Environmental Science and Pollution Research (2020) 27:44427–44439
https://doi.org/10.1007/s11356-020-09901-y

RESEARCH ARTICLE

Effect of temperature on microcystin-LR removal and lysis activity on *Microcystis aeruginosa* (cyanobacteria) by an indigenous bacterium belonging to the genus *Achromobacter*

Melina Crettaz-Minaglia[1,2] · Maximiliano Fallico[1] · Oswaldo Aranda[1] · Ivan Juarez[2,3] · Magdalena Pezzoni[4] · Cristina Costa[4] · Dario Andrinolo[1,2] · Leda Giannuzzi[2,3]

Received: 9 July 2019 / Accepted: 26 June 2020 / Published online: 7 August 2020

Abstract

Microcystis is a frequent cyanobacterium bloom-forming with cosmopolitan distribution which can produce a hepatotoxin group called microcystins (MCs). These MCs are resistant to the traditional processes employed in the water treatment plants and they are often detected after conventional treatments. Because of this, the bio-removal studies have obtained a great interest in the last decades. In this work, a bacterial strain namely LG1 with the ability to remove microcystin-LR (MC-LR) under laboratory conditions was isolated from Rio de la Plata River and it was identified as *Achromobacter* spp. This ubiquitous bacterium was able to remove 79.5% MC-LR in 7 days with average removal time of 3.33 ± 0.08, 3.06 ± 0.05, and 2.77 ± 0.05 days at 28, 32, and 36 ± 1 °C, being higher at high temperature (36 °C) with an activation energy = 16.79 ± 1.99 kJ mol^{-1}. LG1 grew better at higher temperature (from 28 to 36 ± 1 °C) increasing the specific growth rate (μ) and reducing 2-fold the lag phase duration (LPD) without significant differences ($p > 0.05$) between maximum population density (MPD). In addition, LG1 showed a lysis activity on two *M. aeruginosa* native strains in 7 days measured as chlorophyll *a* (Chl-*a*) concentration. The lysis activity increased around 2-fold when increasing the temperature from 28 to 36 ± 1 °C. This is the first report of an indigenous bacterium belonging to the genus *Achromobacter* spp. isolated from the Rio de la Plata River with the capacity to remove MC-LR and lysis activity on *M. aeruginosa*.

Keywords *Achromobacter* spp. · Biological removal · *Microcystis* lysis · Rio de la Plata River

Highlights
- This work provides experimental data and mathematical modeling of the MC-LR removal and *Microcystis aeruginosa* lysis activity on two temperate strains by novel *Achromobacter* spp. isolated from Rio de La Plata River (Argentina).
- It provides data about bacterial growth parameters using Gompertz model. It also provides evidence of the possible environmental biotransformation of this cyanotoxin and bloom senescence.
- Its use as ex situ treatment must be deepened and scaled according to the best conditions assayed in the present work.

Responsible editor: Vitor Manuel Oliveira Vasconcelos

✉ Melina Crettaz-Minaglia
mcrettaz@exactas.unlp.edu.ar

[1] Laboratorio de Toxicología General, Facultad de Ciencias Exactas, UNLP, La Plata, Argentina

[2] CONICET, Buenos Aires, Argentina

[3] Centro de Investigaciones y Desarrollo en Criotecnología de Alimentos, UNLP-CONICET, La Plata, Argentina

[4] Departamento de Radiobiología, Comisión Nacional de Energía Atómica, Buenos Aires, Argentina

Introduction

Cyanobacterial blooms are a global problem for freshwater ecosystems including drinking water and recreational uses (Paerl et al. 2001). The genus *Microcystis* is one of the most important cyanobacteria due to its great ecological importance and public health implications (Sanchis et al. 2004). Its blooms affect fish (Gaete et al. 1994), zooplankton communities (Amé et al. 2010), aquatic plants, and vertebrates (MacKintosh et al. 1990). Moreover, *Microcystis* can produce microcystins (MCs) which are toxic cyclic heptapeptides and they contain an unusual β-amino acid (3-amino-9-methoxy-10-phenyl-2,3,8-trimethyl-deca-4,6-dienoic acid) (-Adda). There are around 250 MCs variants (Foss et al. 2018) being MC-LR the most commonly found (Carmichael 1992). These potent mammalian toxins are known to be responsible for acute and chronic effects which cause liver hemorrhage within few hours and show tumor promotion activity through protein phosphatase inhibition observed in rodents as well as in primary hepatocytes in vitro. The resulting hyperphosphorylation of intracellular protein

leads to the disruption of intermediate filaments that form the cellular scaffold in human and rodent hepatocytes (Carmichael 1992). The International Agency for Research on Cancer (IARC) classifies MC-LR as possibly carcinogenic to humans (group 2B), based on the inadequate evidence that it directly causes cancer in either laboratory animals or humans. Moreover, several authors have reported data that suggest the MCs may be responsible for the high incidence of liver cancer in populations dependent upon MC-contaminated drinking water in China, Serbia, and Florida of the USA (Crettaz-Minaglia et al. 2015). As a result of the reported effects of MCs, the World Health Organization (WHO) proposed a guideline of 1 μg L^{-1} as the maximum concentration in drinking water (WHO 1998).

The contamination of water resources with MC-LR represents a major concern for the drinking water industry because thousands of consumers may be affected (Eleuterio and Batista 2010). Microcystins are resistant to traditional processes employed in the water treatment plants (Lahiti and Hiisvirta 1989) and they are often detected after conventional physicochemical treatments. From December 2004 to April 2005, Echenique et al. (2006) informed MCs in drinking water supply in 10 of 13 samples in the Ensenada and La Plata cities (Argentina) in levels above than guide values (1–7 μg L^{-1}). This showed that the conventional water treatment process was inefficient at least during the critical periods of massive development of cyanobacterial blooms.

Because of this, the microbial removal of contaminants as a cost-effective and efficient way has gained increasing research attention (Kong et al. 2013). Several investigations have been carried out about indigenous bacteria capable of removing MC-LR isolated from water bodies (water and sediment) and filters from water treatment plants (Crettaz-Minaglia et al. 2015). A large group of bacteria has been isolated being Sphingomonadaceae the most studied family of bacteria. Most of these organisms have been identified as *Sphingomonas* (Bourne et al. 1996, 2001; Park et al. 2001; Saito et al. 2003; Saitou et al. 2003; Harada et al. 2004; Ishii et al. 2004; Ho et al. 2006; Amé et al. 2006) and *Sphingopyxis* (Okano et al. 2010; Zhang et al. 2010; Shimizu et al. 2011; Yan et al. 2012a, b). Other studies have focused their efforts on the studies of the lysis activity by bacteria on *M. aeruginosa* strains (Yamamoto et al. 1993; Manage et al. 2000; Zhang et al. 2011; Sun et al. 2015).

Additionally, some authors have studied the factors that influence the removal of MCs in the water such as pH (Chen et al. 2010), initial toxin concentration (Ho et al. 2007), and temperature (Park et al. 2001). Due to the temperature is one of the determining factors for MCs removal (Alamri 2012; Kansole and Lin 2016; Park et al. 2001; Yang et al. 2014; Zhang et al. 2017), for the growth of bacteria, and the expression of certain enzymes, it is possible that an expected temperature increase under future climate scenarios could modify the activity of cell lysis and removal of MCs. Although numerous bacteria have been isolated with the potential to remove MCs and lysis activity; there is little literature with mathematical analysis and comparison of these bacteria.

The aim of this work was to isolate indigenous microorganisms from the Rio de La Plata River that have lytic activity on two temperate and native strains of *M. aeruginosa* and removal of MC-LR activity. In addition, the removal of MC-LR, the lysis capacity, and the growth of the microorganism with greater capacity at three temperatures were modeled.

Methods

Microcystin-LR producing by *M. aeruginosa*

Microcystin-LR produced by a strain of *M. aeruginosa* called CAAT 2005-3 was used as a source of MC-LR. This strain was described by Rosso et al. (2014) and the principal MC that can produce is the [D-Leu^1] MC-LR (Qi et al. 2015). This cyanotoxin has been reported in Brazil (Matthiensen et al. 2000), Canada (Park et al. 2001a), Argentina (Rosso et al. 2014), and more recently, in the USA (Foss et al. 2018) and it can be produced by not only cyanobacterial genus *Microcystis* but also *Phormidium*, and *Nostoc* (Shishido et al. 2013).

M. aeruginosa strain preservation was performed by periodic (every 15 days) passage through modified BG11 culture medium (N:P ratio 10) (Rippka et al. 1979) under controlled laboratory conditions at 28 ± 1 °C, constant aeration with humidified sterile air, and a fluorescent light intensity of 30 μmol photons m^{-2} s^{-1} with a light-dark cycle of 10:14 h (Crettaz-Minaglia et al. 2017).

