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ORIGINAL PAPER

# Biodegradation of the cyanobacterial toxin anatoxin-a by a *Bacillus subtilis* strain isolated from a eutrophic lake in Saudi Arabia

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## Abstract

Anatoxin-a (ATX-a) is a neurotoxin produced by some species of cyanobacteria. Due to its water solubility and stability in natural water, it could pose health risks to human, animals, and plants. Conventional water treatment techniques are not only insufficient for the removal of ATX-a, but they also result in cell lysis and toxin release. The elimination of this toxin through biodegradation may be a promising strategy. This study examines for the first time the biodegradation of ATX-a to a non-toxic metabolite (Epoxy-ATX-a) by a strain of *Bacillus* that has a history of dealing with toxic cyanobacteria in a eutrophic lake. The *Bacillus* strain AMRI-03 thrived without lag phase in a lake water containing ATX-a. The strain displayed fast degradation of ATX-a, depending on initial toxin concentration. At the highest initial concentrations (50 & 100 µg $L^{-1}$), total ATX-a degradation took place in 4 days, but it took 6 & 7 days at lower concentrations (20, 10, and 1 µg $L^{-1}$, respectively). The ATX-a biodegradation rate was also influenced by the initial toxin concentration, reaching its maximum value (12.5 µg $L^{-1}$ $day^{-1}$) at the highest initial toxin concentrations (50 & 100 µg $L^{-1}$). Temperature and pH also had an impact on the rate of ATX-a biodegradation, with the highest rates occurring at 25 and 30 ºC and pH 7 and 8. This nontoxic bacterial strain could be immobilized within a biofilm on sand filters and/or sludge for the degradation and removal of ATX-a and other cyanotoxins during water treatment processes, following the establishment of mesocosm experiments to assess the potential effects of this bacterium on water quality.

**Keywords** Bacteria · Bioremoval · Cyanotoxins · Water treatment

## Introduction

The enrichment of nutrients, particularly, nitrogen and phosphorus, poses a threat to aquatic ecosystems and deteriorate the water quality (Burgan et al. 2013). Due to nutrient enrichment and global warming, the proliferation of harmful cyanobacteria is expected to increase in aquatic ecosystems (Mohamed and Al-Shehri 2012, 2015; Rigosi et al. 2014; Chia et al. 2019). Cyanobacterial blooms present significant environmental and human health hazards as several cyanobacterial species can produce secondary toxic metabolites called cyanotoxins, with different toxicological characteristics including neurotoxicity (e.g., saxitoxins and anatoxins), hepatotoxicity (e.g., microcystins and cylindrospermopsins) to dermatotoxicity (e.g., aplysiatoxin) (O'Neil et al. 2012).

One particularly potent neurotoxin is anatoxin-a (ATX-a), which is a water-soluble secondary bicyclic amine (2-acetyl-9-azabycyclo [4.2.1.] non-2-ene –MW 165 Da, $C_{10}H_{15}NO$) (Osswald et al. 2007). It is an alkaloid neurotoxin produced by cyanobacterial species of the genera *Dolichospermum* (formerly, *Anabaena), Aphanizomenon, Arthrospira, Cylindrospermum, Microcystis, Nostoc, Phormidium, Planktothrix, Raphidiopsis* and *Oscillatoria* (Colas et al. 2021). ATX-a is a potent agonist of nicotinic acetylcholine receptors expressed in synapses involved in cholinergic axon transmission (Bertrand and Terry 2018). Since ATX-a

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cannot be broken down by acetylcholinesterase, it has the potential to cause respiratory arrest, chronic muscular stimulation, paralysis, and death (Plata-Calzado et al. 2022). ATX-a and their cyanobacterial producers can be found in both temperate and tropical freshwaters (Bruno et al. 2017; Yilmaz et al. 2018; Svirče et al. 2019; Christophoridis et al. 2018; Mantzouki et al. 2018; Pitois et al. 2018; John et al. 2019; Karosienė et al. 2020). Furthermore, independent of cyanobacterial growth, temperature and nutrient concentrations, particularly nitrogen and phosphorus, were found to regulate the production of ATX-a (Bashir et al. 2023). Furthermore, the existence of ATX-a in drinking water sources at levels exceeding the WHO guideline limit of 30 µg $L^{-1}$ (WHO 2020) poses health hazards to humans (Liu et al. 2018). Current and most updated treatment methods including activated carbon, membrane filtration and $TiO_2$ photocatalysis were found to efficiently eliminate ATX-a from water (Kumar et al. 2018; Kaminski et al. 2021). However, some of these methods are expensive and others require specialized equipment and expertise.

