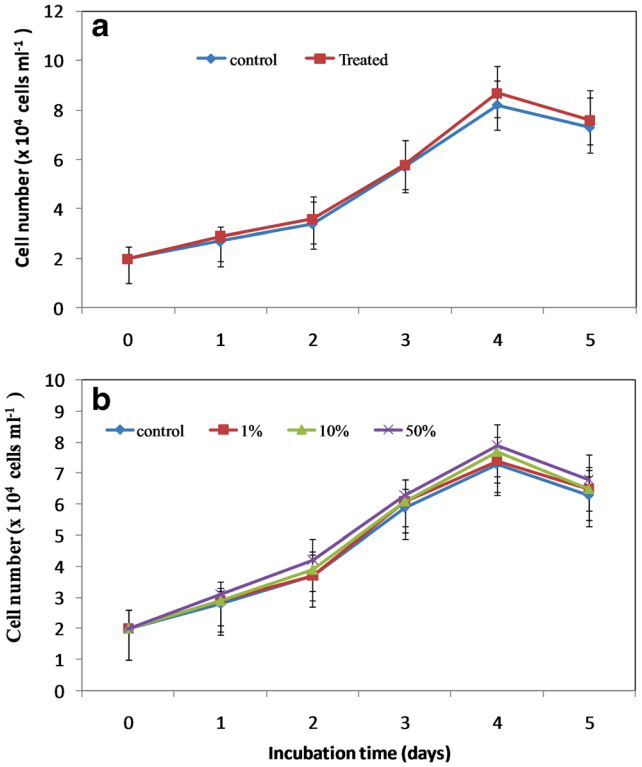

Fig. 4 Changes in the growth of the diatom *Nitzschia* sp. co-cultivated with living cells (**A**) and filtrate (**B**) of *A. pullulans* at different amounts (1%, 10%, 50%, v/v)

adsorb MC toxins released into the medium. Our experiments showed that inoculation of *M. aeruginosa* cultures with yeast cells led to the reduction of *M. aeruginosa* cell density, with complete cell lysis within 72 h. The removal efficiency of *Microcystis* cells by our yeast strain can be compared with that of other algicidal fungi such as *Trichoderma abietinum* 1302BG, *Lopharia spadicea*, and *Trichoderma citrinoviride* kkuf-0955 that were capable for eliminating all *Microcystis* cells within 48 h (Jia et al. 2010b; Mohamed et al. 2014). This

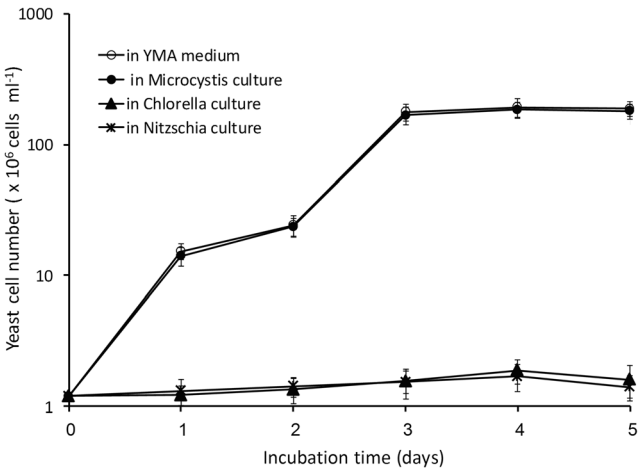

Fig. 5 Cell number of *A. pullulans* ($\times 10^6$ cells mL^{-1}) co-cultivated with living cells of *Microcystis aeruginosa*, *Chlorella* sp., and *Nitzschia* sp.

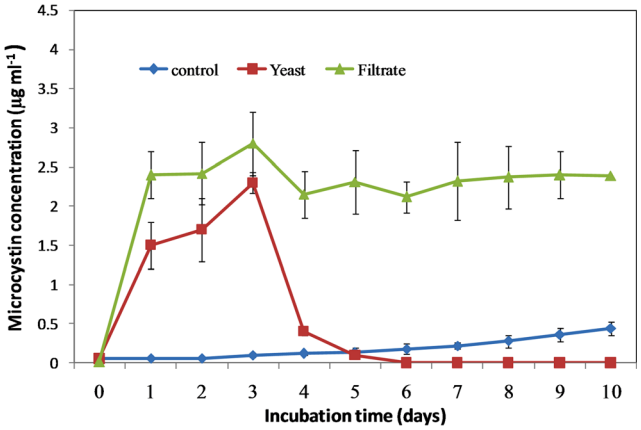

Fig. 6 Changes in concentrations of microcystins released into the medium of *M. aeruginosa* cultures during incubation with living cells and filtrate of *A. pullulans*