Microcystin-LR extraction and quantification

The culture in exponential phase was submitted to ultrasonication for 30 min (Omni Ruptor 400) and then centrifuged for 15 min at 5000 rpm to eliminate cell debris. The supernatant was passed through conditioned (10 mL 100% methanol, 50 mL 100% distilled water) Sep-Pak C18 cartridges (Waters) and the MCs were eluted with 80% methanol. Quantitative chromatographic analysis of MCs was performed by simple quadrupole HPLC/MS Shimadzu LCMS-2020 determining a principal component of [D-Leu^1] MC-LR toxins (*m*/*z* 520) (Qi et al. 2015) using C18 column (Hyperprep HS, 5-μm pore, 250 mm 10 mm) according to Crettaz-Minaglia et al. (2017). The column was equilibrated with a mixture composed of 65% A solution (Milli-Q water + 12.7 mM formic acid) and 35% B solution (acetonitrile + 12.7 mM formic acid). The mobile phase consisted of a discontinuous gradient of A and B solutions which consisted of 30% B solution, then 70% linear gradient of the B solution for

12 min, isocratic elution with 70% of the B solution for 3 min, and finally, a linear gradient of 30% B solution during 5 min (total run time 20 min).

The flow rate was 1.0 mL min^{-1} with a flow division before the entrance to ESI (0.2 mL min^{-1} to the ESI-MS). A standard of MC-LR was purchased from Sigma (St. Louis, MO, USA), and a calibration curve was performed (0.1–1.0 μg L^{-1}). The limit of detection (LOD) and the quantification (LOQ) were 0.05 μg L^{-1} and 0.10 μg L^{-1}, respectively.

Microorganism acclimation and isolation

A water sample from the Rio La Plata River (Ensenada, Buenos Aires) (34° 49′ 58.7″ S– 57° 56′ 54.1″ W) was used as microorganism's source. This sample was obtained during a *Microcystis* bloom registered on 10th January 2014 using a phytoplankton net (pore = 25 μm). In this zone, the cyanobacterial blooms have been observed frequently (Andrinolo et al. 2007; Giannuzzi et al. 2012).

Acclimation and isolation were carried out according to Amé et al. (2006). The initial acclimation of the microorganisms at 1.10^6 CFU mL^{-1} concentration was made in a 250-mL sterile Erlenmeyer flask containing the environmental water sample (20 mL) and sterile mineral salts medium (MSM) adjusted to pH 7.0 supplemented with 200 μg L^{-1} of MC-LR (80 mL) that was added as the sole carbon and nitrogen source. Control was made in the same condition without an environmental water sample. Both were incubated in a dark stove at 32 ± 1 °C, and MC-LR was periodically quantified according to the technique as described above. Acclimated microorganisms were obtained from 5 serial subcultures by transferring 20 mL of the starting culture to a sterile Erlenmeyer flask containing MSM and MC-LR (80 mL) in 70 days. To purify the culture of MC-removal microorganisms obtained from the last subculture, 100 μL of the acclimated sample were transferred to enriched culture and to spread on agarized MSM supplemented MC-LR (200 μg L^{-1}) and incubated in the dark stove at 32 ± 1 °C during 72 h. Then, different types of colonies were isolated in a nutritive agar the same conditions described above according to size, color, and morphology.

Microcystin-LR removal assay

Microorganism inoculum preparation

Every isolated microorganism was grown in a nutritive broth during 12 h (overnight) in the dark stove at 37 ± 1 °C to obtain an exponential phase culture (10^9 CFU mL^{-1}). Then, it was centrifuged at 5000 rpm during 2 min at 4 °C and washed 3 times with sterile MSM in order to eliminate the excess nutritive broth due to it can provide an additional carbon source. Serial dilutions were performed in a phosphate buffer in order to obtain an equivalent concentration of 10^6 CFU mL^{-1} for each microorganism.

Microcystin-LR removal by bacterium LG1 at different temperatures

Microcystin-LR removal assay was performed in a 100-mL sterile Erlenmeyer flask containing 25 mL of MSM supplemented with MC-LR (200 μg L^{-1}) and an inoculum of 10^6 CFU mL^{-1} of the isolated microorganism according to describe above. Control was made in the same condition without adding microorganisms. The treatments and the corresponding control (without bacteria) were incubated at 28 °C, 32 °C, and 36 °C ± 1 °C in the dark stove. In all experiments, 100-μL samples were taken in 3 and 7 days and centrifuged (12,000 rpm, 15 min, 4 °C) for determining the concentrations of MC-LR in the samples using HPLC/MS according to the technique which was described above. All experiments were conducted for triplicate. The microorganism that produces higher MC-LR removal was assigned as LG1.

Microcystin-LR removal was evaluated by linear regression using Eq. (1).

$$C/C_0 = -k \times t \quad (1)$$

where C_0 is the initial concentration of MC-LR, C is the concentration MC-LR at time t, k is a constant of removal (the slope of the linear regression), and t is the time (day). The average removal time (tm) was defined as the necessary time to reduce the MC-LR concentration by half with respect to initial concentration (Eq. 2).

$$\mathrm{tm} = \log_2/k \quad (2)$$

Lysis activity by bacterium LG1 on *M. aeruginosa* at different temperatures

Similar to MC-LR removal assay, every microorganism isolated was tested in order to study its lysis capacity on *M. aeruginosa* strains through reduction chlorophyll *a* (Chl-*a*) concentration (Zhang et al. 2011). Lysis activity test was performed on *M. aeruginosa* CAAT 2005-3 (toxic strain) and on *M. aeruginosa* 24A (nontoxic strain). *Microcystis aeruginosa* strains preservation and inoculum preparation were carried out according to describe above.

An overnight culture of each isolated microorganism (10^6 CFU mL^{-1}) was co-incubated with toxic and nontoxic *M. aeruginosa* culture (1.10^5 cell mL^{-1}) in a 100-mL sterile Erlenmeyer flask containing BG11 medium. Control was made in the same condition without microorganisms. The cultures were incubated at 28, 32, and 36 ± 1 °C in a chamber (Ingelab I-292PF) with intensity irradiation of 30 μm mol photons.m^2 s^1 (light-dark cycle 14:10) during 7 days. The Chl-*a* values were periodically quantified according to Marker (1972). The experiments were conducted for triplicate.

The microorganism that produces higher lysis activity was assigned as LG1.

The lytic activity (La) or the *M. aeruginosa* inhibitory rate was calculated with Eq. 3 (Zhang et al. 2011).

$$\mathrm{La}(\%) = (1-\mathrm{Tt}/\mathrm{Ct}) \times 100 \quad (3)$$

where Tt (treatment) and Ct (control) are the concentrations of Chl-*a* of treated sample and control, respectively.

Bacterial growth at different temperatures

The growth kinetics of the bacterium with greater capacity to remove MC and lysis activity assigned as LG1 was studied. For this, an overnight culture of the bacterium which was grown at 37 °C ± 1 in the nutritive broth was centrifuged at 5000 rpm, and then the pellet was resuspended in phosphate buffer. One milliliter was inoculated in nutritive broth at the level of 10^5 CFU mL^{-1}. The cultures were incubated at 28 °C, 32 °C, and 36 °C ± 1 °C in the dark stove. Periodically, some aliquots were taken in order to perform the viable counts. Serial dilutions were carried out and 1 mL was plated in a nutritive agar according to pour plate procedure. The plates were incubated at 37 ± 1 °C during 48 h, and then the bacterial colonies were counted and the results expressed as CFU mL^{-1}.

The cells number of LG1 in CFU mL^{-1} obtained as a function of time was modeled using the modified Gompertz equation according to Crettaz-Minaglia et al. (2017), Eq. 4).

$$\mathrm{Log}(N) = a + c \times \exp\left(-\exp\left(-b \times (t-m)\right)\right) \quad (4)$$

where Log(*N*) is the decimal logarithm of the colony counts (log (CFU mL^{-1})), *t* is time (h), *a* is the logarithm of the asymptotic counts when time decreases indefinitely (roughly equivalent to the logarithm of the initial levels of bacterium (log (CFU mL^{-1})), *c* is the logarithm of the asymptotic counts when time is increased indefinitely (the number of log cycles of growth) (log (log (CFU mL^{-1})), *b* is the growth rate relative to time (h^{-1}), and *m* is the required time to reach the maximum growth rate (h). The maximum or specific growth rate (μ) value was calculated as $\mu = b \times c / e$ with $e = 2.7182$ as a constant (Euler number), the lag phase duration (LPD) was calculated as $\mathrm{LPD} = m - 1/b$, (h), and the maximum population density as $\mathrm{MPD} = a + c$ (log (CFU mL^{-1})) (Giannuzzi et al. 1998).

The equation was applied to bacterial growth data by nonlinear regression using the program Systat (Systat Inc., version 5.0). The selected algorithm calculates the set of parameters with the lowest residual sum of squares and a 95% confidence interval for bacterial growth.

The effect of temperature on the specific growth rate (μ) of LG1 bacterial growth was described by the Eq. 5 Arrhenius model.

$$\mu = A \exp\left(\mathrm{Ea}/RT\right) \quad (5)$$

where μ is the specific growth rate (log (CFU mL^{-1}) h^{-1}), *T* the absolute temperature, Ea is the activation energy (kJ mol^{-1}), *A* is the pre-exponential factor (log (CFU mL^{-1}) h^{-1}), and *R* the gas constant (8.31 J K^{-1} mol^{-1}).