According to earlier studies, biodegradation is a useful strategy for cyanotoxin elimination without harmful byproducts (Mohamed and Alamri 2012; Mohamed 2016; Li et al. 2015; Lezcano 2016; Mohamed et al. 2022). However, little is known about ATX-a biodegradation by bacteria in comparison to other cyanotoxins. Reduction of ATX-a levels was linked to bacteria found in sediments (Rapala et al. 1994). Kiviranta et al. (1991) found that a strain of *Pseudomonas* sp. can degrade ATX-a at a rate of 2–10 µg $mL^{-1}$ $d^{-1}$. Additionally, it has been asserted that ATX-a biodegradation occurred by an assemblage of bacteria related to the renowned order Sphingomonadales, which contains several species known to degrade microcystins (Kormas and Lymperopoulou 2013; Mou et al. 2013; Briand et al. 2016; Ding et al. 2022; Mugani et al. 2024). Therefore, it has been proposed that indigenous cyanotoxin-degrading bacteria can form biofilms under environmentally relevant conditions, and thereby could contribute to the self-purification of the ecosystem from such potent toxins (Smith et al. 2008; Mohamed and Alamri 2012). These bacteria can also be utilized to established biofilm within sand filter to help degrade cyanotoxins in wastewater treatment plants (Ho et al. 2007; Wu et al. 2019). Therefore, it is necessary to search for pure bacterial strains that have potential to degrade ATX-a in order to establish a viable biodegradation approach for this toxin that might be used in water treatment plants. Given that the indigenous *Bacillus* strain (AMRI-03) was isolated from a eutrophic Saudi lake dominated by microcystin-producing blooms of *Anabaenopsis arnoldi* (Mohamed and Al-Shehri 2009) and was discovered to be capable of degrading microcystin and cylindrospermopsin toxins (Alamri 2010; Mohamed and Alamri 2012; Mohamed et al. 2022), it might also be able to degrade ATX-a in natural waters. Therefore, the main novelty of the present study was to examine the biodegradation of ATX-a for the first time by a pure bacterial strain. The potential effects of pH and temperature on ATX-a biodegradation by this bacterial strain were also studied. Finally, the toxicity of the bacterial strain and ATX-a biodegradation byproducts was evaluated for possible application in water treatment plants.

## Materials and methods

### Materials

The *Bacillus* strain (AMRI-03) employed in this study was previously isolated from Tendaha Lake, Saudi Arabia (18º30′N and 42º20′E) and deposited in the Gene bank with an accession number of GU294753 (Alamri 2010). The lake has been characterized by alkaline pH (8-8.4), higher temperatures (28–30ºC), and dominance of microcystin-producing blooms of *Anabaenopsis arnoldi* (Mohamed and Al-Shehri 2009). The strain demonstrated highest homology with *Bacillus subtilis* (Alamri 2010). The strain was found to degrade microcystin and cylindrospermopsin toxins (Alamri 2010; Mohamed and Alamri 2012). ATX-a was purchased from Abraxis (Warminster, PA, USA). The toxin was dissolved in sterilized lake water and the resulting solution was then diluted to reach the desired experimental concentrations of 1, 10, 20, 50 and 100 µg $L^{-1}$.

### Biodegradation of anatoxin-a

The strain AMRI-03 was cultivated in liquid nutrition broth (NB) medium (Tiedje 1982) at 30 °C for 48 h while being shaken at 120 rpm. The bacterial cells were then collected by centrifugation (6000x*g*, 15 min, 4 °C). The pellet was washed twice with sterile 0.02 M sodium phosphate buffer (pH = 7.2). Bacterial suspension of AMRI-03 cells at a final concentration of $10^5$ cfu $mL^{-1}$ was added into a 250-mL sterile conical flask containing 100 mL of filtered (0.2 µm) and sterilized lake water (pH7). To examine the capacity of strain AMRI-03 to utilize ATX -a as a nutrient source, we used NB medium without nitrogen source in this experiment. ATX-a toxin was spiked separately to the flasks at concentrations of 1, 10, 20, 50 and 100 µg $L^{-1}$. These concentrations were chosen based on that ATX-a levels may reach up to 100 µg $L^{-1}$ in the natural aquatic environment when blooms occur (Colas et al. 2021). Two controls were used in our experiments. Control 1, which contained bacterial cells of strain AMRI-03 and filtered and sterilized lake water but no ATX-a, was utilized to compare bacterial growth in the absence and presence of ATX-a in cultures.

Control 2 contained filtered and sterilized lake water and 100 µg $L^{-1}$ ATX-a but no bacterial cells to ensure that it was not destroyed by abiotic conditions. Treated and control flasks were incubated at 30 °C with shaking (140 rpm) in the dark to avoid photodegradation of ATX-a by light. In order to determine bacterial growth and analyze ATX-a, samples (2mL) were aseptically taken from the flasks at regular intervals for 8 days. Bacterial growth was monitored by spectrophotometrically measuring optical density (OD) at a wavelength of 600 nm. The specific flowchart of the experimental design of ATX-a biodegradation by strain AMRI-03 under different conditions of temperature, pH and initial toxin concentration are shown in Fig. 1. Each experiment was conducted in triplicate, and each assay such as optical density and toxin concentration was run in duplicate.

## Effect of temperature and pH on ATX–a biodegradation

Bacterial cells were grown in filtered and sterilized lake water amended with 50 µg $L^{-1}$ ATX –a at 10, 20, 25 and 30ºC to examine the influence of temperature on ATX -a degradation by the AMRI-03 strain. The impact of pH on ATX-a biodegradation was also examined by growing AMRI-03 cells in sterilized lake water supplemented with 50 µg $L^{-1}$ ATX-a at various pHs (6, 7, 8, 9 and10, with 0.1 M phosphate buffer). These experiments were executed with the same protocol as described in the previous section of biodegradation of anatoxin-a. The concentration 50 µg $L^{-1}$ ATX-a was used in the pH and temperature experiments because there was no difference in the biodegradation rate of ATX- a by *Bacillus* strain at 50 µg $L^{-1}$ and 100 µg $L^{-1}$.

