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Antagonistic effect of *Anabaena fertilissima* CCC597 on pathogenic *Vibrio cholerae* propagating in association with cyanobacterial community in freshwater bodies of Eastern Madhya Pradesh

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Various biodiversity indices revealed that *Microcystis aeruginosa* is a major bloom forming colonial cyanobacterium dominantly present in the examined two districts of Eastern Madhya Pradesh. *Microcystis viridis, Microcystis panniformis* and *Microcystis botrys* along with filamentous cyanobacteria *Anabaena* spp., *Arthrospira major* and *Oscillatoria limosa/O. laetevirens* were the other species present. Amplification of VCO1 and VCO139 choleragenic *Vibrio cholerae* strains in phytoplankton material revealed their association with cyanobacteria. VCO1 gene was amplified in five water bodies, and among them, one reservoir also displayed amplification of VCO139 gene. VCO1 and VCO139 genes were not amplified in three water bodies. All of them were infested with *Anabaena* spp. as the second largest phytoplankton constituent. It was hypothesized that *Anabaena* spp. produced some antibacterial metabolites with antagonistic property against *V. cholerae*. To prove this, colonies of *V. cholerae* on TCBS agar were isolated from those water bodies which displayed VCO1 and VCO139 gene amplification. Methyl Red test, Voges-Prauskaur test and arginine dehydrolase tests confirmed *Vibrio*. Further identification of *V. cholerae* was carried out by amplification of VCO1 and VCO139 genes in genomic DNA isolated from *V. cholerae* colonies. A hexane extractable metabolite extracted from lab culture of *Anabaena fertilissima* CCC597, a native of these lakes, was tested for its antagonizing effect on growth of *V. cholerae* strains O1 and O139. A "closed water system" was used to examine the effect of *A. fertilissima* cell mass on time-dependent population size of *Vibrio*. Upon such incubation, there was a steady decrease in the viable colony counts of *V. cholerae*.

Key words: Antibacterial effect, cyanobacterial population, *Vibrio cholerae* O1 and 139, important value index.

INTRODUCTION

Cyanobacteria are primitive prokaryotic organisms dwelling in both freshwater as well as marine ecosystems. They are goldmines as they produce a wide variety of economically important compounds (Whitton and Potts, 2000). In the course of evolution, these ancient organisms have undergone many adaptations (Kumar et al., 2010; Khairy and El-Kassas, 2010; Sethubathi and Prabu, 2010; Battu et al., 2011; Mhadhebi et al., 2012). A large number of microalgal compounds have been found to exhibit antibacterial activity, which includes an array of alkaloids, depsipeptides, undecapeptides, linear and lipopeptides and fatty acids (Swain et al., 2017). Recently, amongst the biologically active peptides, microginins have been shown originating from planktic cyanobacteria

that inhibits growth of a number of bacteria (Silva-Stenico et al., 2010). Studies further indicate the presence of bioactive compounds in freshwater cyanobacteria, that exhibit anticancer, antimicrobial, anti-inflammatory and other pharmacological activities (Borowitzka and Borowitzka, 1992; Gul and Hamann, 2005; Mayer and Hamann, 2005). Mundt et al. (2003) observed fatty acids produced by *Oscillatoria redekei* to possess antibacterial activity. Pedersen and Dasilva (1973) reported bromophenols with antibacterial activity, produced from a cyanobacterium *Calothrix brevissima*. In the application front, some of the bioactive metabolites were used as biocontrol agents. Chaudhary et al. (2012), for e.g., reported about eco-friendly bio-control options against soil borne fungal diseases of tomato and evaluated the fungicidal potential of a cyanobacterium, *Anabaena*.

