Biotransformation and detoxification of saxitoxin by Bacillus flexus in batch experiments

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Abstract

Saxitoxins (STXs) are carbamate alkaloid neurotoxins produced by some species of cyanobacteria. They are water soluble and relatively stable in the natural environment, and thereby represent a risk to animal and human health through a long-time exposure. STXs cannot be sufficiently removed by conventional water treatment methods. Therefore, this study investigates the potential STX biodegradation and detoxification by bacteria as a promising method for toxin removal. STX biodegradation experiments were conducted using Bacillus flexus SSZ01 strain in batch cultures. The results revealed that SSZ01 strain grew well and rapidly detoxified STX, with no lag phase observed. STX detoxification by SSZ01 strain was initial-toxin-concentration-dependent. The highest biotransformation rate (10µg STX L\(^{-1}\) day\(^{-1}\)) was obtained at the highest initial toxin concentrations (50µg L\(^{-1}\)) and the lowest (0.06µg STX L\(^{-1}\) day\(^{-1}\)) was recorded at the lowest initial concentration (0.5µg L\(^{-1}\)). STX biotransformation rate increased with temperature, with highest occurred at 30 ºC. This rate was also influenced by pH, with highest obtained at pH8 and lowest at higher and lower pH values. HPLC chromatograms showed that STX biotransformation peak is corresponding to the least STX analogue (disulfated sulfocarbamoyl-C1 variant). The Artemia-based toxicity assay revealed that this biotransformation byproduct was nontoxic. This suggests the potential application of this bacterial strain in slow sand filters for cyanotoxin removal in water treatment plants. Being nontoxic, this byproduct needs to be assayed for its therapeutic effects towards neurodegenerative diseases.

Introduction

Saxitoxins are a group of neurotoxins produced by cyanobacteria and dinoflagellates in marine and freshwaters (Burford et al.2016; Mohamed 2018). They are carbamate alkaloids (MW = 299), that block sodium channels in nerves and muscle cells and prevent electrical impulses transmission, with eventual respiratory failure and death, particularly at high toxin levels (O'Neill et al., 2016). STXs are produced by cyanobacteria species belonging to the genera Aphanizomenon, Dolichospermum (formerly Anabaena), Lyngbya, Planktothrix, Raphidiopsis (formerly Cylindrospermopsis) and Scytonema (Christensen and Khan 2020). STX is water soluble (Trainer and Hardy, 2015; Kamp et al., 2016), and stable at pH3 with exponential decrease at pH7-9 (Pereira et al., 2004). STXs have been detected in drinking water (Etheridge 2010; Merel et al.2013), and thereby presenting a threat to human upon consumption of such contaminated water. Therefore, the drinking-water guideline values for STX 3µg/L has been established (WHO 2020). Conventional methods in drinking water treatment plants can sufficiently remove intracellular toxins (i.e., cells with associated toxins), but are ineffective for removing extracellular/dissolved toxins (Merel et al.2013). Additionally, removal of cyanotoxins by chemical methods can produce secondary byproducts that damage the environment (Nishu et al. 2019). As a result, an efficient cost-effective and eco-friendly methods of cyanotoxin elimination are required. In this respect, it has been reported that biodegradation is cost effective method with low risk for removing cyanotoxins from water (Mohamed and Alamri 2012; Li et al., 2015; Lezcano 2016; Mohamed et al. 2022). However, little is known about biodegradation and biotransformation of STXs by bacteria,
compared to microcystin and cylindrospermopsin. Nevertheless, Kayal et al. (2008) reported the biotransformation of the less toxic C-toxins to more toxic analogues (GTX and STX) by bacteria present in bioactive filler beds in a treatment plant. Donovan et al. (2008) has also demonstrated the biodegradation of STXs by seven unidentified marine bacteria, isolated from the digestive tract of blue mussels. Taken that *Bacillus flexus* was referred as an environment-friendly bioagent for elimination of arsenic and other pollutants from wastewater (Jebeli et al. 2017) and its capability for degradation of microcystin and cylindrospermopsin toxins (Alamri 2012; Mohamed et al. 2022), it could also have the potentiality to degrade STXs in aquatic environment. Hence, the present study adopted the evaluation of biodegradation capability of STXs by *Bacillus flexus* SSZ01 strain isolated from decayed cyanobacterial blooms in a eutrophic Saudi lake (Alamri 2010). Furthermore, our study also investigated the effects of temperature and pH on STX biotransformation by this bacterium, where STXs can be found under changeable conditions of pH and temperature in the natural environment.