## Anatoxin-a analysis

To determine the ATX–a concentrations remaining (i.e., not-degraded) during biodegradation experiments, samples from bacterial cultures were collected in real time and centrifuged (6000xg, 10 min, 4ºC). Concentrations of ATX –a in the supernatant were then determined using ELISA kit (Abraxis, Warminster, PA, USA) according to the manufacturer instructions. ATX-a quantity was estimated from calibration curve of semi-log relationship between relative absorbance and toxin concentration using ATX-a standard provided with ELISA kit. All assays were performed on duplicate for each sample. Detection limit for ATX-a was 0.15 µg $L^{-1}$.

## Estimation of anatoxin-a biodegradation rate

The average biodegradation rate of ATX-a by AMRI-03 strain was computed by dividing the initial ATX concentration spiked into the bacterial cultures by the number of days until ATX-a was no longer detectable by ELISA.

## Toxicity of anatoxin-a and degradation products

The toxicity of ANX-a and its bacterial degradation products was assessed using a commercially available Thamnotox kit F™ following the manufacturer's instructions. The basis for this assay is the percentage mortality of the larvae

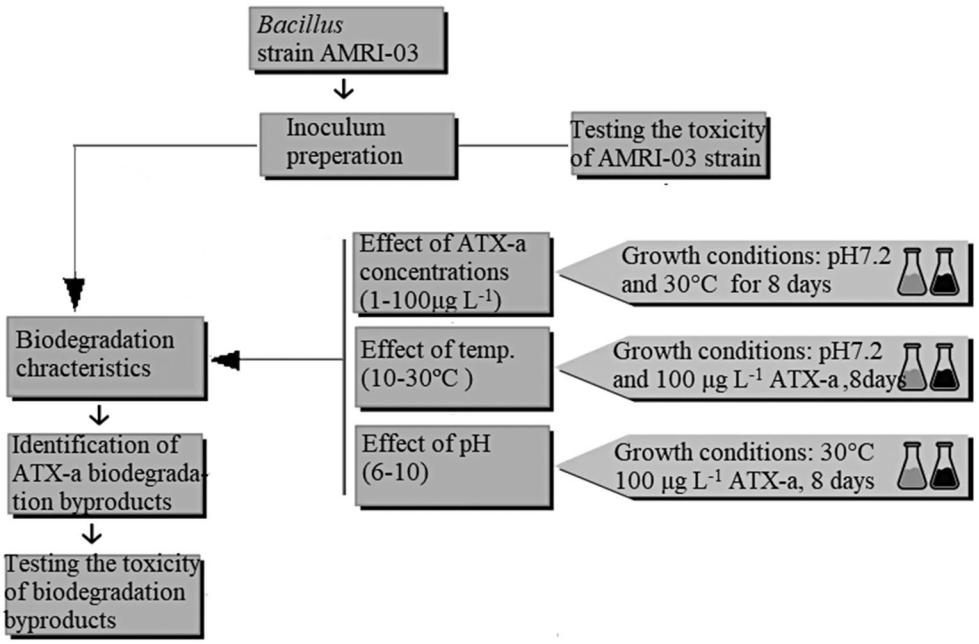


**Fig. 1** Flowchart of the experimental methodology applied in this study

of the crustacean *Thamnocephalus platyurus*. This assay is simple, commonly available and has been widely employed to test the toxicity of several cyanotoxins (Törökné et al. 2007; Sierosławska et al. 2014). The test was performed in microplate wells, and each well contained 10 larvae that were either exposed to various ATX-a concentrations (1, 10, 20, 30, 40, and 50 µg L-1) or to an aliquot from the medium of treated cultures with 50 µg ATX-a $L^{-1}$ at the point of complete toxin degradation (i.e., 96 h). The expression "complete toxin degradation" used through the whole manuscript means that ATX-a concentration was below the detection limit. The test organisms were grown at room temperature (25 °C ± 2) in the dark for 24 h. Thereafter, dead larvae were counted, and the % mortality was calculated and expressed as the difference (%) between mortalities in the tested and control samples. The lethal concentration that causes death in 50% of animals ($LC_{50}$) with 95% confidence limits was calculated by the probit analysis method (Finney 1971).

### Toxicity of *Bacillus* AMRI-03 strain

The potential toxicity of AMRI-03 strain was tested by the same assay used for evaluating ATX-a toxicity using a commercially available Thamnotox kit F™. The assay was performed in the cell-free supernatant of the bacterial culture grown in liquid NB medium for 72 h. A strain of *Bacillus cereus*, which was found to produce both emetic and enterotoxins (Ahmed et al. 2018), was used as a positive control. The standard freshwater provided in the Thamnotox kit served as a negative control. The percentage mortality and the 24 h $LC_{50}$ for AMRI-03 strain and the killing *B. cereus* (control bacterium) was evaluated by the same method as mentioned above.

### Identification of ATX-a biodegradation intermediates

Bacterial cultures exposed to ATX-a at a concentration of 100 µg $L^{-1}$ (i.e., before complete biodegradation), were collected after 2 days and 4 days (time of complete ATX-a biodegradation) and centrifuged (10,000 ×g, 10 min). After being combined, the supernatants were dried in the evaporator and redissolved in 10% aqueous methanol. The solution was centrifuged (10,000×g., 10 min) and 50µL of the supernatant was transferred to HPLC vials. Biodegradation products in this supernatant and the ATX-a standard were both analyzed by using a Waters Acquity ultra-high-performance supercritical fluid chromatography coupled to quadrupole-time-of-flight mass spectrometry (UHPSFC/QTOF-MS) following the same conditions and procedures described in Kaminski et al. (2021). The mass spectrometer was operated in the positive-ion mode was applied with a scan range of 50–400 *m/z*. Data acquisition and processing was achieved using MassLynx software (Waters Corporation, Milford, MA).