Vibrio cholerae is a gamma Proteobacteria present in freshwaters and marine waters. *V. cholerae* strains VCO1 and VCO139 are predominantly responsible for the cause of cholera epidemic (Sack et al., 2004). *V. cholerae* are known to attach with phytoplankton, zooplankton and other aquatic organisms develop in many freshwaters resources such as ponds, lakes and reservoirs (Ahmad et al., 2007; Berg et al., 2009; Chaturvedi et al., 2015). *Vibrio* is reported to produce extracellular enzymes chitinase and mucinase for adherence and attachment to obtain nutrients for the rapid growth on phytoplankton, zooplankton and other aquatic organism (Schets et al., 2011; Neogi et al., 2012). Chitin is a major constituent of the exoskeleton in zooplankton and many species of bloom forming cyanobacterial phytoplankton. *Vibrio* develops biofilm on the plankton for survival in the aquatic environment (Cottingham et al., 2003; Bag et al., 2008; Givens et al., 2014).

The present study was aimed to understand the underlying mechanism of interaction between cyanobacterial populations and associated heterotrophic bacteria. A biodiversity parameter, Important Value Index was used to examine cyanobacterial dominance in the water bodies of some locations in Eastern Madhya Pradesh. Distribution of phytoplankton-anchored *V. cholerae* was also profiled. Finally, antagonistic effect of *A. fertilissima* CCC597 was demonstrated on isolated colonies of *V. cholerae*.

MATERIALS AND METHODS

Chemicals

All general purpose chemicals were procured from HiMedia (India) and Sigma-Aldrich (USA). The primers were procured from Imperial Life Sciences Pvt. Ltd. (India).

Survey and sampling

The study was conducted in the Eastern Madhya Pradesh. Jabalpur and Dindori districts (longitude 79ºE - 81ºE and latitude 22ºN - 24ºN) were surveyed and eight water bodies were examined for prevalence of planktic cyanobacterial populations within January, 2013 to January, 2015. These phytoplankton materials were collected by skimming over the surface of water and transferred to sterilized wide-mouth plastic bottles. The buoyant bloom/scum floated on the surface were collected and brought to the laboratory in ice box.

Identification and diversity of cyanobacterial species present in water bodies

The cyanobacteria present in the bloom/scum samples were identified up to species level by following the keys as described by Desikachary (1959), Via-Ordorika et al. (2004) and Jain (2015). The various biodiversity indices namely abundance, frequency and biovolume were calculated according to Jayatissa et al. (2006). The diversity of cyanobacteria species was denoted in terms of Important Value Index (IVI) which is sum of the percentages of the abundance, frequency and biovolume (Jayatissa et al., 2006).

DNA extraction from bloom/scum

The bloom/scum materials were lyophilized at -20ºC until it turned into powder and became brittle. This was stored in cryo-vials at 4ºC. Samples collected at locations were dried and stored. In a method described by Jungblut and Neilan (2006), 25 mg of lyophilized bloom material was heated at 65ºC for 2 h in 3.0 ml of DNA extraction buffer containing 800 mM ammonium acetate, 20 mM EDTA, 100 mM Tris-HCl (pH 8.0), 1% SDS and 1% freshly prepared lysozyme. Thereafter, 50 µl of RNase from a stock of 10 mg ml^{-1} was added and further incubated at 37ºC for 30 min. To stop the reaction, mixture was chilled in ice bath for 10 min and centrifuged at 12000 × g for 10 min at 4ºC. To one volume of cell extract was added one volume of ice cold isopropanol and 0.1 volume of 4 M ammonium acetate and centrifuged at 12000 × *g* for 10 min at 4ºC to precipitate the DNA. The precipitated DNA was resuspended in 100 µl of sterile water. Approximately 10 µl of DNA sample obtained as above was added to 990 µl of sterile double distilled water. Their purity was checked by taking the ratio of their absorbance at A_{260}/A_{280} nm. The yield of each sample was also calculated by using the following formula:

A_{260} × dilution factor × 50 µg ml^{-1}

PCR amplification reaction for detecting VCO1/ VCO139 genes of *V. cholera*

Amplification reaction was carried out for *V. cholerae* O1 and O139 strains associated with bloom/scum materials using above DNA preparations and primer pairs specific for VCO1 and VCO139 genes as detailed subsequently (Binsztein et al., 2004), procured from Imperial Life Sciences, India. Reaction mixture was prepared according to Jungblut and Neilan (2006) and Kumar et al. (2011) and thermal cycling was performed according to the Binsztein et al. (2004) with an initial denaturation step at 94°C for 5 min, followed