**Materials And Methods**

**Materials**

*Bacillus flexus* SSZ01 strain was previously isolated from decayed cyanobacterial blooms in eutrophic lake in Saudi Arabia. It was identified by 16S rRNA gene analysis and deposited in the Gene bank with an accession of GU112451 (Alamri 2012). The strain was found to degrade other cyanotoxins such as microcystin and cylindrospermopsin (Alamri 2012; Mohamed et al. 2022). STX was purchased from Abraxis (Warminster, PA, USA). The toxin was dissolved in a small volume of sterilized lake water, and the solution was diluted to give final concentrations of 1, 5, 10, 20, 50 and 100µg L$^{-1}$.

**Bacterial Degradation Of Saxitoxin**

Strain SSZ01 was re-cultured for 48 hours in liquid nutrient broth (NB) medium at 30°C while shaking (120 rpm). Bacterial pellets were collected by centrifugation (6000xg, 15 min, 4°C). The pellet was washed twice with sterile 0.02 M sodium phosphate buffer (pH = 7.2), re-suspended in sterilized water to the required concentration of bacterial cells and assigned as viable bacteria. Another portion of pellets was heat-inactivated by boiling at 95°C for 1h and centrifuged (6000xg, 15 min). The supernatant was discarded and non-viable bacteria were used for potential STX adsorption. Viable bacterial cells of strain SSZ01 at a final concentration of $10^5$ cfu ml$^{-1}$ was inoculated into a 250-ml sterile conical flask containing 100 ml of filtered (0.2 µm) and autoclaved lake water (pH7) and different concentrations of STX (0.5, 1, 5, 10, 20, and 50 µg L$^{-1}$). These concentrations were chosen based on the range (1 − 26 µg L$^{-1}$) of environmentally relevant concentrations of extracellular STXs (Munoz et al. 2021; Li and Persson 2021). In our experiments, two control groups were used. Control 1 contained bacterial cells of strain SSZ01 and lake water, but without STX, which was used to compare the bacterial growth in absence and presence of STX in cultures. Control 2 contained lake water and 100 µg L$^{-1}$ STX without bacterial cells, and was used ensure that is no degraded by abiotic conditions. Control and treated flasks were incubated
in a shaker (140 rpm) at 30°C. Subsamples (2ml) were aseptically taken from each flask at daily intervals for 10 days for toxin determination and bacterial growth. Bacterial growth was estimated as optical density (OD) at 600 nm wavelength.

**Effect Of Temperature And Ph On Stx Biodegradation**

To investigate the effect of temperature on STX degradation by strain SSZ01, bacterial cells were grown in lake water with 50µg L⁻¹ STX and incubated at different temperatures (10, 20, 25 and 30°C). The effect of pH on STX biodegradation was studied by cultivation of strain SSZ01 in lake water with 50µg L⁻¹ STX with different pH levels (6, 7, 8, 9 and 10) adjusted by phosphate buffer (0.1M).

**Saxitoxin Analysis By Elisa**

Concentrations of STX remaining in the cultures (i.e., not-degraded) during biodegradation experiments, were determined by centrifugation (6000xg, 10 min, 4°C) of subsamples withdrawn from bacterial cultures at real time. STX concentrations were then directly determined in the supernatants using the STX ELISA kit (Abraxis, Warminster, PA, USA) according to the manufacturer’s instructions. The absorbance of the colorimetric product of antibody-conjugated enzymes was read at 450 nm on a Synergy™ hybrid multi-mode microplate reader (BioTek, USA). STX quantity was calculated from calibration curve of semi-log relationship between relative absorbance and toxin concentration using STX standard provided with ELISA kit. Each sample was run in duplicate for each assay. Detection limit for STX was 0.02 µg L⁻¹. The STX biodegradation rate by strain SSZ01 was estimated by dividing the initial STX concentration spiked into the bacterial cultures by the number of days until the toxin was no longer detected.