### 4.1. Statistical analysis

Differences in ATX-a biodegradation by AMRI-03 strain along different initial ATX concentrations, different temperatures and different pH levels were tested statistically using one-way ANOVA ($P < 0.05$), followed by Tukey post-hoc multiple comparison test to show which groups differed from each other ($P < 0.05$).

The data analysis was done using SPSS version 17.

## Results

### ATX-a biodegradation

Figure 2 shows the growth curve of AMRI-03 strain grown with different concentrations of ATX -a (1–100 µg $L^{-1}$). During the first three days of incubation, the growth of this bacterium exhibited insignificant variation between control and ATX-a-treated cultures ($P > 0.05$). Subsequently, the bacterial growth declined sharply in control cultures, but markedly increased in ATX-a-treated cultures with a decline phase obtained at day 7 for all treatments. Moreover, this strain's growth varied considerably ($F3.48 = 4.3$, $P = 0.02$) among ATX-a concentrations, reaching its maximum growth at 50 µg $L^{-1}$. The bacterial growth varied considerably ($F3.48 = 4.3$, $P = 0.02$) between ATX-a concentrations used in the experiment, reaching its maximum growth at 50 µg $L^{-1}$. On the first day of incubation with bacterial strain, ATX-a exhibited a minor degradation. Thereafter, depending on its initial concentration, ATX-a steady degraded, reaching below the detection limit of ELISA kit by day 8 at most.

Meanwhile, ATX-a concentrations in control 2 (i.e., without bacteria) showed no remarkable change along the incubation period, verifying that ATX-a degradation occurred by bacteria rather than abiotic factors. The reduction in ATX-a concentrations by AMRI-03 strain differed considerably ($F3.48 = 790$, $P < 0.0001$) along the initial ATX-a concentrations. Moreover, the ATX-a biodegradation rate by AMRI-03 strain increased proportionally ($F3.48 = 158.7$, $p < 0.0001$) to the initial ATX –a concentrations (Tables 1 and 2). However, no significant variation in this rate was observed between the highest two ATX-a initial concentrations ($F = 7.7 = 0$, $P = 1$). The highest biodegradation rate (12.5 µg $L^{-1}$ $day^{-1}$) was recorded at higher initial ATX-a concentrations (50 & 100 µg $L^{-1}$) (Table 1). Additionally,

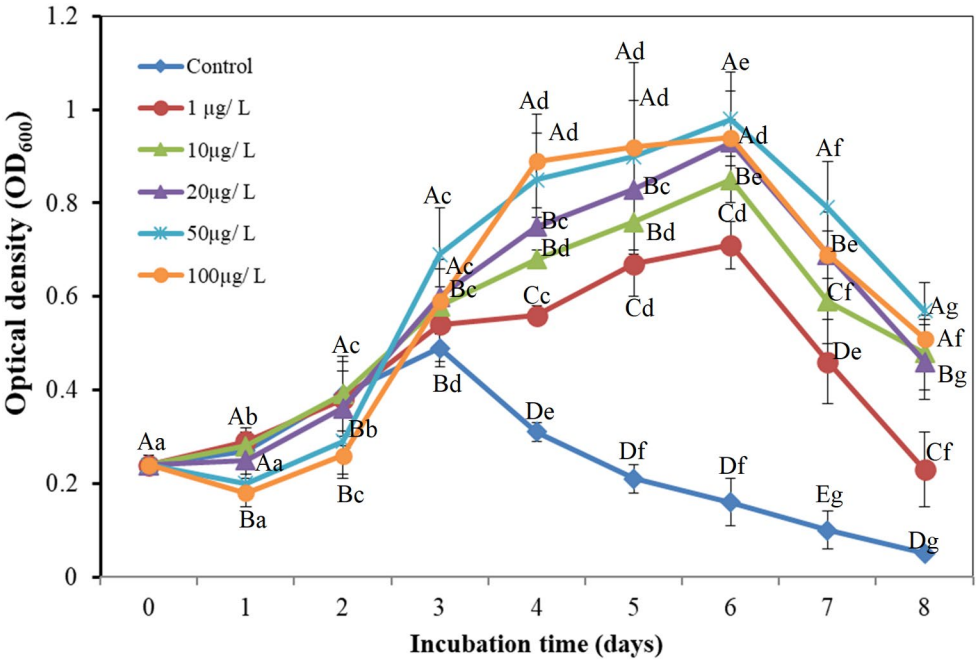


**Fig. 2** Growth curve of *Bacillus* strain AMRI-03 at different initial concentrations of anatoxin-a in batch cultures. Values are expressed as mean ± SD ($n = 6$, $P < 0.05$). Different uppercase letters indicate significant differences in the optical density among different treatments at the same incubation time (i.e., vertical track). Different lowercase letters indicate significant differences in the optical density for each treatment between different incubation times (i.e., horizontal track)