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by 40 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 3 min, with a final extension for 10 min at 72°C. The amplified product was then subjected to agarose gel electrophoresis. VCO1: Forward 5'-CAACAGAATAGACTCAAGAA-3'; reverse 5'-TATCTTCTGATACTTTTCTAC-3'. VCO139: Forward 5'-TTACCAGTCTACATTGCC-3'; reverse 5'-CGTTTCGGTAGTTTTTCTGG-3'.

Extraction of DNA from colonies of *V. cholerae*

For the isolation of *V*. *cholerae*, the water bodies, wherein the amplification of VCO1/ VCO139 genes was observed in blooms/scum material, were shortlisted. The fresh phytoplankton material was collected from these water bodies as above and was diluted by 1/10 and poured on to Petri dishes containing agar-solidified thiosulfate-citrate-bile-salts-sucrose (TCBS) medium (Tulip Diagnostics) and incubated at 37°C for 48 h. One representative isolated colony from each plate were picked using inoculation loop and used for extraction of DNA according to Bag et al. (2008). The colony was homogenized in 100 µl of autoclaved normal saline. Bacterial suspension was pelleted by centrifugation at 12000 × g for 10 min at 4°C. The pellet was re-suspended in 100 µl of double distilled autoclaved water and it was boiled for 10 min. Debris was removed by centrifugation at 12000 × *g* for 10 min at 4°C. The supernatant containing DNA was transferred in fresh microcentrifuge tubes (Eppendorf) and stored at 4°C in a refrigerator for further use.

PCR amplification reaction was carried out using DNA isolated from *V*. *cholerae* grown on TCBS agar medium. Reaction mixture was prepared according to Jungblut and Neilan (2006) and Kumar et al. (2011). Thermal cycling and subsequent electrophoresis of amplified products was carried out according to the Binsztein et al. (2004) using the primer pairs selective for VCO1 and VCO139 genes.

Biochemical tests for identification of *V. cholera*

The colonies as recovered above were first characterized by colony appearance and colour according to the Handbook of Culture Media (Atlas and Parks, 1997), and the colony forming units were also calculated. These were subjected to the following standard examinations: For Methyl Red-Voges Proskauer (MR-VP) test (Aneja, 2010), 10 ml MRVP broth (peptone 7 g l^{-1}, glucose 5.0 g l^{-1}, potassium phosphate 5 g l^{-1} and 1000 ml of distilled water; pH 6.9) was placed in sterilized test tubes in two sets. The tubes were inoculated with colonies of *Vibrio*. One tube was left uninoculated and kept as control. The cultures/control were incubated at 35°C for 48 h. The tubes were divided in two sets. In the first set, 5 drops of methyl red reagent was added. In the other set, 12 drops of V-P reagent I (naphthol solution) and subsequently 2 to 3 drops of V-P reagent II (40% potassium hydroxide) were added. Appearance of red colour within 15 min gives positive test for MR or VP.

For L - arginine dehydrolase test (Choopun et al., 2002), Luria Bertani (LB) broth containing 1% arginine was prepared, autoclaved and poured in tubes. The medium was inoculated with colonies of *Vibrio,* and one tube was left uninoculated as control. This culture/control was incubated at 37°C for 48 h. Five to six drops of phenol red was added in the tubes. Appearance of red colour indicates positive test, whose visible intensity was arbitrarily determined as pale, moderate or intense.