**Hplc Analysis Of Stx And Biotransformation Products**

STX and potential biotransformation products were determined in bacterial culture supernatant using reverse phase HPLC with post-column derivatization and fluorescence detection according to the methods of Oshima (1995). A C8 column (150 mm × 4.6 mm) with a C8 guard cartridge was used for the analysis of all STX analogues (STXs, GTXs and C-toxins). The temperature for column oven was 40°C for STX and 20°C for GTXs and C-toxins. The flow rate was 0.8 mL/min with injection volume varying from 5 to 200 µL. The flow rate for post column reagents was 0.4 mL/min. The excitation and emission wavelengths were 330 nm and 390 nm, respectively. All samples were filtered through 0.45µm filters before analysis. Specifically, the mobile phase used for the analysis of STXs consisted of sodium heptane sulfonate (2 mM), diammonium hydrogen orthophosphate (25 mM, respectively) and 5% acetonitrile (pH 7.1 with phosphoric acid). For GTXs analysis, the mobile phase consisted of sodium heptane sulfonate (2 mM) and diammonium hydrogen orthophosphate (10 mM, pH 7.1 with phosphoric acid). The post column oxidant solution was 7 mM periodate in 50 mM diammonium hydrogen orthophosphate, adjusted to pH 7.8 with 5 M sodium hydroxide, and the post column acid solution was
0.5 M acetic acid. The oxidant solution was 7 mM periodate in 50 mM diammonium hydrogen orthophosphate (pH 7.8 with 5 M sodium hydroxide) and the acid solution was 0.5 M acetic acid. For the analysis of sulfocarbamoyl toxins C1 and C2, the mobile phase was tetrabutyl ammonium phosphate (1 mM, pH 6 with 1 M sodium hydroxide). The oxidant solution was 7 mM periodate in 50 mM potassium dihydrogen phosphate (pH9 with 5 M sodium hydroxide) and the acid solution was 0.5 M acetic acid. Toxin standards including gonyautoxin 2 and 3 (GTX2, GTX3), gonyautoxin 5 (GTX5), decarbamoylsaxitoxin (dcSTX), and N-sulfocarbamoyl toxins (C1, C2), were purchased through local company from the Institute for Marine Bioscience, National Research Council, Canada

**Artemia salina toxicity assay**

The *Artemia salina* assay was conducted to test the toxicity of parent STX (i.e., STX standard without bacteria) and STX biotransformation byproducts obtained during biodegradation experiments. Potential toxicity of STX biotransformation byproducts was carried out for bacterial cultures showing complete biotransformation of STX (i.e., not-detectable by ELISA). The assay was performed using freshly hatched *A. salina* L nauplii according to Kiviranta et al. (1991) and Tokodi et al. (2018). The mortality was estimated after 48h and expressed as the difference (%) between mortalities in the tested and control samples following this formula—%mortality = [(Test − Control)/(1 − Control)] × 100. The lethal concentration causing death of 50% (LC$_{50}$) with 95% confidence limits was calculated by the probit analysis method (Finney, 1971)

**Statistical analysis**

All experiments were performed in triplicate. Variations in STX biotransformation by strain SSZ01 between different initial toxin concentrations, temperatures and pH levels were evaluated using one-way ANOVA (P < 0.05).