**Table 1** Effect of ATX-a concentration, temperature and pH on the degradation rate of anatoxin-a *Bacillus* strain AMRI-03 during batch experiments. Values are expressed as mean ± SD. Different lowercase letters indicate significant differences in the mortality among different treatments for each experiment

| Factor | Degradation rate (µg $L^{-1}$ $d^{-1}$) | Time of complete degradation (day) |
|---|---|---|
| ATX-a concentration (µg/L) | | |
| 1 | $^{a}$7.14 ± 1.3 | 7 |
| 10 | $^{a}$8.33 ± 1.2 | 7 |
| 20 | $^{a}$8.33 ± 1.4 | 6 |
| 50 | $^{b}$12.5 ± 2.3 | 4 |
| 100 | $^{b}$12.5 ± 2.5 | 4 |
| Temperature (°C) | | |
| 10 | $^{a}$1.2 ± 0.2 | *NO |
| 20 | $^{b}$4.9 ± 0.9 | NO |
| 25 | $^{c}$10 ± 1.7 | 5 |
| 30 | $^{c}$10 ± 1.5 | 5 |
| pH | | |
| 6 | $^{a}$0.44 ± 0.1 | NO |
| 7 | $^{b}$8.33 ± 1.6 | 6 |
| 8 | $^{c}$10 ± 2.1 | 5 |
| 9 | $^{b}$7.14 ± 1.3 | 7 |
| 10 | $^{d}$1.56 ± 0.4 | NO |

*NO means that complete degradation did not occur

complete ATX-a degradation by this strain also depended on the initial toxin concentration; at higher concentrations of 50 and 100 µg $L^{-1}$, it took 4 days to completely degrade ATX-a, whereas at lower concentrations (20, 10, and 1 µg $L^{-1}$, respectively), it took 6 and 7 days (Fig. 3).

**Table 2** Results of One-way analysis of variance (ANOVA) for rate of ATX-a biodegradation by *Bacillus* strain AMRI-03 at different conditions of initial ATX-a concentrations, temperature, and pH

| Variable | Df | MS | F-value | *P*-value | F-crit |
|---|---|---|---|---|---|
| Initial ATX-a concentration | 4 | 19.83 | 158.72 | < 0.0001 | 3.47 |
| Temperature | 3 | 5812.31 | 1223.64 | < 0.0001 | 4.06 |
| pH | 4 | 6140.23 | 990.36 | < 0.0001 | 3.47 |

## Effect of temperature and pH on ATX-a biodegradation

The effects of temperature and pH on the growth and biodegradation rate of ATX –a by AMRI-03 strain are shown in Fig.S1 and Table 1. The optimal conditions for the growth of this strain were 25ºC and at pH7-8 (Fig.S1). The ATX–a degradation rate increased with increasing temperatures ($F4.07 = 34.3$, $P < 0.0001$), and the highest rates were obtained at 25 and 30ºC without significant difference between them (Tables 1 and 2). ATX-a degradation also occurred at lower temperatures (10 & 20ºC), but the toxin was not completely removed, with 81 and 21% of ATX-a remaining on day 8, respectively (Table 1). Conversely, ATX -a was entirely eliminated in 5 days at 25ºC and 30ºC.

Our results also showed that ATX-a degradation activity of AMRI-03 strain differed markedly with the change of the pH of growth cultures ($F3.48 = 990.4$, $P < 0.0001$) (Tables 1 and 2). The highest degradation rates were obtained at pH7 (8.33 µg $L^{-1}$ $d^{-1}$), pH8 (10 µg $L^{-1}$ $d^{-1}$) and pH9 (7.14 µg $L^{-1}$ $d^{-1}$), but this rate decreased sharply at lower pH6 (0.44 µg $L^{-1}$ $d^{-1}$) and higher pH10 (1.56 µg $L^{-1}$ $d^{-1}$)

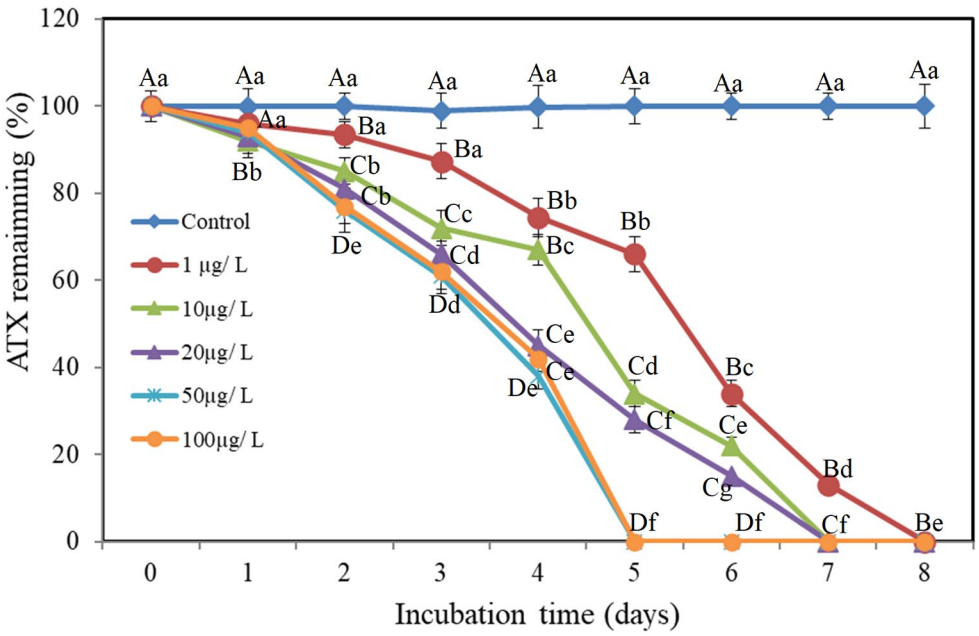


**Fig. 3** Influence of initial anatoxin-a concentrations on anatoxin-a biodegradation (% of ATX-a remaining in the medium) by *Bacillus* strain AMRI-03 during batch experiments. Values are expressed as mean ± SD ($n = 6$, $P < 0.05$). Different uppercase letters indicate significant differences in the parentage of ATX-a remaining in the culture medium among different treatments at the same incubation time (i.e., vertical track). Different lowercase letters indicate significant differences in the parentage of ATX-a remaining in the culture medium for each treatment between different incubation times (i.e., horizontal track)