Extraction of antibacterial metabolite from *A. fertilissima* lab cultures

Large scale cultivation of a rice field cyanobacterium from Jabalpur, *A*. *fertilissima* in BG-11 medium was carried out under the conditions as described in Banerjee et al. (2013). About 1 L of culture was centrifuged at 1,000 × *g* for 15 min to harvest the cells which were air dried at 37°C. The pellet was homogenized in 10 ml of 10% aqueous methanol. This crude extract was passed through previously equilibrated LiChrolut RP-18 (ODS) cartridges (Merck, Germany, 500 mg sorbent). After having washed with 10% methanol the bound material was eluted in 100% methanol. Methanolic extract was then mixed with equal volume of hexane in a separating funnel and the hexane phase was separated. Hexane phase was subjected to evaporation at room temperature and the final residue was dissolved in 10% of methanol. This extract was passed through 1 g of animal charcoal in Whatman filter paper. The filtrate was termed as hexane-extractable metabolite.

Screening of the hexane-extractable metabolite on *V. cholerae* lawns

V. cholerae was subcultured on TCBS medium by repeated streaking. A lawn was prepared by mixing one such segregated colony with agar medium and was poured on the Petri dishes. The antibacterial activity of the hexane-extractable metabolite was determined by well-diffusion method. For this, wells were dug using sterilized cork borer and inside about 0.5 ml of 10% methanolic solution of *A. fertilissima* extract was poured. After allowing the bacterium to grow, the antibacterial activity was determined as diameter of the clearing zone produced around the wells. 10% methanol (0.5 ml) at equivalent volume was used as a negative control. For positive control 0.5 ml solution of 150 mg ml^{-1} of azithromycin in 10% methanol was used.

Antibacterial activity was determined by following well diffusion technique. Suspension of *V. cholerae* wells (5 mm) was prepared in these plates using cork-borer, by maintaining sterile conditions. 0.5 ml of the extract (concentration 125 mg ml^{-1}) was poured into the wells. All the plates were incubated at 37°C for 24 h. Growth inhibition zones produced by the extract were examined and the diameter (mm) was measured.

In situ* analysis of the effect *of A. fertilissima* cell mass on colony counts of *V. cholera

First a "closed water system" was fabricated to conduct the experiment. For this, about 50 L of clear water from Pariyat reservoir (Jabalpur) was poured into 61 cm × 30 cm × 38 cm aquarium covered with acrylic cover, and placed under regular day-night regime. In batches, *A*. *fertilissima* cells from about 10 L of cultures in BG 11 medium were harvested by coarse filtration and pooled up for building a large mass of the cells. The inoculum was raised in 1 L of BG 11 medium in 5-L Erlenmeyer flasks kept under growth conditions as described previously (Banerjee et al., 2013). After 25-days of growth the cell mass equivalent to 10 g fresh weight was air-dried and placed in pouches prepared of three layers of muslin cloth and then tied from top. These pouches were suspended in the aquarium and were left for 20 days. Control sets were without the pouches being dipped in the water. Manually water was percolated twice a day for aeration. Aliquots of water from the tanks were diluted and from a series, *V. cholera* cells were enumerated on TCBS agar medium from colony specific viable counts.

RESULTS

Diversity of cyanobacterial population

During this study, eight water bodies were found to be

Table 1. Cyanobacterial diversity in eight water bodies of Eastern Madhya Pradesh (India) in terms of relative frequencies and mean biovolume.

Water body	Cyanobacterial frequencies (%)	Mean biovolume ($mm^3 L^{-1}$)
Khairy lake, Jabalpur	*Microcystis aeruginosa* (92), *Arthrospira major* (8)	16.2
Shahpura lake, Dindori	*Oscillatoria limosa/ O. laetevirens* (100)	16.0
Gangasagar lake, Jabalpur	*O. limosa* (68), *Nostoc* spp. (32)	6.0
Salondi lake, Jabalpur	*O. limosa* (76), *Anabaena* spp. (24)	204.0
Pariyat reservoir, Jabalpur	*M. aeruginosa* (57), *M. viridis* (22), *M. panniformis* (21)	10.0
Bahela lake, Jabalpur	*O. limosa* (64), *Nostoc* spp. (36)	33.2
Mahegong lake, Jabalpur	*O. limosa* (78), *Anabaena* spp. (22)	98.2
Samnapur lake, Jabalpur	*O. limosa* (87), *Anabaena* spp. (13)	27.8