**Results**

**STX biodegradation**

The growth curve of strain SSZ01 cultivated with different concentrations of STX (0.5–50µg L$^{-1}$) is shown in Fig. 1. The bacterial strain showed no lag period either in STX-treated or control 1 cultures. The growth of this bacterium increased in both STX-treated cultures and control 1 (P > 0.05) until the day 3 of incubation period. Subsequently, the bacterial growth decreased sharply in control 1 cultures, but increased in all STX-treated cultures with a decline phase obtained at day 6 (Fig. 1). The growth of this strain also varied significantly (F3.1 = 110.9, P < 0.0001) and increased with initial STX concentrations, with no remarkable difference between the highest two STX concentrations (20 & 50µg L$^{-1}$). In addition to its survival, *Bacillus* strain SSZ01 was also able to degrade STX with varying extent depending on initial toxin concentration (Fig. 2). STX concentrations showed a slight decrease during the first day of incubation in treated bacterial cultures. Thereafter, these concentrations declined gradually and they
became undetectable by day 8 at most. At the same time, STX concentrations in control 2 cultures (i.e., without bacteria) showed no remarkable change along the incubation period, confirming that STX biotransformation occurred by bacteria rather than abiotic factors. Additionally, non-viable heat-treated bacteria showed no significant decrease in STX concentration during all the experiment period (therefore data are not shown), indicating that STX removal does not occur through adsorption on bacterial surface. The decrease in STX concentrations by strain SSZ01 differed significantly (F3.1 = 111, P < 0.0001) with the initial STX concentrations. Additionally, the STX biotransformation rate by strain SSZ01 also increased (F3.1 = 318.7, p < 0.0001) with the increase in the initial STX concentration. The highest biotransformation rate (10µg STX L⁻¹ day⁻¹) was obtained at the highest initial STX concentration (50µg L⁻¹) (Fig. 3A), while the lowest rate (0.06µg STX L⁻¹ day⁻¹) was recorded at the lowest initial STX concentration (0.5µg L⁻¹). Complete biotransformation of STX by strain SSZ01 also depended on the initial toxin concentration, as it occurred more rapidly within 5 days at higher STX concentrations (10, 20 & 50µg L⁻¹), compared with 6, 7 and 8 days at lower concentrations (5, 1, and 0.5µg L⁻¹, respectively) (Fig. 2).

**Effect Of Temperature And Ph On Stx Biodegradation**

Figure 3 shows the biotransformation curves of STX by strain SSZ01 incubated at various temperatures (10, 20, 25 and 30°C). The STX biotransformation rate increased with increasing temperatures (F4.07 = 179, P < 0.0001), with highest biotransformation ratios obtained at 25 and 30 °C (83% and 1005, respectively) (Fig. 3). STX was completely removed by this bacterium within 4 days at 30°C, compared to 6 days at 25°C. On the other hand, the biotransformation activity of strain SSZ01 decreased sharply at lower (10 & 20°C) and the toxin was not completely removed until day 8, with biotransformation ratios of 29% and 75%, respectively (Fig. 3). In addition to temperature, the pH of growth cultures also affected significantly STX biotransformation by strain SSZ01 (Fig. 4). The biotransformation ratio varied significantly (F4.07 = 55, P < 0.0001) between the pHs tested during the present study (Fig. 5). After 5 days of incubation, complete biotransformation of STX (i.e., 100% degradation ratio) was observed at pH8. This ratio decreased at lower pHs (6, 7) (19 and 62%, respectively) and higher pHs (9, 10) (88 and 58%, respectively) (Fig. 4). Similarly, the STX biotransformation rate differed markedly with the pH of culture medium (Fig. 5). The highest biotransformation rate was obtained at pH8 (10 µg L⁻¹ d⁻¹), while it decreased sharply at slightly acidic - pH6 (2.4 µg L⁻¹ d⁻¹), neutral-pH7 (6.3µg L⁻¹ d⁻¹) as well as pHs higher than pH8 (7.1µg L⁻¹ d⁻¹ at pH9 and 6.3µg L⁻¹ d⁻¹ at pH10).