is the first study reporting the algicidal and lytic activity of yeast against cyanobacteria. Compared with bacteria, our yeast strain is similar to the strongest algicidal bacteria, which removed and lysed 100% of algal cells within 72 h (Lee et al. 2018). The inhibitory effects of algicidal bacteria and fungi against *M. aeruginosa* may occur through direct cell contact (Jia et al. 2010b) or were mediated by extracellular substances (Mu et al. 2007; Mohamed et al. 2014). In our experiments, the filtrate of *A. pullulans* inhibited the growth of *M. aeruginosa*, possibly involving active compounds secreted by the yeast. Consistent with our study, Mohamed et al. (2014) reported that *T. citrinoviride* degrades *Microcystis* cells through the excretion of algicidal compounds into the medium rather than direct attack. In our study, the biomass of *A. pullulans* yeast in *Microcystis* cultures increased after decaying all cells after 3 days of co-cultivation. The growth of *A. pullulans* in cyanobacterial cultures not-containing carbon source indicates that this yeast is able to lyse *Microcystis* cells, decompose, and utilize their constituents for its growth. Previously, it has been found that the yeast *Saccharomyces cerevisiae* can use cyanobacterial bloom material as

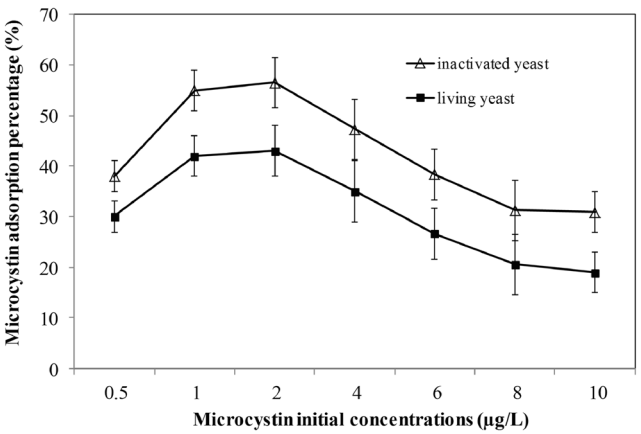

Fig. 7 Adsorption capacity (9%) of microcystin by living and heat-inactivated cells of *A. pullulans*

Table 1 Fitting results of isotherms (K and n = empirical constants of Freundlich equation) for microcystin adsorption by living and inactivated cells of *A. pullulans*

	K	$1/n$	R^2
Inactivated yeast	2.9	0.56	0.967
Living yeast	2.5	0.45	0.846

carbohydrate and nutrient source (Möllers et al. 2014). Furthermore, our results indicated that the algicidal activity of *A. pullulans* was highly specific towards *Microcystis* with no significant effect on the growth of other eukaryotic green algae and diatoms. The selectivity in the antagonistic activity of fungi towards cyanobacterial species has been previously reported for *T. citrinoviride* (Mohamed et al. 2014). The specific activity of *A. pullulans* against cyanobacteria may be due to its production for lytic enzymes such as *N*-β-acetylglucosaminidase (Di Francesco et al. 2015) which are able to digest bacterial peptidoglycan, the main constituent of cyanobacterial cell wall (Salazar and Asenjo 2007), without any effect on cellulosic or silicified cell wall of eukaryotic algae. Thus, our results indicate that the yeast lytic activity against phytoplankton is a species-specific. This agrees with the suggestion of Meyer et al. (2017) stating that lytic bacteria target specific species within phytoplankton community, and the species that exhibit resistance to lytic bacteria might have a structural and/or physiological trait preventing this lysis. The resistance of the green alga *C. vulgaris* to yeast lytic activity revealed in our study was probably due to the presence of lysis resistant substances in the cell wall of this organism. Previous studies demonstrated that most of the microalgae belonging to Chlorococcales (e.g., *C. vulgaris*) are resistant to several lytic enzymes, lysozyme, and chemical agents, due to the presence of glycoproteins, biopolymers containing glucosamine, and acetolysis-resistant biopolymers in their cell walls (Rashidi and Trindade 2018). More specifically, the existence of an acetolysis-resistant biopolymer, called algaenan in the cell wall of some green algae, was found to contribute to the high resistance of cell walls to enzymatic lysis (Burczyk et al. 2014). Additionally, the presence of sporopollenin in the cell wall of some species of *Chlorella* has also been associated with resistance to enzymatic lysis (He et al. 2016). Diatoms have also been found to produce oxylipins and proteases to resist and counteract microbial lytic enzymes (Paul and Pohnert 2013; Meyer et al. 2018). However, further research is needed to isolate and characterize compounds responsible for such inhibitory and lytic activities of *A. pullulans*. Biological control measures based on antagonistic yeast would be also evaluated in the natural environment, to ensure if it is appropriate and ecologically feasible for application owing to the high selectivity for harmful algae.