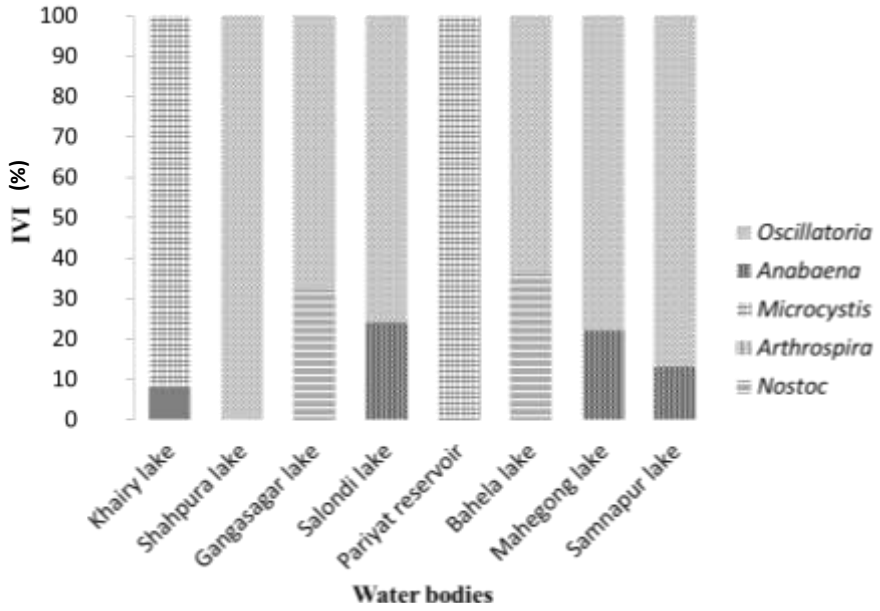

Figure 1. Important value index (IVI) for all cyanobacterial genera present in the water bodies.

heavily infested with cyanobacterial bloom/scum. Analysis of indices that determine cyanobacterial diversity showed that the *Microcystis aeruginosa* was dominantly present as bloom forming cyanobacteria in water bodies. Maximum frequency of *M. aeruginosa* (92%) was in Khairy lake, Jabalpur (Table 1). *Oscillatoria limosa*/*O*. *laetevirens* were identified as being the sole or major proportion of scum material collected from different lakes. The other forms of colonial cyanobacteria present sub-dominantly were *Microcystis viridis, M. botrys, M. panniformis* and some filamentous cyanobacteria, *viz*. *Arthrospira major* and different species of *Nostoc* and *Anabaena.* Cyanobacterial diversity showed that *A*. *major* was present in one water body; *O. limosa* and *O*. *laetevirens* in six water bodies and *Anabaena* in three water bodies (Table 1).

In terms of biovolume of total cyanobacteria, the highest biomass was recovered from Salondi lake (Jabalpur), while minimum from Gangasagar lake (Jabalpur) both predominantly harbouring *O. limosa*/*O. laetevirens* scums (Table 1). The IVI scores (Figure 1) clearly indicate that Pariyat reservoir (Jabalpur) and Shahpura lake (Dindori) had unicyanobacterial populations of *Microcystis* and *Oscillatoria* respectively, whereas the rest of the surveyed lakes exhibited presence of other cyanobacteria as sub-dominant genera.

Amplification of VCO1 and VCO139 genes of *V. cholerae* adhered to cyanobacterial blooms/scum

For PCR amplification reaction, the recovery of DNA in the dried bloom/scum material was between 100 to 200 µg ml^{-1}, as determined from ratio $A_{260/280}$ which was 1.6 to 1.7. Subsequent PCR amplification results (Figure 2)