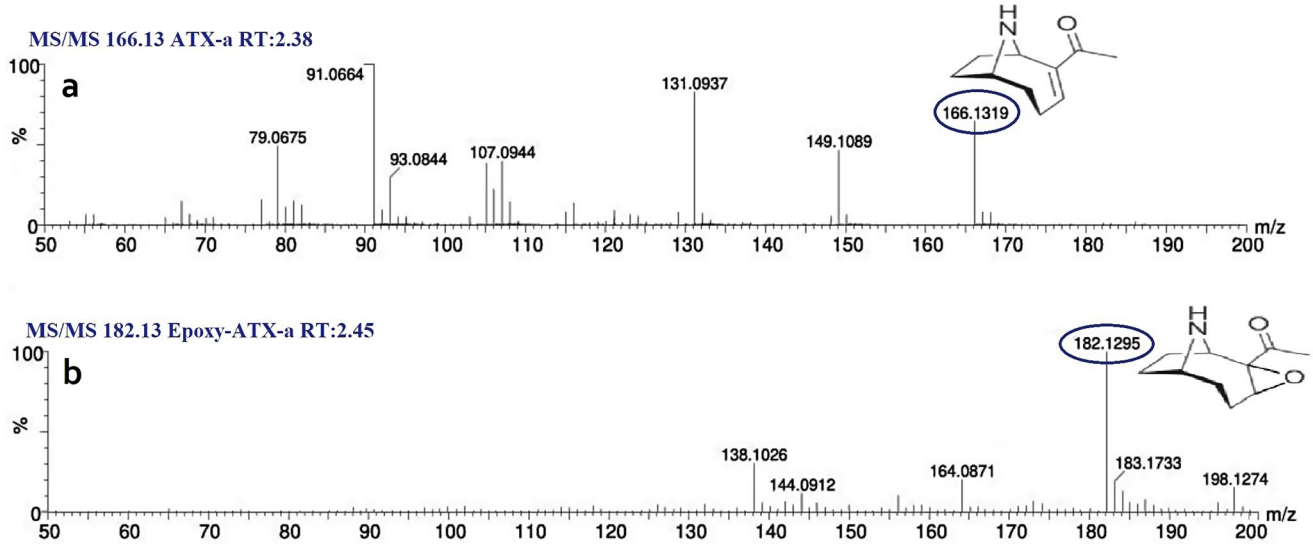


**Fig. 4** Fragmentation mass spectra (MS/MS) of ATX-a with product ion *m/z*166.13 (**a**) and Epoxy-ATX-a with product ion *m/z* 182.13 (**b**)

(Table 1). ATX-a completely degraded at pH 8 in 5 days, pH 7 in 6 days, and pH 9 in 7 days (Table 1). Conversely, the bacterial strain showed no complete degradation at pH 6 and pH10 with 93% and 75% of ATX-a remaining on day 8, respectively.

## Identification and toxicity of degradation products

The MS/MS spectra of ATX-a standard showed a fragment ion at *m/z* 166.13, while degradation product in bacterial supernatants showed a fragment ion at m/z 182.13 (Fig. 4). This is in accordance with previous studies of the MS fragmentation product for the epoxy-ATX-a molecular ion ($[M+H]^+$, *m/z* 182) (Kaminski et al. 2021; Liu et al. 2022). The structure was also searched via the Mass Frontier™ software (Thermo Fisher Scientific Inc.) using its exact mass to ensure proper identification. This demonstrates that the product ion with *m/z* 182 (i.e., degradation product) detected by LC-MS/MS analysis is epoxy-anatoxin-a (epoxy-ATX-a).

Table 3 displays the mortality of *T. platyurus* resulting from ATX-a standard and the bacterial degradation product of this toxin. ATX-a exhibited severe mortality to *T. platyurus*, and this mortality increased with the increase of ATX-a concentrations with an $LC_{50}$ value of 13.8 µg $L^{-1}$.

**Table 3** Mortality (%) of fairy shrimp larvae *(Thamnocephalus platyurus)* exposed to anatoxin-a and anatoxin-a degradation product by *Bacillus* strain AMRI-03 for 24 h. Values are expressed as mean ± SD. Different lowercase letters indicate significant differences in the mortality among different treatments

| ATX-a concentrations (μg $L^{-1}$) | ATX-a mortality (%) | Mortality of degradation products (%) |
|---|---|---|
| 0 | 0 | *NM |
| 1 | [a]6±1 | NM |
| 10 | [b]29±4 | NM |
| 20 | [c]58±6 | NM |
| 50 | [d]87±8 | NM |
| 100 | [e]100±7 | NM |

*NM indicates that no mortality was observed

**Table 4** Mortality (%) of fairy shrimp larvae *(Thamnocephalus platyurus)* exposed to supernatants of toxic *Bacillus cereus* as a positive control and *Bacillus* AMRI-03 strain used in anatoxin-a degradation. Values are expressed as mean ± SD. Different lowercase letters indicate significant differences in the mortality among different treatments

| Supernatant Proportion | Bacillus cereus | Bacillus AMRI-03 |
|---|---|---|
| | % Mortality | % Mortality |
| Culture medium | 0 | *NM |
| 25% | 31 ± 6 | NM |
| 50% | 69 ± 8 | NM |
| 75% | 86 ± 8 | NM |
| 100% | 100 ± 7 | NM |

*NM indicates that no mortality was observed

Conversely, bacterial cultures showing complete ATX-a degradation showed no toxicity to *T. platyurus*.