Bacterial identification

The strain the microorganism that had the highest removal rate and lysis activity was identified. Gram stain was made in the isolated bacterium LG1 and observed in an Olympus® microscope at 1000 X. The pure culture was grown in a soy tryptone agar during 24 h at 37 ± 1 °C in a dark stove. Then, the following biochemical tests were carried out: oxidase, citrate test, mobility, nitrate reduction, indole, urease, Voges Proskauer, *ortho*-nitrophenyl-β-galactoside (ONPG), glucose, mannitol, and xylose utilization (Diaz et al. 1995; MacFaddin 2003; Koneman and Allen 2008).

In order to identify the LG1 strain by 16S rRNA sequencing, the genomic DNA was isolated with the commercial kit Invitek®, and the 16S rRNA gene was amplified and sequenced by Macrogen Korea by using the primers 27F (5′ AGA GTT TGA TCM TGG CTC AG 3′) and 1492R (5′ TAC GGY TAC CTT GTT ACG ACT T 3′). The sequence obtained was compared to entries of the National Center for Biotechnology Information nucleotide sequence database using the BLASTN algorithm (Altschul et al. 1997).

Data analysis

The statistical computer program Systat (Systat Inc., version 5.0) was used. Analysis of variance (ANOVA) and Tukey test comparison were applied with significance levels of 0.01 and 0.001. The statistical requirements for the ANOVA (normal distribution and homogeneity of variance) were performed. The statistical requirements for the ANOVA (normal distribution, homogeneity of variance) were performed. Fitting was carried out for each temperature condition by nonlinear regression, minimizing the sum of the squares of the deviations between the experimental and predicted values. The goodness-of-fit was evaluated by two parameters: coefficient of determination (R^2) and the root-mean-square error (RMSE) defined as Eq. (6).

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i:1}^{n}(\mathrm{experimentaldata}_i - \mathrm{predicted\ data}_i)2}{n-k}} \quad (6)$$

where experimental are the experimental data, predicted are

the values predicted by the model, n is the number of experimental data, and k is the number of parameters of the assessed model. Lower RMSE values indicate a better fit of the model to describe the data (Snedecor and Cochran 1969).

Results and discussion

Bacterial identification

The last subculture obtained then 70-day acclimation was composed by 15 bacteria (2 Gram (+), and 13 Gram (−)), and 1 yeast. Every microorganism isolated was tested in order to study its ability to remove MC-LR and Chl-*a*. It was observed that the LG1 isolated had both the higher capacity of MC-LR removal and lysis activity. The biochemical tests indicated that LG1 was as follows: Gram (−), oxidase (+), citrate (+), mobility (+), nitrate reduction (+), glucose and xylose oxidation (+), mannitol oxidation (−), ONPG (+), indole (−), urease (−), and Voges Proskauer (−). The sequence of its 16S rRNA (partial sequence, 1302 bp) showed 99% identity with strains of *Achromobacter xylosoxidans* (Sakurad 2012). The nucleotide sequence data of LG1 was deposited in the GenBank under accession number MF959519 *A. xylosoxidans.*

Previously, it is known as *Alcaligenes xylosoxidans* has demonstrated to have as algicidal activity on *M. aeruginosa* (Yamamoto et al. 1993; Manage et al. 2000; Sun et al. 2015). However, its ability to bio-remove MC-LR has not been reported, although a *Burkholderia* order was reported by Lemes et al. (2008). In this way, the Genome Sequencing Project *Achromobacter insuavis* AXXA (NCBI: txid1003200) (Subhraveti et al. 2019) enabled the identification of four genes coding for enzymes with microcystinase activity: AXXA_06763, AXXA_ 06793, AXXA_ 21793, and AXXA_25015 (Szklarczyk et al. 2019, available in STRING online database). All these are mlrC, one of the 4 genes responsible for the enzymatic degradation of MCs (Bourne et al. 1996, 2001; Dziga et al. 2013) with -Adda as unusual amino acid found only in cyanobacterial toxins (Shimizu et al. 2012) and commonly in the Proteobacteria family (Edwards and Lawton 2009). This gene mlrC is induced by only MC-LR presence (Shimizu et al. 2011) and encodes the MlrC protein synthesis (Dziga et al. 2013) that is able to hydrolyze the tetrapeptide and the linear MC (Dziga et al. 2012; Shimizu et al. 2012). This process may occur with no previous processing of acyclic MC by MlrB, and -Adda is the main product of such MlrC activity (Dziga et al. 2013).

Most of the genomes available for the genus today are of clinical origin, while a few were sequenced in the context of bioremediation research (Jeukens et al. 2017). *Achromobacter* genus was previously reported for its ability to several pollutant-degrading such as polychlorinated biphenyls (Ahmed and Focht 1973), endosulfan (*A. faecalis* strain JBW4, Kong et al. 2013), phenol (Rehfuss and Urban 2005), and fluoranthene (*A. xylosoxidans* strain DNoo2, Ma et al. 2015).

Microcystin-LR removal by bacterium LG1

Fifteen bacterial strains and 1 yeast that showed distinct morphological characteristics were selected from the nutritive agar medium and their MCs removal capabilities were evaluated. A bacterium with a high capacity to remove MC-LR could be isolated and it was namely LG1, and an important removal of MC-LR at 32 ± 1 °C is showing in Fig. 1. After 7 days, a maximum decrease in the initial toxin concentration of 79.5% (41 μg L^{-1} residual MC-LR) was observed. In Fig. 1, it can be seen the application of linear regression at the bacteria data. Data from other authors have been included in the same figure in order to compare the bacterium LG1 under similar conditions. In addition, in Table 1, the parameters that were obtained with Eqs. 1 and 2 can be seen. A good agreement between experimental data and predicted values were obtained with a determination coefficient (R^2) ranging between 0.800 and 0.998.

ANOVA and comparison tests allowed separating the tm (Eq. 2) into 4 groups (Table 1). *Bacillus* sp. and *Stenotrophomonas* sp. strain EMS were the bacteria that most quickly removed [D-Leu1] MC-LR according to Eq. 2 (tm = 0.41– 0.42 day). Then, the *Sphingomonas* sp. group had tm values between 1.12–1.27 days and 10.5– 11.0 days. These differences may be due to different species and strains that were used. Meanwhile, LG1 presented tm value of 3.06 days.

There is no scientific literature comparing different strains of bacteria that remove MC-LR. Although in this work we did not study the mechanisms by which LG1 removes MC-LR, our premise is that this bacterium uses the toxin as a carbon and nitrogen source as has been reported in other bacteria (Park et al. 2001; Eleuterio and Batista 2010; Giaramida et al. 2013). Singh and Singh (2011) mentioned that the difference in removal capability of various strains may be due to the difference in enzyme system and/or difference in their growth rate but not all MC degradations are genes Mlr-dependent (Zhang et al. 2020). Moreover, several authors have reported the importance of the environmental conditions in the bio-removal process (Ren et al. 2020) such as temperature (Park et al. 2001), pH (Chen et al. 2010), and initial toxin concentration (Ho et al. 2007). High temperatures and pH values were similar to those of blooms of cyanobacteria in natural environments (Okano et al. 2015; Ren et al. 2020). Therefore, it is possible that the bacteria are adapted to these conditions and that exposure to MCs allows them to be used as a carbon source according to the observations Another important factor is the initial inoculum of bacteria. In this regard, Kang et al. (2012) noted that a concentration lower than 10^6 CFU/mL decreases the ability to remove MCs. Despite differences in experimental conditions, observations indicate that MCs are biotransformed in the environment mediated by

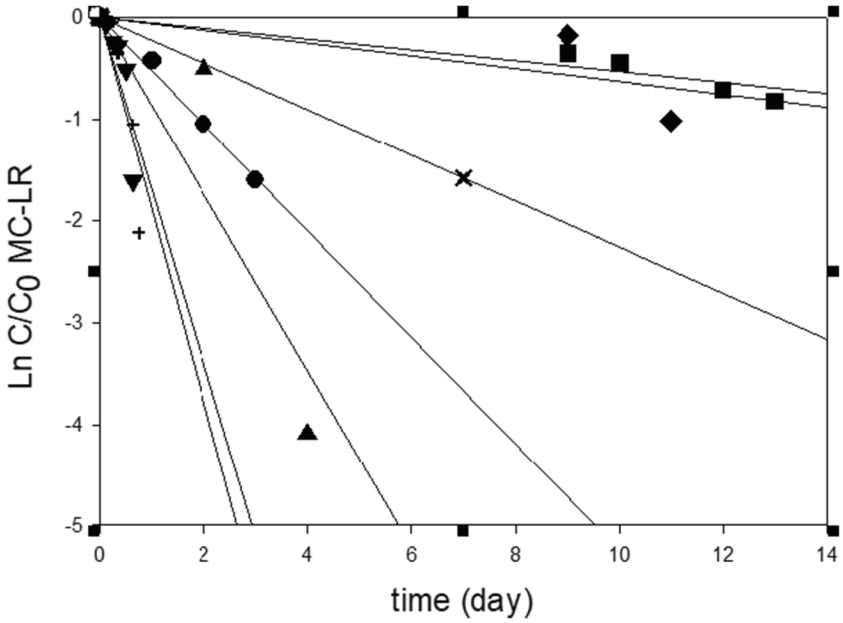

Fig. 1 Linear regression model of MC-LR removed by bacteria calculated with Eq. 1. *Bacillus* sp. (Hu et al. 2012) (plus sign), *Stenotrophomonas* sp. (Chen et al. 2010) (black down-pointing triangle), *Sphingomonas* sp. (Ishii et al. 2004) (black up-pointing triangle), *Sphingomonas* sp. (Park et al. 2001) (black circle), LG1 (X mark), *Sphingomonas* ACM-3962 (Ho et al. 2007) (black square), and *Sphingopyxis* LH21 (Ho et al. 2007) (black diamond)