Table 2 Potential release of microcystin adsorbed by living and inactivated yeast upon washing with BG-11 medium

Adsorbent	Bound MC (μg g^{-1})	Released MC (μg g^{-1})
Living yeast	2.54	2.03 ± 0.6 (79.8%)
Inactivated yeast	4.61	ND*

*ND = non-detectable at ELISA limit

Besides growth inhibition and cell lysis of *Microcystis*, *A. pullulans* was able to eliminate MC toxin released into the medium after the decay of *Microcystis* cells. The decrease in released MC concentrations occurred in cultures containing yeast cells rather than those containing yeast filtrate, indicating that these toxins could be removed from the medium either via biodegradation by living yeast cells or adsorption onto yeast cell wall. Our adsorption experiments showed that both living and heat-inactivated yeast cells caused a reduction in MC concentrations in the solution. The decrease in MC concentrations by inactivated yeast was insignificantly higher than by living yeast, suggesting that the cell and enzymatic activities nearly had no effect on the reduction of MC concentrations. This indicates that the decrease in toxin concentration was due to its adsorption on yeast cell wall rather than metabolism or enzymatic reactions (i.e., biodegradation). These results are consistent with other studies on the adsorption of mycotoxins by yeast strains (Luo et al. 2015). The Freundlich isotherm modeling parameters, K (related to adsorption capacity) and $1/n$ (related to the affinity of the adsorbent), are highly correlated, suggesting that MC adsorption on yeast cells is favorable. The values of Freundlich isotherm parameters ($K = 2.9$, and $1/n = 0.56$), obtained in our study, can also be compared with those (1.73, 0.48, respectively) recorded in the literature for MC adsorption by activated carbon (Albuquerque et al. 2008). We also observed that the adsorption capacity increases rapidly with the increase of initial MC concentrations, up to some value (2 μg mL^{-1}) and then slowed at high initial concentrations (4–10 μg mL^{-1}), indicating that binding of MC onto yeast cells is a process reaching saturation. The maximum adsorption capacity of MC by inactivated yeast (56.5%) was obtained at an initial concentration of 2 μg mL^{-1}, which corresponds to 15.1 μg toxin g^{-1}yeast. Given that the maximum environmentally relevant concentration of MC during most cyanobacterial blooms in lakes is 10 μg L^{-1} (Jiang et al. 2011), and the World Health Organization recommended a maximum allowable level of 1 μg L^{-1} MC in drinking water, it requires removing 90% of MC present in lake water which can be achieved by using 0.6 g of our inactivated yeast to clean 1 L of water. Additionally, the binding of MC to inactivated yeast was very stable, but its binding to living yeast cells was not stable, as 80% of adsorbed MC released under the influence of washing with BG-11 medium. This is in accordance with previous studies reporting that inactivated yeast does not lose their mycotoxin adsorption capability (Luo et al. 2015; Baptista et al. 2004). To our knowledge, our study is the first attempt to show the ability of

yeast *A. pullulans* to adsorb MC toxins. However, several studies demonstrated the ability of *S. cerevisiae* yeast to adsorb different mycotoxins such as aflatoxins, ochratoxins, zearalenone, and patulin (Armando et al. 2012; Bovo et al. 2015; Luo et al. 2015). It is important to note that the adsorption capacity of yeast for mycotoxins has been attributed to the presence of β-D-glucans located in the yeast cell wall (Bovo et al. 2015).

In conclusion, this study provided promising results of using the yeast *A. pullulans* or its filtrate to selectively control the growth of *M. aeruginosa* in water sources without any remarkable effect on beneficial algae. Being non-toxic, pathogenic, or infective, the inactivated yeast cells of *A. pullulans* could be used to remove cyanotoxins in drinking water treatment plants. However, there is an urgent need for in situ studies to make the availability of inactivated yeast as a potential biosorbent at a commercial level. Additionally, different kinds of yeast should be evaluated for the ability to adsorb and eliminate other cyanobacterial toxins, e.g., anatoxins, cylindrospermopsin, and saxitoxins from water.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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