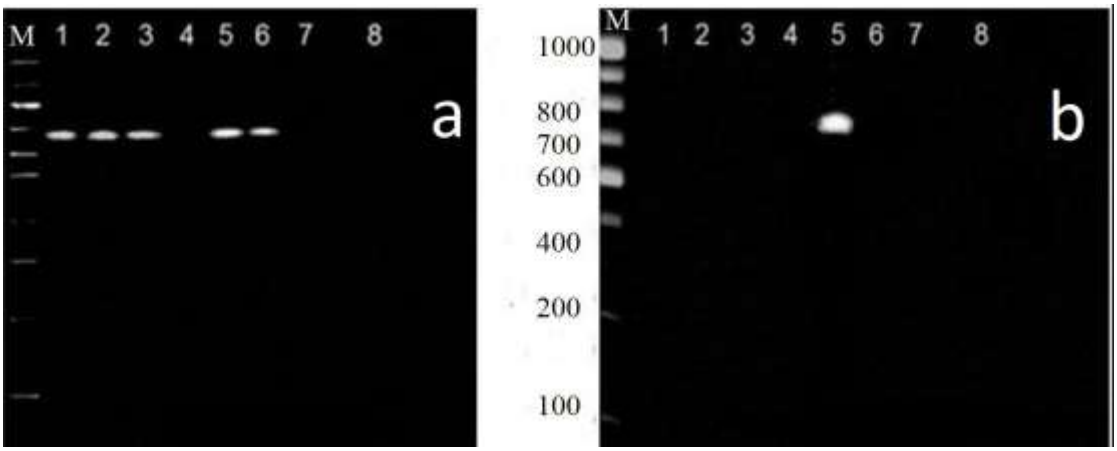

Figure 2. Amplification of (a) VCO1 and (b) VCO139 genes in dry bloom/scum samples. (M) 1000-100 bp DNA ladder (1) Khairy lake, Jabalpur; (2) Shahpura lake, Dindori; (3) Gangasagar lake, Jabalpur; (4) Salondi lake, Jabalpur; (5) Pariyat reservoir, Jabalpur; (6) Bahela lake, Jabalpur; (7) Mahegong lake, Jabalpur; (8) Samnapur lake, Jabalpur.

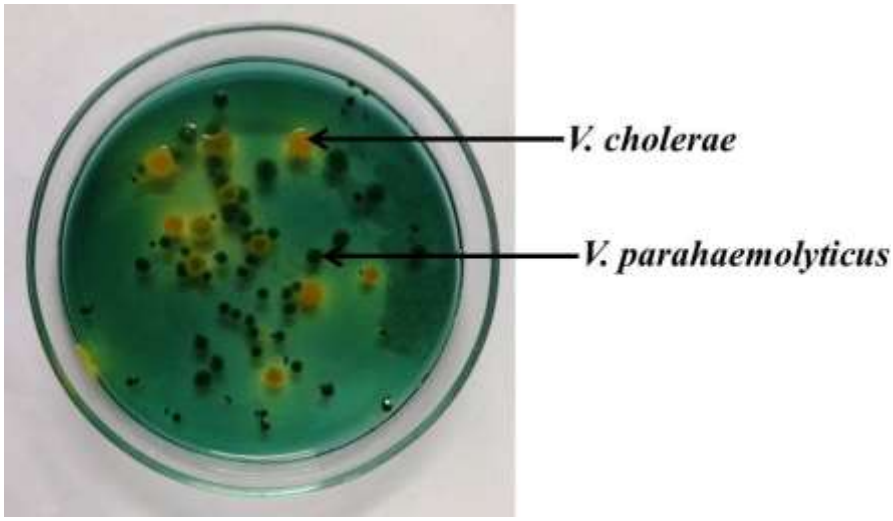

Figure 3. Bacterial colonies grown on TCBS agar medium isolated from Pariyat reservoir, Jabalpur. Large yellow colonies represent growth of *V. cholerae* and small green colonies represent *V. parahaemolyticus*.

indicate that VCO1 gene was amplified at 647 bp position from the DNA isolated from the scum processed from five lakes, namely Khairy lake (Jabalpur), Shahpura lake (Dindori), Gangasagar lake (Jabalpur), Pariyat reservoir (Jabalpur) and Bahela lake (Jabalpur). VCO139 gene was resolved at 741 bp position only in one water body, Pariyat reservoir (Jabalpur). However, in comparison to the above water resources, when DNA preparations from the remaining three water bodies, that is, Salondi lake (Jabalpur), Mahegong lake (Jabalpur), and Samnapur lake (Jabalpur) were used for amplification, there was no band discernable at either 647 bp or at 741 bp positions, suggesting that neither VCO1 nor VCO139 gene was amplified.