heterotrophic bacteria (Christoffersen et al. 2002; Ishii et al. 2004). In addition, this not required biotic intrusion because the microorganisms are already present (Chen et al. 2006; Eleuterio and Batista 2010). The biological removal can be very effective, although the factors that influence are difficult to control. For this reason, biological methods have been proposed in combination with other physical adsorption methods (Drikas et al. 2001) such as those practiced routinely in the drinking water treatment process (Jones et al. 1994; Hoeger et al. 2005; Ho et al. 2007). More recently, Li and Pan (2015) used soil microorganisms combined with chitosan in a column as a filter and observed a high efficiency of removal of MCs by combining flocculation (eliminating intracellular MCs) and the filtering process.

Effect of temperature on MC-LR removal by bacterium LG1

The effect of temperature on MC-LR removal by bacterium LG1 (Eq. 3) is shown in Fig. 2a. The best conditions for the removal of MC-LR by LG1 occurred at 36 °C, while toxin concentration did not decrease during the experiment in the control samples.

The constants of MC-LR removal (k) at different temperatures were calculated by linear regression. Data in Fig. 2 show that 200 μg L^{-1}MC-LR were removed by bacterium LG1 with k values of 0.208 ± 0.018 $days^{-1}$, 0.226 ± 0.013 $days^{-1}$, and 0.250 ± 0.012 $days^{-1}$ at 28 °C, 32 °C, and 36 °C ± 1 °C, respectively. Significant differences ($p < 0.05$) were found in the k values between 28 °C and 36 °C ± 1 °C being higher at high temperature. The tm values were 3.33 ± 0.08, 3.06 ± 0.05, and 2.77 ± 0.05 days at 28 °C, 32 °C, and 36 °C ± 1 °C, respectively.

Plot ln k vs 1/T the slope is the Ea (Fig. 2b). There is a linear relation between ln(k) and 1/T, a corresponding correlation coefficient $R^2 = 0.99$ with $A = 5.15 \pm 0.80$, slope = -2.02 ± 0.24, and Ea = 16.79 ± 1.99 kJ mol^{-1}.

The Ea can be considered as the sensitivity of the k parameter to thermal changes and it permits to calculate the k value at a temperature different from those tested in this work. It can permit to characterize the LG1 bacterium respect to its ability

Table 1 Estimated linear model parameters to fitting experimental data to Eq. 1 (k), coefficient of determination (R^2), and average removal time (tm, Eq. 2)

Bacteria	References	k (day^{-1})	R^2	tm (day)
Bacillus sp.	Hu et al. (2012)	− 1.703 ± 0.06	0.94	0.41 ± 0.05^{a}**
Stenotrophomonas sp. EMS	Chen et al. (2010)	− 1.659 ± 0.05	0.94	0.42 ± 0.04^{a}**
Sphingomonas sp.	Ishii et al. (2004)	− 0.637 ± 0.04	0.87	1.12 ± 0.05^{b}**
Sphingomonas sp.	Park et al. (2001)	− 0.545 ± 0.04	0.99	1.27 ± 0.06^{b} *
LG1	This work	− 0.226 ± 0.03	0.99	3.06 ± 0.07^{c} **
Sphingomonas AMC-3962	Ho et al. (2007)	− 0.066 ± 0.01	0.80	10.5 ± 0.06^{d} **
Sphingopyxis LH21	Ho et al. (2007)	− 0.063 ± 0.01	0.91	11.0 ± 0.08^{d}**

Different letters in each column indicate significant differences between bacteria (*$p < 0.01$, **$p < 0.001$)

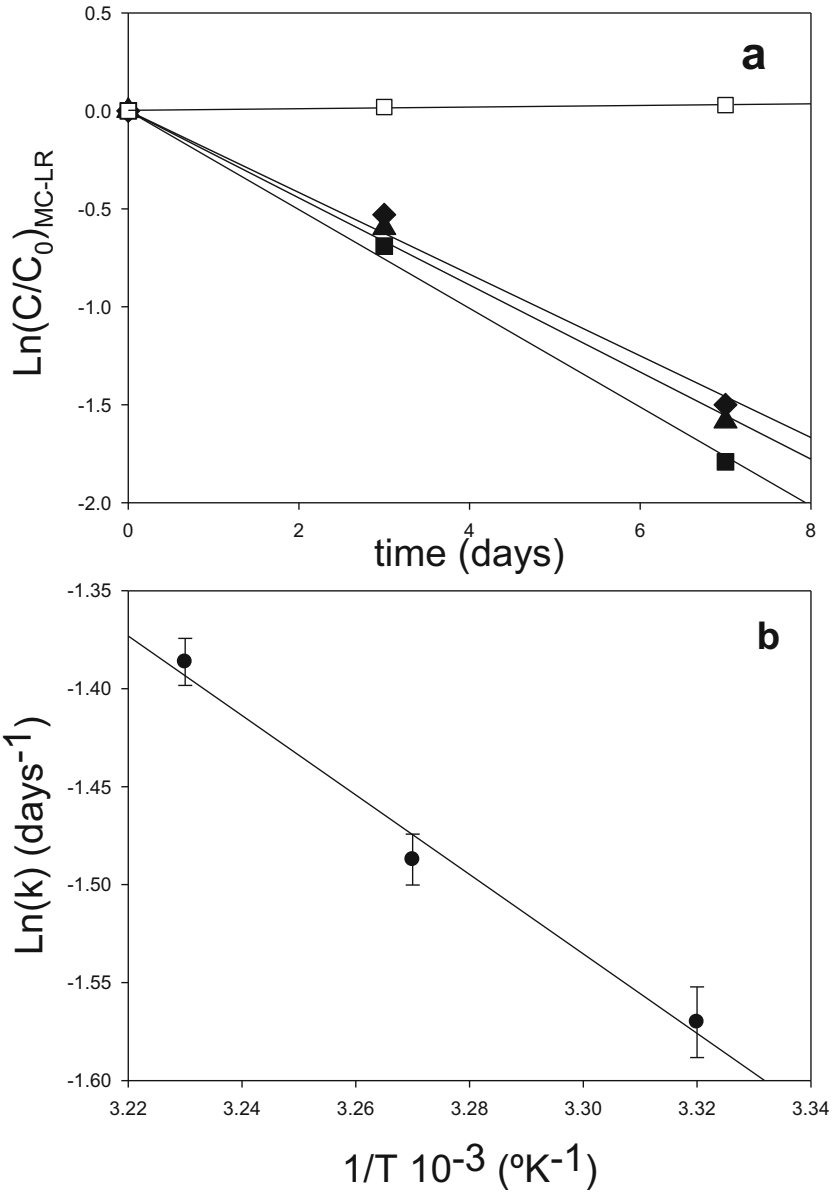

Fig. 2 **a** Decrease in the concentration of MC-LR by the bacterium strain LG1 at different temperatures black diamond—28 °C, black up-pointing triangle—30 °C, and black square—36 °C; control at white diamond—28 °C, white up-pointing triangle—32 °C, and white square—36 °C. **b** Arrhenius plot of *k* values and 1/T for determination of activation energy on LG1

to remove MC-LR. No information is available in the literature on the activation energy of k on *Achromobacter* spp. However; previously Park et al. (2001) also found degradation rates by *Sphingomonas* Y2 were strongly dependent on temperature. Degradation at 5 °C was very slow, and the highest degradation rate occurred at 30 °C.

On the other hand, some investigations about pollutant-removing by genus *Achromobacter* have studied the environmental conditions that improve the bacterium performance. Singh and Singh (2011) reported that the degradation of endosulfan also corresponded to the growth of the bacterial population (*A. xylosoxidans* strain C8B) in soils being the best conditions at 28 °C and pH 6.8. Ma et al. (2015) reported that optimal fluoranthene-degrading conditions were 30 °C and pH = 7.0, Kong et al. (2013) reported the optimal endosulfan-degrading conditions at 40 °C and pH = 7.0. Additionally, Rehfuss and Urban (2005) informed phenol-degrading by *Alcaligenes* at 28 °C. This background on *Achromobacter* would indicate that this bacterium can use diverse sources of carbon as well as other macro and/or micronutrients so it would be effective in the removal of a variety of contaminants depending on the source of the inoculum. Likewise, bacteria better degrade contaminants at relatively high temperatures (between > 28 °C and < 45 °C) and neutral pH (around 7.0).