**Potential Biodegradation Byproducts**

Figure 6 shows the HPLC analysis of bacterial supernatants of strain SSZ01 incubated with STX of concentration of 50 µg L⁻¹ at 72 and 120 h. HPLC chromatogram of control samples containing bacterial cells incubated in the absence of STX did not show any peak (Fig. 7a). HPLC chromatograms showed that the retention time for STX was 6.3 min (Fig. 6b). An unknown peak was observed at retention time of
2.8 min as a probable biotransformation product of STX by strain SSZ01. This peak increased with the incubation period, in concomitance with the decrease in the peak of the parent STX (Fig. 6b,c). After 120 h of incubation (i.e., the time of complete biotransformation of STX), the STX peak vanished totally with increase in the unknown peak (Fig. 6d). With continued incubation, no other change in byproduct profile was seen (data not shown). Compared to HPLC and MS/MS chromatograms of STX analogues used as standards in our study (Fig. 7,8), the unknown peak (i.e., biotransformation byproduct peak) was corresponding to C1.

**Toxicity Of Stx And Stx Biotransformation Byproducts**

Table 1 shows the mortality of *A. salina* caused by parental STX and its biotransformation byproducts obtained during degradation experiments. The parent STX exhibited severe mortality to Artemia, and this mortality increased with the increase of STX concentrations with an LC$_{50}$ value of 9.4µg L$^{-1}$. On the other hand, bacterial cultures showing complete STX biotransformation showed no toxicity to Artemia.

<table>
<thead>
<tr>
<th>Saxitoxin concentrations (µg L$^{-1}$)</th>
<th>Saxitoxin standard Mortality (%)</th>
<th>biodegradation product Mortality (%)</th>
</tr>
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<tbody>
<tr>
<td>Seawater + shrimps</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Bacterial cultures without STX</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>9</td>
<td>0</td>
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<tr>
<td>1</td>
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<td>5</td>
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<td>20</td>
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<td>50</td>
<td>100</td>
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<tr>
<td>LC$_{50}$ (µg L$^{-1}$)</td>
<td>9.4</td>
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**Discussion**
The present study provides evidence of bacterial biotransformation of STX by a *Bacillus* strain, isolated from eutrophic lake. STX biotransformation has been previously attributed to a mixed bacterial population found in the bioactive filtration beds of water treatment plant (Kayal et al., 2008). However, to our knowledge, STX biodegradation by a typical strain of bacteria has been assigned to *Pseudoalteromonas haloplanktis* (Donovan et al., 2009). Therefore, our study is the second to recognize and identify a bacterial strain (*Bacillus flexus* SSZ01) as STX-degrader. Strain SSZ01 grew well and showed no lag phase in the presence of STX in bacterial cultures. The absence of lag phase was also observed during CYN biodegradation by this strain (Mohamed et al. 2022). These results support the hypothesis that biodegradation of cyanotoxins takes place rapidly by bacterial strains with a previous history of these toxins in the aquatic environment (Smith et al. 2008; Mohamed and Alamri 2012; Mohamed et al. 2022). By co-existing with toxic cyanobacterial blooms in the same environment, our strain could have been frequently exposed to this toxin produced by cyanobacteria. Additionally, strain SSZ01 went into the decline phase earlier (after 3 days) in control 1 cultures (i.e., without STX) compared to 5 days in STX-treated cultures. Similarly, earlier studies have shown that *Bacillus* strains can grow in sediments and lake water supplemented with other cyanotoxin, cylindrospermopsin, but without any addition of natural organic matter (Mohamed and Alamri 2012; Mohamed et al. 2022). It is interesting to note that, strain SSZ01 showed a more rapid complete biotransformation of STX at higher initial concentrations (10, 20 and 50 µg L\(^{-1}\)) than at lower concentrations (0.5-5 µg L\(^{-1}\)). Similar bacterial biodegradation results were also reported for another cyanotoxin (CYN) by strain SSZ01 (Mohamed et al. 2022), and by different bacterial strains (Smith et al. 2008; Mohamed and Alamri 2012), where complete degradation occurred at higher initial toxin concentrations but not observed at lower levels.

This apparently the most common feature of the biodegradation of many organic pollutants. It has been found that genes involved in the biodegradation of aniline by *Pseudomonas* sp. have been induced by aniline (Thomas and Peretti 1998), and the biphenyl degradation genes have been induced by biphenyl (Ohtsubo et al. 2000).