### Toxicity of *Bacillus* AMRI-03 strain

Table 4 presents the findings of the toxicity assay conducted on *T. platyurus*. The results indicate that the toxic B. cereus supernatant exhibited a high mortality rate against *T. platyurus* nauplii. This mortality increased as the proportion of bacterial supernatant with the highest mortality (100%) increased, which was obtained when using bacterial supernatant without dilution. However, T. platyurus nauplii was not affected in any way by the supernatant of our *Bacillus* strain (AMRI-03).

## Discussion

The present study unequivocally showed that our Bacillus strain, isolated from a eutrophic lake, is capable of degrading the cyanotoxin ATX-a. To date, only one study identified a *Pseudomonas* strain responsible for ATX-a degradation (Kiviranta et al. 1991). Our study is, therefore, the second one to recognize and identify a bacterial strain as ATX-a degrader. In our study, AMRI-03 strain showed short lag phase (one day) when was grown with ATX-a. Previously, Kiviranta et al. (1991) reported ATX-a degradation by bacterial populations with a lag period of 4 days. AMRI-03 strain was similarly found to have short lag phase during CYN biodegradation (Mohamed and Alamri 2012). This agrees with the suggestion that cyanotoxin biodegradation occurs rapidly by strains with a previous history of cyanotoxins in water bodies (Smith et al. 2008; Mohamed and Alamri 2012). This could be the situation of our strain which could have been previously exposed to this cyanotoxin contained in cyanobacterial blooms.

Additionally, our findings demonstrated that strain AMRI-03 entered a decline phase in cultures not treated with ATX-a (i.e., control 2) after 3 days as opposed to 6 days in ATX-treated cultures. This indicates that this bacterium could employ ATX-a as a carbon and nitrogen source for its growth, after the depletion of the natural organic matter found in lake water used in our batch experiments. Accordingly, previous studies demonstrated that bacteria could grow in preconditioned sediments (Klitzke et al. 2010) and lake water with CYN without any addition of natural organic matter (Mohamed and Alamri 2012), possibly using of CYN as a carbon source.

In our study, the statistical analysis revealed a positive correlation ($r = 0.89$) between the initial ATX–a concentrations and its degradation rate. The maximum rate (12.5 μg $L^{-1}$ $day^{-1}$) was obtained at higher initial ATX-a concentrations (50 and 100 μg $L^{-1}$). Our results thus support the evidence that cyanotoxin biodegradation by bacterial populations is initial-concentration-dependent (Smith et al. 2008; Alamri 2010; Mohamed and Alamri 2012). However, no significant difference in ATX-a degradation rate was observed between the highest two initial concentrations of ATX-a (50 and 100 μg $L^{-1}$). This finding can be due to the possible existence of a threshold ATX-a concentration that is required to induce ATX-a biodegradation by bacteria. The degradation rate of ATX-a by our strain (12.5 μg $L^{-1}$ $day^{-1}$) can be compared to that (6–30 μg $mL^{-1}$ per 3days) by *Pseudomonas* sp. (Kiviranta et al. 1991) and by bacterial consortium in sediment (2.4 μg $mL^{-1}$ per 4 days, Rapala et al. 1994). This discrepancy could be due to the difference in bacterial strains involved in ATX-a biodegradation. On the other hand, the ATX-a degradation rate obtained in the present study, is comparable to that of CYN toxin (1.25–50 μg $L^{-1}$ $day^{-1}$) but less than that of MC toxin (145 μg $L^{-1}$ $day^{-1}$) obtained in our previous studies by the same strain (Alamri 2010; Mohamed and Alamri 2012).

In addition to toxin concentrations, our study demonstrated that ATX-a degradation by strain AMRI-03 was similarly affected by temperature and pH changes as bacterial growth was. The highest degradation rate (10 μg $L^{-1}$ $day^{-1}$)

was obtained at higher temperatures (25 & 30ºC), and the decrease in temperature below 20ºC slowed down ATX-a degradation rate by a factor of 2–10. Given that strain AMRI-03 was isolated from a warm lake, it makes sense that this strain would have a high optimal temperature for growth and ATX-a degradation. Similar temperature effects for the biodegradation of other cyanotoxins, such cylindrospermopsin and microcystin, have also been shown in other research (Smith et al. 2008; Ho et al. 2007, 2010; Klitzke and Fastner 2012; Mohamed and Alamri 2012). Regarding the effects of pH, the highest degradation rates (8.33and 10 µg $L^{-1}$ $day^{-1}$, respectively) were obtained at pH 7 and 8 (the optimal pH range for bacterial growth). This degradation rate decreased sharply at pH 6 and pH 10, reaching levels of 0.44 and 1.56 µg $L^{-1}$ $day^{-1}$, respectively. This indicates that ATX-a degradation may have an optimum pH for the activity of its degradation enzyme. Previously, Smith and Sutton (1993) found that the half-life of ATX-a in sediments containing microbial communities was 21 days at pH 4, 14 days at pH 8 and 10, and 5 days at neutral pH. Additionally, the strain AMRI-03 showed similar effects of pH on CYN degradation, with highest rates (16.7 and 15.6 µg $L^{-1}$ $d^{-1}$) obtained at pH7 and 8 and lowest recorded at pH10 and 11 (Mohamed and Alamri 2012).