Lysis activity of LG1 on *Microcystis aeruginosa*

Under laboratory conditions, the lysis activity of LG1 on *M. aeruginosa* was observed indirectly measured as cellular Chl-*a* concentration. In Fig. 3, the Chl-*a* concentration of *M. aeruginosa* CAAT-2005-3 and 24A in the control and treatment samples can be seen. Initial Chl-*a* concentrations

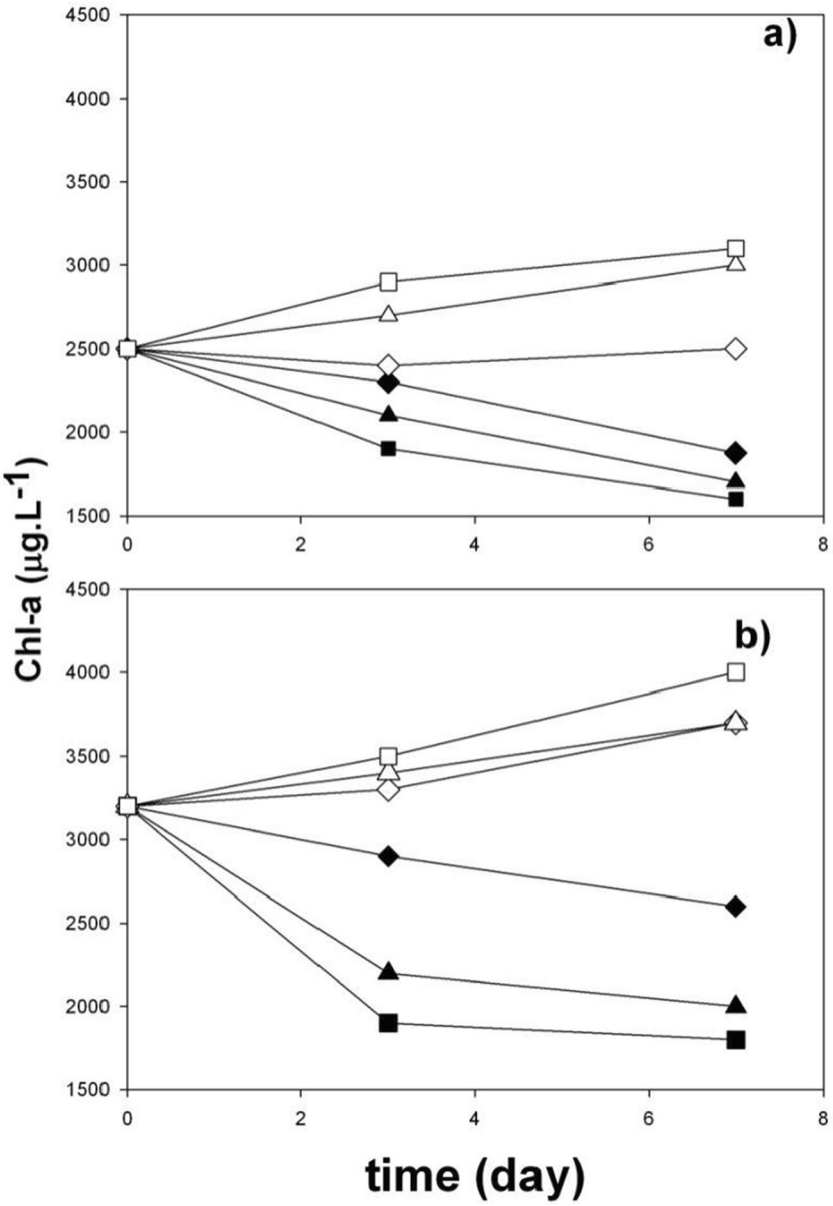

Fig. 3 Chl-*a* lysis by bacteria LG1 on **a** *M. aeruginosa* strain 24A at different temperatures black diamond—28 °C, black up-pointing triangle—30 °C, and black square—36 °C; control at white diamond—28 °C, white up-pointing triangle—32 °C, and white square—36 °C. **b** *M. aeruginosa* strain CAAT-2005-03 at different temperatures black diamond—28 °C, black up-pointing triangle—30 °C, and black square—36 °C, and control at white diamond—28 °C, white up-pointing triangle—32 °C, and white square—36 °C

varied between 2500 and 3200 μg L^{-1} (10^5 cell mL^{-1}). At the end of the experiments, the treatments had a concentration between 1900 and 2100 μg L^{-1} (10^4 cell mL^{-1}). The same figure shows that the Chl-*a* decreased in the treatment samples respect to controls in both strains of *M. aeruginosa*. Additionally, Table 2 shows % lysis activity that was obtained using Eq. 3.

In this figure, it can be observed that when the temperature increased from 28 °C to 36 °C, the concentration of Chl-*a* decreased in two strains of *M. aeruginosa* with respect to the control samples (Table 2), and no-degradation occurs in the control samples. During the assays, it was observed that the lysis activity was maximum at 36 °C showing 48 and 55% for *M. aeruginosa* strain CAAT-2005-03 and 24A respectively, in 7 days (Table 2).

Analysis of variance and comparison tests (Table 2) for lysis activity according to the temperature incubation of *M. aeruginosa* showed no significant differences between

Table 2 Percentage lysis activity in 7 days (Eq. 3) on *M. aeruginosa* strain CAAT-2005-03 and 24A at 3 temperatures by LG1 bacterium

	% lyses activity in 7 days		
Strain	28 °C	32 °C	36 °C
24A	25.3 ± 2.2^{a}**	43.0 ± 2.0^{b}*	48.3 ± 5.5^{c}*
CAAT -2005-03	29.0 ± 1.8^{a}**	40.9 ± 1.5^{b}*	55.0 ± 3.8^{c}*

Different letters in each column indicate significant differences between bacteria (*$p < 0.01$, **$p < 0.001$)

strains ($p = 0.415$). However, for each strain, significant differences between temperatures were observed and lysis activity at 28 °C was very significantly different to 32 °C and 36 °C ($p < 0.001$), and 32 °C was different at 36 °C ($p < 0.05$).

Several investigations have been conducted in order to study the activity of lysis on *Microcystis* species by bacteria. Some of them were isolated from aquatic environments and reported as *Alcaligenes denitrificans* (Manage et al. 2000), *Bacillus cereus* N-14 (Nakamura et al. 2003), *Pseudomonas stutzeri* B2 and *Bacillus mycoides* B16 (Gumbo et al. 2010), *Pseudomonas aeruginosa* R219 (Ren et al. 2010), *Rhodococcus* sp. KWR2 (Lee et al. 2010), *Aeromonas* LTH-1 (Yang et al. 2013), and *Acinetobacter* sp. J25 (Su et al. 2016).

A few works found that dually, a bacterium could produce cell lysis and removal MC-LR such as *Pseudomonas* (Zhao et al. 2005), and *Acinetobacter* (Li et al. 2016). Both bacteria had greater capacity than *Achromobacter* LG1. However, LG1 had similar % lysis activity than *P. stutzeri B2* (Gumbo et al. 2010) with a 48% reduction in the *Microcystis* cell number.

Similarly, only a few studies investigated environmental factors that affect lysis activity. Nakamura et al. (2003) found that as the temperature increases (from 25 °C to 30 °C), the cells were lysed more effectively in alkaline pH conditions. Moreover, Su et al. (2016) reported that the best temperature for lysis activity was 30.5 °C. Like *Achromobacter*, the lysis activity would increase to high temperatures and pH from neutral to alkaline. This is because in natural environments, there is usually a direct correlation between the concentration of cyanobacteria and bacteria (Daft et al. 1975) where the blooms occur in the summer (high temperatures), and during the photosynthesis, the inorganic carbon decreases increasing the pH values (De León 2002). Although if the equilibrium changes markedly as a result of a change in environmental conditions situations may arise where the bacteria may play an important role in the lysis of algal blooms (Daft et al. 1975). Some of these investigations studied the mechanisms by which lysis activity occurs indicating that some bacteria produced extracellular algicidal substances (Lee et al. 2010; Yang et al. 2013) and others did not produce them (Manage et al. 2000). However, Daft et al. (1975) mentioned that the bacteria require contact with the host for lysis to occur. Although in the present work the lysis activity mechanisms were not studied, it seems that they can vary according to the type of bacteria and the bacteria-cyanobacterial relationship that is established.

Growth bacterium LG1 at different temperatures

The bacterial LG1 growth was studied at 3 temperatures on a broth culture. The growth is shown in Fig. 3. The number of CFU mL^{-1} obtained as a function of time was modeled using the modified Gompertz (Eq. 4). Fitting of the Gompertz model to experimental data of bacterium growth at 28 °C, 32 °C, and 36 °C is shown in Fig. 4a. At least 8 data points were obtained per curve and each point was the mean of two microbial counts. In all cases, a good agreement between experimental and predicted data was obtained (Table 3).

The Gompertz parameters and the derived parameters (μ, LPD, and MPD) at 28 °C, 32 °C, and 36 °C ± 1 °C are shown in Table 3. Bacterium growth at 36 °C had higher μ values and the lowest LPD values with significant differences ($p < 0.05$). No significant differences were obtained in MPD values at different temperatures.