In our study, the regression analysis revealed a positive correlation (\(R^2 = 0.97\)) between STX biotransformation rate and its initial concentrations. The highest rate (10 µg L\(^{-1}\) day\(^{-1}\)) was achieved at highest initial STX concentrations (50 µg L\(^{-1}\)), and this rate declined markedly (0.06–0.83 µg L\(^{-1}\) day\(^{-1}\)) at lower initial toxin concentrations (0.5-5µg L\(^{-1}\)). This finding is in agreement with that obtained by Donovan et al. (2008) demonstrating that complete degradation of STX and neo-STX by marine bacteria strains occurred within 1–3 days, with a rate following first-order kinetics. Our results therefore confirm the suggestion that cyanotoxin biodegradation by bacterial populations is initial-concentration-dependent (Smith et al. 2008; Alamri 2010; Mohamed and Alamri 2012). This could be explained by that STX can induce the expression of STX biotransformation genes in this bacterium. However, a further study is needed to characterize STX-biotransformation genes and the factors affecting their expression in this bacterium.
Our study also found that STX biotransformation rate by strain SSZ01 was temperature-dependent. The highest biotransformation rate (12.5µg L\(^{-1}\) day\(^{-1}\)) was achieved at the highest temperature (30ºC), and the temperature decline below 25ºC slowed this rate by a factor of 1.5-8. Such a high optimum temperature for STX biotransformation by strain SSZ01 is rational, since this strain has been isolated from a warm lake. Similarly, temperatures between 25 to 35 ºC have been shown to be favorable for the biotransformation of other cyanotoxins including microcystin (Ho et al., 2007, 2010) and CYN (Smith et al. 2008; Klitze and Fastner 2012; Mohamed and Alamri 2012). These studies suggested that low temperature may negatively affect the biotransformation rate of cyanotoxins by delaying the expression of cyanotoxin biotransforming genes, decreasing the activity of the enzymes involved in cyanotoxins biotransformation and/or decreasing the rate of cyanotoxin diffusion into the cell. In addition to temperature, pH also influenced the biodegradation rate of STX by strain SSZ01. The highest biotransformation rate was recorded at pH8 (10 µg L\(^{-1}\) day\(^{-1}\)), and this rate decreased significantly at lower pH values (pH6 and pH 7) and higher pHs (9 and 10). This reflects that STX biotransformation by this bacterial strain has an optimum pH for enhancing the expression of its biotransformation genes. Similar effects of pH on toxin biodegradation were also demonstrated for CYN by another *Bacillus* strain (AMRI-03) (Mohamed and Alamri 2012), with highest biodegradation rate obtained at pH7 and 8, and lowest recorded at higher pH levels (pH10 and 11). Also, Smith and Sutton (1993) showed that ATX-a biodegradation by sediment microbial populations occurred more rapidly at neutral pH (5 days), compared to 14 days at pH8 and 10, and 21 days at pH4. Although STX did not undergo a complete degradation by strain SSZ01 at higher pH levels in our study, this bacterium nevertheless makes a contribution to STX degradation under alkaline conditions, in particular at pH9 and 10. The biodegradation of STX at pH9 and 10 might have important implications in water sources with heavy cyanobacterial blooms, which increase water column pH to alkaline levels (pH9-11) (Zepernick et al. 2021).

In our study, strain SSZ01 was found to transform STX to C1 toxin, which was verified using an authentic standard by HPLC. C1 toxin is doubly-sulphated sulfocarbamoyl derivative of STX and is considered the least toxic variant of STX analogues (Donovan e al. 2008; Ho et al.2012; Lukowski et al. 2019; Raposo et al. 2020). C1 toxin is 165 times less toxic than STX (Wiese et al., 2010). It seems that C1 toxin is the only product generated from STX degradation by strain SSZ01, where no other degradation products of STX were identified. Previous studies reported the capability of some bacteria to sulfate STX analogues to less toxic sulfocarbamoyl derivatives via sulfotransferases (Sako et al., 2001). The generation of the disulfated C-toxins from STX by the sulfotransferases was also demonstrated cyanobacteria (Lukowski et al.2019)