Similar results of bacterial biodegradation were also reported for another cyanotoxin (CYN), where complete degradation occurred at higher initial concentrations but not observed at lower concentrations (Smith et al. 2008; Mohamed and Alamri 2012). It seems that this is the most common trait of the biodegradation of many organic pollutants. In this respect, the presence of aniline was found to induce the genes involved in biodegradation of aniline by *Pseudomonas* sp. (Thomas and Peretti 1998), and the presence of biphenyl induced the genes involved in their degradation (Ohtsubo et al. 2000). This reflects that ATX-a can enter the bacterial cell and acts as an inducer activating the genes expressing the enzymes involved in the toxin degradation. In this respect, it has been reported that the cyanotoxin, microcystin after uptake into *Sphingopyxis* cells, is firstly hydrolyzed from cyclic form to linear intermediate by the microcystin-degrading enzyme, MlrA, in the periplasmic space, and this linear microcystin is then degraded in the periplasmic space by the enzyme MlrB into tetrapeptide, which in turn is degraded by the enzyme, MlrC in the cytoplasm (Maseda et al. 2012).

Given that the periplasm is exclusive to gram-negative bacteria, it is unlikely to be the site of ATX-a degradation by our *Bacillus* strain AMRI-03 (Gram positive bacteria). Instead, Gram-positive bacteria secrete degradative enzymes into the surrounding environment, and these enzymes act beyond the cell to digest or alter toxic substances to forms that are harmless to cells (Zuber et al. 2006).

In our study, the results of MS/MS analysis revealed the presence of degradation product with *m/z* 182 in the supernatant of *Bacillus* culture (AMRI-03) exposed to ATX-a for 2 days and after ATX-a had completely degraded (4 days). Based on other publications describing ATX-a and its analogues, this degradation product could be identified as epoxy-ATX-a (James et al. 2005; Kaminski et al. 2021; Liu et al. 2022). Previous studies have demonstrated that epoxy-ATX-a is formed by oxidation of ATX-a with UV-A/$TiO_2$ photocatalysis (Kaminski et al. 2021) or $Fe^{III}$ –$B^*$/$H_2O_2$ catalytic oxidation system (Liu et al. 2022). In our study, the *Bacillus* strain (AMRI-03) could oxidize ATX-a by generating extracellular enzymes e.g., monooxygenases, which add one oxygen atom to ATX-a (Arora et al. 2010) to transform it into epoxy-ATX-a. Notably, some strains of *B. subtilis* were found to produce P450 monooxygenase which catalyzed the epoxidation of linoleic acid without further conversion of the epoxidation product (Hou 2006). However, additional research on the *Bacillus* strain (AMRI-03) is needed to identify the genes and enzymes responsible for ATX-a degradation.

What is also noteworthy here in our study is that the degradation product, epoxy-ATX-a resulting from the biodegradation of ATX-a by strain AMRI-03, was not toxic to *T. platyurus*. Our results are thus in agreement with previous studies that epoxy-ATX-a is nontoxic (James et al. 2005; Kaminski et al. 2021; Liu et al. 2022). Moreover, strain AMRI-03, which exhibited the highest similarity with *Bacillus subtilis* (Alamri 2010), was shown to be non-toxic to *T. platyurus* during the present study. This is consistent with previous studies showing that most *B. subtilis* strains are non-pathogenic and non-toxigenic to humans (Environmental Protection Agency, EPA 1997; Lefevre et al. 2017). This has led to the widespread use of *B. subtilis* in the manufacture of compounds and enzymes for application in biotechnology and other industries (Su et al. 2020). Since our *Bacillus* strain (AMRI-03) is nontoxic, it might be used in a slow sand filter to remove ATX-a in drinking water treatment facilities. However, microcosm or mesocosm experiments should be set up to study the potential effects of this bacterial strain on water quality. Previously, Eleuterio and Batista (2010) demonstrated the feasibility of using drinking water biofilters containing microcystin degrading bacteria to remove microcystins from waters.

## Conclusions

This study provides evidence of ATX-a biodegradation for the first time by a *Bacillus* strain into a non-toxic byproduct (Epoxy-ATX-a). The *Bacillus* strain degraded ATX-a at different rates, and the degradation rates were dependent

on initial ATX-a concentration, temperature, and pH. The highest degradation rates were obtained at higher initial ATX-a concentrations (50&100 µg $L^{-1}$), pH8, and 25ºC and 30ºC. Under these circumstances, strain AMRI-03 degraded ATX-a completely in five days. The results of present study have two possible application sides. First, the *Bacillus* strain AMRI-03 has a great deal of potential for application in the bioremediation of water bodies contaminated with ATX-a, and it may even be implicated in the degradation of ATX-a during the collapse of cyanobacterial blooms. Second, since strain AMRI-03 and its ATX-a biodegradation byproduct are nontoxic, this bacterial strain could be immobilized within a biofilm on sand filters and or/drinking water sludge for degradation and removal of ATX-a and other cyanotoxins during water treatment processes. However, prior to taking this move, mesocosm experiments should be set up to assess the potential effects of this bacterium on water quality.

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**Data availability** No datasets were generated or analysed during the current study.

## Declarations

**Ethics approval and consent to participate** Not applicable.

**Consent to publish** Not applicable.

**Competing interests** The authors declare no competing interests.

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