In the same way as in this work, some studies coinciding with the fact that bacteria that remove MC and lyse *Microcystis* cells grow better at high temperatures and neutral-alkaline pH values. Ma et al. (2015) reported optimal conditions for *A. xylosoxidans* strain DN002 growth at 37 °C and pH = 7.2. Previously, Daft et al. (1975) had reported that a set of bacteria that lysate cyanobacteria grow well at temperatures between 28 °C and 32 °C and up to 37 °C, and pH = 7– 9. On the other hand, *A. xylosoxidans* strain C8B had grown very fast at 35 °C initially without any lag phase while it was 4 and 8 h respectively at 28 °C and 20 °C using a Luria-Berniti medium (Singh and Singh 2011). In addition, the optimal pH was 6.8 (Singh and Singh 2011).

Additionally, Fig. 4b shows the effect of temperature on μ values of LG1 bacterium. There is a linear relation between $\ln(\mu)$ and 1/T a corresponding correlation coefficient $R^2 = 0.89$ with $A = 18.70 \pm 6.77$, slope = -5.93 ± 2.01, and Ea = 49.28 ± 16 kJ mol^{-1}.

Table 3 Gompertz equation parameters *a*, *c*, *b*, *m* and derived kinetics parameters (μ, LPD, and MPD) for LG1 bacterium growth in culture conditions at 28 °C, 32 °C, and 36 °C

Temperature (°C)	*c*	*b*	*m*	*a*	μ (h^{-1})	LPD (h)	MPD (CFU mL^{-1})	R^2	RMSE
28	3.81 ± 0.19	0.25 ± 0.03	7.81 ± 0.26	5.02 ± 0.12	$0.35 \pm 0.06^{a*}$	$3.81 \pm 0.74^{b*}$	$8.83 \pm 0.31^{a*}$	0.999	0.128
32	4.95 ± 0.37	0.30 ± 0.05	7.07 ± 0.43	4.35 ± 0.24	$0.55 \pm 0.17^{b*}$	$3.72 \pm 0.98^{b*}$	$9.30 \pm 0.61^{a*}$	0.985	0.387
36	3.99 ± 0.10	0.40 ± 0.05	4.31 ± 0.16	5.03 ± 0.07	$0.59 \pm 0.11^{b*}$	$1.81 \pm 0.47^{a*}$	$9.02 \pm 0.17^{a*}$	0.997	0.104

Different letters in each column indicate significant differences between bacteria (*$p < 0.01$, **$p < 0.001$)

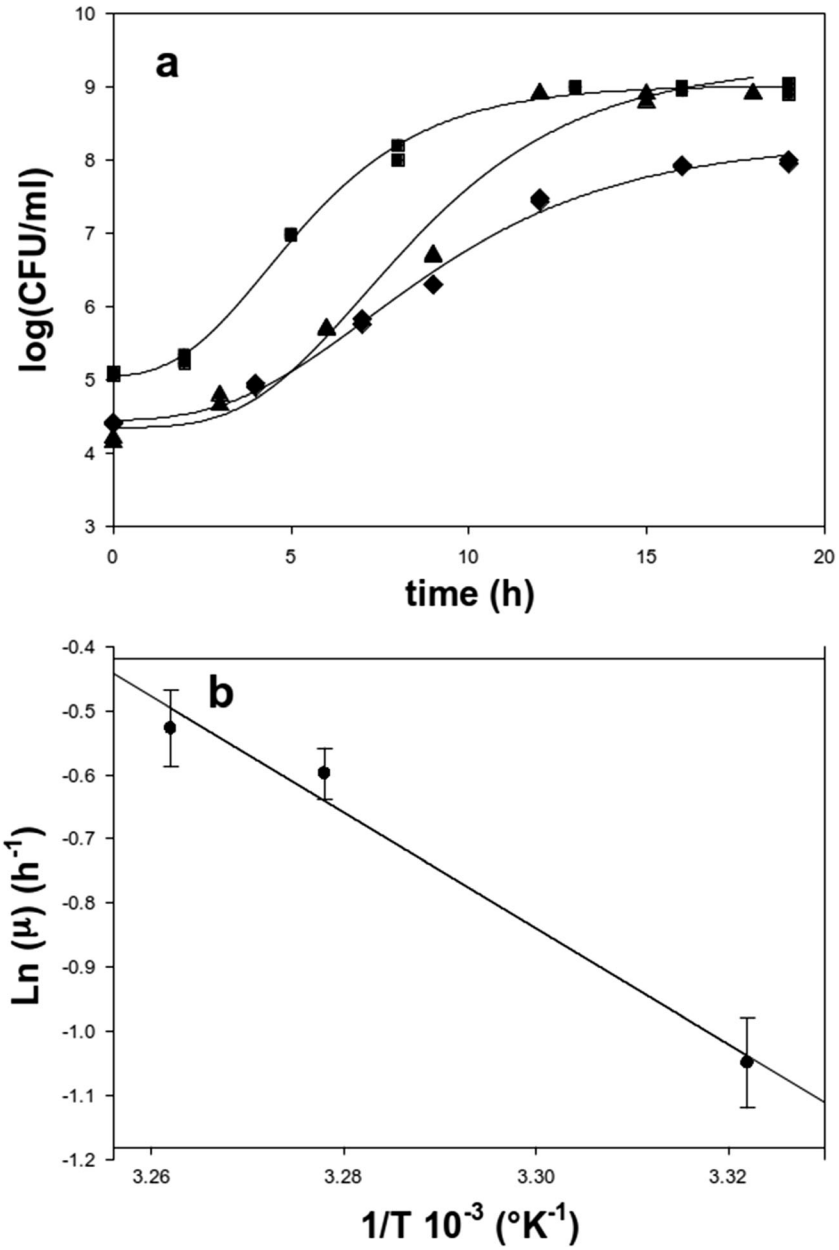

Fig. 4 **a** Growth of LG1 in broth culture at different temperatures black diamond—28 °C, black up-pointing triangle—32 °C, and black square—36 °C. **b** Arrhenius plot of μ values and 1/T for determination of activation energy

The effect of temperature on the removal of MC-LR evaluated by Ea = 16.79 kJ mol^{-1} resulted to be 3 times less than the effect of temperature on the growth of the bacterium whose value was Ea = 49.28 kJ mol^{-1}. This means that the growth of LG1 bacteria is more sensitive to thermal changes than the removal of MC-LR.

The present work allowed predicts microbial growth of LG1 bacteria at different temperatures, by means of activation energy values for and *m*, derived from Arrhenius type models. Besides, the obtained results will help to develop a software to predict the growth of LG1 submitted to thermal variations within 28 °C–36 °C range in scenarios of climate change where a significant increase in temperature is expected. Under these conditions, this bacterium shows greater lysis of *M. aeruginosa* and removal of MC-LR. On the basis that microbial testing in water is expensive and time-consuming, mathematical models become a useful tool to provide a matrix of microbial growth responses to a broad range of conditions.

Conclusion

Microcystin-LR bio-removal was studied as biological methods that it can use as complementary pollution water treatment. The bacteria were demonstrated a high ability for MC-LR removal mainly the *Sphingomonas* group. This work

was the first to record the ability MC-LR removal by indigenous microorganisms and a strain belonging to the genus *Achromobacter* isolated from the Rio de la Plata River. These showed good results, although these did not rich the levels suggested by the WHO (1 μg L^{-1}) in the MC-LR removal assay. In addition, this bacterium was able to remove Chl-*a* as lysis activity being previously reported by other authors. We provided the experimental and predictive data through mathematical modeling of removal MC-LR, lysis activity, and growth *Achromobacter* spp. at 3 temperatures in laboratory conditions considering the scenarios of global change.

The isolation of new bacterial strains degrading MCs could be a simple, low-cost, and effective water treatment method that can be combined with other methods such as adsorption.

Funding information This study was financially supported by the University National of La Plata (UNLP X526), National Agency of Scientific and Technical Research (PICT0861-2013), and CONICET(PIP0959).