The toxicity as estimated by *A. salina* assay of this byproduct was eliminated, indicating that the product of bacterial biotransformation of STX by strain SSZ01 is benign. *A. salina* assay proved to be suitable method to gives a value of the total acute toxicity of many cyanotoxins including microcystin (Kiviranta et al., 1991; Tokodi et al. 2018), cylindrospermopsin (Metcalf et al., 2002), anatoxin-a (Lahti et al., 1995)
and saxitoxin (Mohamed and Al-Shehri 2015; Tokodi et al. 2018). Recently, the disulfated C-toxins derived from STX by sulfotransferases have greater potential as safe pharmaceutical agents for the treatment of numerous neurodegenerative diseases due to voltage-gated sodium channels (VGSCs) disorders (Lukowski et al. 2019).

Conclusions

The present study clearly demonstrated the ability of the bacterial strain SSZ01 to transform STX to disulfated sulfocarbamoyl derivative (C1). STX biotransformation rate increased with the increase of initial STX concentrations. The study also revealed that STX biotransformation by strain SSZ01 was greatly influenced by temperature, with highest biotransformation rate obtained at 30 °C. Moreover, pH of the cultures played a significant role in STX biotransformation by this strain. The highest STX biotransformation rate was achieved at pH8, and this rate underwent significant decrease at lower and higher pH values. The biotransformation of STX by bacteria under alkaline conditions (pH9,10) is of important implications in water sources containing heavy cyanobacterial blooms causing pH to rise to alkaline levels. Based on A. salina toxicity assay, our study also showed that the byproduct of STX biotransformation (C1) by strain SSZ01 was nontoxic. Therefore, this bacterium could be applied in slow sand filter to remove STXs in drinking water treatment plants. However, this necessitates setting up microcosm or mesocosm experiments to study the potential effects of this bacterial strain on water quality. Furthermore, such a nontoxic biotransformation product of STX would be assayed in a further study for its potential therapeutic effects towards VGSC disorders.

Declarations

Authors Contributions: MH, SA, YM, SAL cultured and determined the growth of Bacillus strain. ZM designed STX degradation experiments, and was a major contributor in writing the manuscript. All authors took part in data analysis and interpretation, read and approved the final manuscript.

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

References


41. Screening of cyanobacterial cultures originating from different environments for cyanotoxicity and cyanotoxins, Toxicon 154: 1-6. https://doi.org/10.1016/j.toxicon.2018.09.001


**Figures**
Figure 1

Growth curve of *Bacillus* strain SSZ01 at different saxitoxin initial concentrations in batch cultures. Values are expressed as mean ±SD.
Figure 2

Effect of initial saxitoxin concentrations on saxitoxin biotransformation (% of STX remaining in the medium) by *Bacillus* strain SSZ01 during batch experiments. Values are expressed as mean ±SD.
Figure 3

Effect of temperature on saxitoxin biotransformation (% of STX remaining in the medium) by *Bacillus* strain SSZ01 during batch experiments. Values are expressed as mean ±SD.
Effect of pH on saxitoxin biotransformation (% of STX remaining in the medium) by *Bacillus* strain SSZ01 during batch experiments. Values are expressed as mean ±SD.
Figure 5

Saxitoxin biotransformation rate at different initial STX concentrations (Top panel), different temperatures (middle panel) and different pH values (bottom panel). Values are expressed as mean of 3 readings.
Figure 6

HPLC chromatogram of (a) supernatant of control bacterial culture without saxitoxin, (b) saxitoxin standard, and (c, d) supernatants of bacterial cultures exposed to saxitoxin for 72 and 120 min, respectively.
Figure 7

HPLC chromatogram of (a) mixed standards of, GTX2,3, GTX5 and dcSTX, (b) N-sulfocarbamoyl toxins C1 and C2, and (c) byproduct of STX biotransformation by strain SSZ01.
Figure 8

LC-MS/MS chromatogram of STX and C1 standards and byproduct of STX biotransformation by strain SSZ01