References

Ahmed M, Focht DD (1973) Degradation of polychlorinated biphenyls by two species of Achromobacter. Can J Microbiol 19(1):47–52

Alamri S A (2012) Biodegradation of microcystin-RR by Bacillus flexus isolated from a Saudi freshwater lake. Saudi J Biol Sci 19(4):435–440

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped blast and psi-blast: a new generation of protein database search programs. Nucleic Acids Res 25(17):3389–3402

Amé MV, Ricardo EJ, Stephan P, Alberto WD (2006) Degradation of microcystin-RR by *Sphingomonas* sp. CBA4 isolated from San Roque reservoir (Córdoba–Argentina). Biodegradation 17(5):447–455

Amé MV, Galanti LN, Menone ML, Gerpe MS, Moreno VJ, Wunderlin DA (2010) Microcystin–lr,–rr,–yr and–la in water samples and fishes from a shallow lake in Argentina. Harmful Algae 9(1):66–73

Andrinolo D, Pereira P, Giannuzzi L, Aura C, Massera S, Caneo M, Caixach J, Barco M, Echenique R (2007) Occurrence of *Microcystis aeruginosa* and microcystins in Río de la Plata river (Argentina). Acta Toxicol Argent 15(1):8–14

Bourne DG, Jones GJ, Blakeley RL, Jones A, Negri AP, Riddles P (1996) Enzymatic pathway for the bacterial degradation of the cyanobacterial cyclic peptide toxin microcystin-LR. Appl Environ Microbiol 62(11):4086–4094

Bourne DG, Riddles P, Jones GJ, Smith W, Blakeley RL (2001) Characterization of a gene cluster involved in bacterial degradation of the cyanobacterial toxin microcystin-LR. Environ Toxicol 16(6):523–534

Carmichael W (1992) Cyanobacteria secondary metabolites—the cyanotoxins. J Appl Microbiol 72(6):445–459

Chen J, Xie P, Zhang D, Ke Z, Yang H (2006) In situ studies on the bioaccumulation of microcystins in the phytoplanktivorous silver carp (*Hypophthalmichthys molitrix*) stocked in Lake Taihu with dense toxic microcystis blooms. Aquaculture 261(3):1026–1038

Chen J, Hu LB, Zhou W, Yan SH, Yang JD, Xue YF, Shi ZQ (2010) Degradation of microcystin-LR and -RR by a *Stenotrophomonas* sp. strain EMS isolated from Lake Taihu, China. Int J Mol Sci 11(3):896–911

Christoffersen K, Lyck S, Winding A (2002) Microbial activity and bacterial community structure during degradation of microcystins. Aquat Microb Ecol 27(2):125–136

Crettaz-Minaglia MC, Andrinolo D, Giannuzzi L (2015) Advances in microbiological degradation of microcystins. Cyanobacteria: Ecological Importance, Biotechnological Uses and Risk Management, pages 113–124

Crettaz-Minaglia MC, Rosso L, Aranda O, Goñi S, Sedan D, Andrinolo D, Giannuzzi L (2017) Mathematical modeling of *Microcystis aeruginosa* growth and [D-leu^1] microcystin-LR production in culture media at different temperatures. Harmful Algae 67:13–25

Daft MJ, McCord SB, Stewart WDP (1975) Ecological studies on algal-lysing bacteria in fresh waters. Freshw Biol *5*(6):577–596

De León L (2002) Floraciones de cianobacterias en aguas continentales del Uruguay: causas y consecuencias. Perfil Ambiental del Uruguay 2002, Domínguez A, Prieto RG, Nordan-Comunidad, Montevideo, pp. 28–37

Diaz R, Gamazo C, Lopez-Goñi I (1995) Manual práctico de Microbiología. Allergol Immunopathol 23:47–47

Drikas M, Chow CW, House J, Burch MD (2001) Toxic cyanobacteria. Am Water Works Assoc J 93(2):100

Dziga D, Wasylewski M, Szetela A, Bocheńska O, Wladyka B (2012) Verification of the role of MlrC in microcystin biodegradation by studies using a heterologously expressed enzyme. Chem Res Toxicol 25:1192–1194

Dziga D, Wasylewski M, Wladyka B, Nybom S, Meriluoto J (2013) Microbial degradation of microcystins. Chem Res Toxicol 26(6):841–852

Echenique R, Rodríguez J, Caneo M, Giannuzzi L, Barco M, Rivera J, Caixach J, Andrinolo D (2006) Microcystins in the drinking water supply in the cities of Ensenada and La Plata (Argentina). In Congresso Brasileiro de Ficología & Simposio Latino-Americano de Algas Nocivas

Edwards C, Lawton LA (2009) Bioremediation of cyanotoxins. Adv Appl Microbiol 67:109–129

Eleuterio L, Batista JR (2010) Biodegradation studies and sequencing of microcystin-LR degrading bacteria isolated from a drinking water biofilter and a freshwater lake. Toxicon 55(8):1434–1442

Foss AJ, Miles CO, Samdal IA, Løvberg KE, Wilkins AL, Rise F et al (2018) Analysis of free and metabolized microcystins in samples following a bird mortality event. Harmful Algae *80*:117–129

Gaete V, Canelo E, Lagos N, Zambrano F (1994) Inhibitory effects of *Microcystis aeruginosa* toxin on ion pumps of the gill of freshwater fish. Toxicon 32(1):121–127

Giannuzzi L, Pinotti A, Zaritzky N (1998) Mathematical modelling of microbial growth in packaged refrigerated beef stored at different temperatures. Int J Food Microbiol 39(1–2):101–110

Giannuzzi L, Carvajal G, Corradini M, Araujo Andrade C, Echenique R, Andrinolo D (2012) Occurrence of toxic cyanobacterial blooms in Rio de la Plata estuary, Argentina: field study and data analysis. J Toxicol, 2012

Giaramida L, Manage PM, Edwards C, Singh BK, Lawton LA (2013) Bacterial communities response to microcystins exposure and nutrient availability: linking degradation capacity to community structure. Int Biodeterior Biodegradation 84:111–117

Gumbo JR, Ross G, Cloete TE (2010) The isolation and identification of predatory bacteria from a Microcystis algal bloom. Afr J Biotechnol, 9(5)

Harada K-i, Imanishi S, Kato H, Mizuno M, Ito E, Tsuji K (2004) Isolation of ADDA from microcystin-LR by microbial degradation. Toxicon 44(1):107–109

Ho L, Meyn T, Keegan A, Hoefel D, Brookes J, Saint CP, Newcombe G (2006) Bacterial degradation of microcystin toxins within a biologically active sand filter. Water Res 40(4):768–774

Ho L, Hoefel D, Saint CP, Newcombe G (2007) Isolation and identification of a novel microcystin-degrading bacterium from a biological sand filter. Water Res 41(20):4685–4695

Hoeger SJ, Hitzfeld BC, Dietrich DR (2005) Occurrence and elimination of cyanobacterial toxins in drinking water treatment plants. Toxicol Appl Pharmacol 203(3):231–242

Hu L, Zhang F, Liu C, Wang M (2012) Biodegradation of microcystins by *Bacillus* sp. strain EMB. Energy Procedia 16:2054–2059

Ishii H, Nishijima M, Abe T (2004) Characterization of degradation process of cyanobacterial hepatotoxins by a gram-negative aerobic bacterium. Water Res 38(11):2667–2676

Jeukens J, Freschi L, Vincent AT, Emond-Rheault JG, Kukavica-Ibrulj I, Charette SJ, Levesque RC (2017) A pan-genomic approach to understand the basis of host adaptation in *Achromobacter*. Genome Biol Evol 9(4):1030–1046

Jones GJ, Bourne DG, Blakeley RL, Doelle H (1994) Degradation of the cyanobacterial hepatotoxin microcystin by aquatic bacteria. Nat Toxins 2(4):228–235

Kang Y-H, Park C-S, Han M-S (2012) *Pseudomonas aeruginosa* UCBPP-pa14 a useful bacterium capable of lysing *Microcystis aeruginosa* cells and degrading microcystins. J Appl Phycol 24(6): 1517–1525

Kansole MM, Lin TF (2016) Microcystin-LR biodegradation by Bacillus sp.: reaction rates and possible genes involved in the degradation. Water 8(11):508

Koneman EW, Allen S (2008) Koneman. Diagnostico Microbiologico/ Microbiological diagnosis: Texto Y Atlas En Color/Text and Color Atlas. Ed. Médica Panamericana

Kong L, Zhu S, Zhu L, Xie H, Su K, Yan T et al (2013) Biodegradation of organochlorine pesticide endosulfan by bacterial strain *Alcaligenes faecalis* JBW4. J Environ Sci 25(11):2257–2264

Lahiti K, Hiisvirta L (1989) Removal of cyanobacterial toxins in water treatment processes: review of studies conducted in Finland. Water Supply 7(4):149–154

Lee YK, Ahn CY, Kim HS, Oh HM (2010) Cyanobactericidal effect of Rhodococcus sp. isolated from eutrophic lake on Microcystis sp. Biotechnol Lett *32*(11):1673–1678

Lemes GA, Kersanach R, Pinto L d S, Dellagostin OA, Yunes JS, Matthiensen A (2008) Biodegradation of microcystins by aquatic *Burkholderia* sp. from a south Brazilian coastal lagoon. Ecotoxicol Environ Saf 69(3):358–365

Li H, Pan G (2015) Simultaneous removal of harmful algal blooms and microcystins using microorganism-and chitosan-modified local soil. Environ Sci Technol 49(10):6249–6256

Li H, Ai H, Kang L, Sun X, He Q (2016) Simultaneous Microcystis algicidal and Microcystin degrading capability by a single Acinetobacter bacterial strain. Environ Sci Technol *50*(21):11, 903–11,911

Ma YL, Lu W, Wan LL, Luo N (2015) Elucidation of fluoranthene degradative characteristics in a newly isolated Achromobacter xylosoxidans DN002. Appl Biochem Biotechnol 175(3):1294–1305

MacFaddin JF (2003) Pruebas bioquímicas para la identificación de bacterias de importancia clínica. Ed. Médica Panamericana

MacKintosh C, Beattie KA, Klumpp S, Cohen P, Codd GA (1990) Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2a from both mammals and higher plants. FEBS Lett 264(2):187–192

Manage PM, Kawabata Z, Nakano S (2000) Algicidal effect of the bacterium *Alcaligenes denitrificans* on *Microcystis* spp. Aquat Microb Ecol 22(2):111–117

Marker A (1972) The use of acetone and methanol in the estimation of chlorophyll in the presence of phaeophytin. Freshw Biol 2(4):361–385

Matthiensen A, Beattie KA, Yunes JS, Kaya K, Codd GA (2000) [D-Leu[1]] Microcystin-LR, from the cyanobacterium Microcystis RST 9501 and from a Microcystis bloom in the Patos Lagoon estuary, Brazil. Phytochemistry *55*(5):383–387

Nakamura N, Nakano K, Sugiura N, Matsumura M (2003) A novel cyanobacteriolytic bacterium, *Bacillus cereus*, isolated from a eutrophic lake. J Biosci Bioeng *95*(2):179–184

Okano K, Shimizu K, Kawauchi Y, Maseda H, Utsumi M, Zhang Z, Neilan BA, Sugiura N (2010) Characteristics of a microcystin-degrading bacterium under alkaline environmental conditions. J Toxicol, 2009

Okano K, Shimizu K, Maseda H, Kawauchi Y, Utsumi M, Itayama T et al (2015) Whole-genome sequence of the microcystin-degrading bacterium Sphingopyxis sp. strain C-1. Genome Announc 3(4): e00838–e00815

Paerl HW, Fulton RS, Moisander PH, Dyble J (2001) Harmful freshwater algal blooms, with an emphasis on cyanobacteria. Sci World J 1:76–113

Park H-D, Sasaki Y, Maruyama T, Yanagisawa E, Hiraishi A, Kato K (2001) Degradation of the cyanobacterial hepatotoxin microcystin by a new bacterium isolated from a hypertrophic lake. Environ Toxicol 16(4):337–343

Park H, Namikoshi M, Brittain SM, Carmichael WW, Murphy T (2001a) [D-Leu[1]] microcystin-LR, a new microcystin isolated from waterbloom in a Canadian prairie lake. Toxicon 39(6):855–862

Qi Y, Rosso L, Sedan D, Giannuzzi L, Andrinolo D, Volmer DA (2015) Seven new microcystin variants discovered from a native *Microcystis aeruginosa* strain–unambiguous assignment of productions by tandem mass spectrometry. Rapid Commun Mass Spectrom 29(2):220–224

Rehfuss M, Urban J (2005) *Alcaligenes faecalis subsp. phenolicus* subsp. nov. a phenol-degrading, denitrifying bacterium isolated from a graywater bioprocessor. Syst Appl Microbiol 28(5):421–429

Ren H, Zhang P, Liu C, Xue Y, Lian B (2010) The potential use of bacterium strain R219 for controlling of the bloom-forming cyanobacteria in freshwater lake. World J Microbiol Biotechnol *26*(3):465–472

Ren G, He X, Wu P, He Y, Zhang Y, Tang S, ..., Yang F (2020) Biodegradation of microcystin-RR and nutrient pollutants using Sphingopyxis sp. YF1 immobilized activated carbon fibers-sodium alginate. Environ Sci Pollut Res, 1–11

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Microbiology 111(1):1–61

Rosso L, Sedan DY, Kolman MdLA, Caixach J, Flores C, Oteiza JM, Salerno GL, Echenique R, Giannuzzi L, Andrinolo D (2014) *Microcystis aeruginosa* strain [d-leu1] mcyst-LR producer, from Buenos Aires province, Argentina. J Coast Life Med, pages 287–296

Saito T, Okano K, Park H-D, Itayama T, Inamori Y, Neilan BA, Burns BP, Sugiura N (2003) Detection and sequencing of the microcystin LR-degrading gene, mlra, from new bacteria isolated from Japanese lakes. FEMS Microbiol Lett 229(2):271–276

Saitou T, Sugiura N, Itayama T, Inamori Y, Matsumura M (2003) Degradation characteristics of microcystins by isolated bacteria from lake Kasumigaura. J Water Supply Res Technol AQUA 52(1):13–18

Sakurad A (2012) *Achromobacter xylosoxidans*. Rev Chil Infectol 29(4): 453–454. https://doi.org/10.4067/S0716-10182012000400016

Sanchis D, Carrasco D, Quesada A (2004) The genus *Microcystis* (microcystaceae/cyanobacteria) from a Spanish reservoir: a contribution to the definition of morphological variations. Nova Hedwigia 79(3–4):479–495

Shimizu K, Maseda H, Okano K, Itayama T, Kawauchi Y, Chen R, Utsumi M, Zhang Z, Sugiura N (2011) How microcystin-degrading bacteria express microcystin degradation activity. Lakes Reserv Res Manag 16(3):169–178

Shimizu K, Maseda H, Okano K, Kurashima T, Kawauchi Y, Xue Q et al (2012) Enzymatic pathway for biodegrading microcystin LR in Sphingopyxis sp. C-1. J Biosci Bioeng 114(6):630–634

Shishido TK, Kaasalainen U, Fewer DP, Rouhiainen L, Jokela J, Wahlsten M, ... Sivonen K (2013). Convergent evolution of [D-Leucine 1] microcystin-LR in taxonomically disparate cyanobacteria. BMC Evol Biol 13(1):1–16

Singh NS, Singh DK (2011) Biodegradation of endosulfan and endosulfan sulfate by Achromobacter xylosoxidans strain C8B in broth medium. Biodegradation 22(5):845–857

Snedecor C, Cochran M (1969) Statistical methode 6th ed. The Iowa State Univ., Press, Iowa

Su FJ, Ma M, Wei L, Ma F, Lu SJ, Shao S (2016) Algicidal and denitrification characterization of Acinetobacter sp. J25 against *Microcystis aeruginosa* and microbial community in eutrophic landscape water. Mar Pollut Bull *107*(1):233–239

Subhraveti P, Midford P, Keseler I, Kothari A, Caspi R, Karp PD (2019) Summary of *Achromobacter insuavis*, Strain AXX-A, version 23.5. https://biocyc.org/organism-summary?object=GCF_000219745.

Sun P, Lin H, Wang G, Zhang X, Zhang Q, Zhao Y (2015) Wheat bran enhances the cytotoxicity of immobilized *Alcaligenes aquatilis* F8 against *Microcystis aeruginosa*. PLoS One 10(8):e0136429

Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J et al (2019) STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res 47(D1): D607–D613

WHO (1998) Guidelines for drinking-water quality. Vol. 2, health criteria and other supporting information: addendum

Yamamoto Y, Niizuma S, Kuroda N, Sakamoto M (1993) Occurrence of heterotrophic bacteria causing lysis of cyanobacteria in a eutrophic lake. Jpn J Phycol. Sapporo 41(3):215–220

Yan H, Wang H, Wang J, Yin C, Ma S, Liu X, Yin X (2012a) Cloning and expression of the first gene for biodegrading microcystin LR by *Sphingopyxis* sp. ustb-05. J Environ Sci 24(10):1816–1822

Yan H, Wang J, Chen J, Wei W, Wang H, Wang H (2012b) Characterization of the first step involved in enzymatic pathway for microcystin-RR biodegraded by *Sphingopyxis* sp. ustb-05. Chemosphere 87(1):12–18

Yang F, Li X, Li Y, Wei H, Yu G, Yin L et al (2013) Lysing activity of an indigenous algicidal bacterium Aeromonas sp. against Microcystis spp. isolated from Lake Taihu. Environ Technol *34*(11):1421–1427

Yang F, Zhou Y, Yin L, Zhu G, Liang G, Pu Y (2014) Microcystin-degrading activity of an indigenous bacterial strain Stenotrophomonas acidaminiphila MC-LTH2 isolated from Lake Taihu. PloS one 9(1):e86216

Zhang D, Xie P, Chen J (2010) Effects of temperature on the stability of microcystins in muscle of fish and its consequences for food safety. Bull Environ Contam Toxicol 84(2):202–207

Zhang H, Yu Z, Huang Q, Xiao X, Wang X, Zhang F, Wang X, Liu Y, Hu C (2011) Isolation, identification and characterization of phytoplankton-lytic bacterium CH-22 against *Microcystis aeruginosa*. Limnologica 41(1):70–77

Zhang L, Gu L, Wei Q, Zhu X, Wang J, Wang X, Yang Z (2017) High temperature favors elimination of toxin-producing Microcystis and degradation of microcystins by mixotrophic Ochromonas. Chemosphere 172:96–102

Zhang X, Yang F, Chen L, Feng H, Yin S, Chen M (2020) Insights into ecological roles and potential evolution of Mlr-dependent microcystin-degrading bacteria. Sci Total Environ, 136401

Zhao CP, Pu YP, Yin LH, Lu XW, Li XN (2005) Isolation and algicidal effect of a lytic Microcystis bacterium from Taihu Lake. J Southwest Univ 35(4):602–605