Morphological appearance of colonies of *Vibrio*

In Figure 3, two different morphologically distinct colonies were observed on TCBS agar. Based on the colony morphology (flat, diameter 3 to 4 mm, yellow) the isolates were designated as *V. cholerae*. The other colonies were small green with dark green center, and were identified as of *V. parahaemolyticus*.

The results of the biochemical tests are shown in Table 2, in which green coloured *V. parahaemolyticus* colonies gave pink red colour in methyl red test and dark pink colour in arginine dihydrolase test, suggestive of positive reactions. VP test turned out to be negative. On the other hand, yellow coloured *V. cholerae* colonies presented

Table 2. Biochemical tests' results for identification of *V. cholerae*.

Name of the test	Uninoculated	Inoculated with			
	Control	Yellow colour colonies		Green colour colonies	
Methyl Red test	Yellow	–	Yellow	+	Pink - red
Voges Proskauer test	Yellow	+	Pink red	–	Yellow
L-arginine dehydrolase test	Light pink	–	Light pink	+	Dark pink

+, Positive test (colour change to pink to red); -, Negative test (no change in colour).

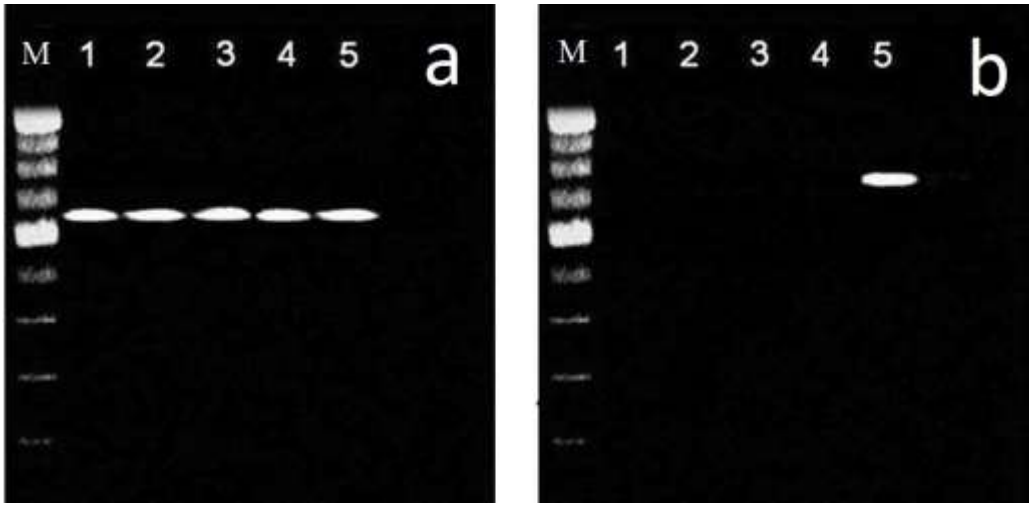

Figure 4. Amplification of (a) VCO1 and (b) VCO139 genes for identification of *V. cholerae* growing in TCBS agar medium cultivated from bloom/scum samples. (M) DNA ladder (1) Khairy lake, Jabalpur; (2) Gangasagar lake, Jabalpur; (3) Shahpura lake, Dindori; (4) Bahela lake, Jabalpur; (5) Pariyat reservoir, Jabalpur.

pink red colouration in VP test, and for the remaining two tests the strain gave negative results.

Amplification of VCO1 and VCO139 genes of isolated *V. cholerae* strains

Isolated colonies of *V. cholerae* were obtained from five water bodies, namely, Khairy lake (Jabalpur), Shahpura lake (Dindori), Gangasagar lake (Jabalpur), Pariyat reservoir (Jabalpur), and Bahela lake (Jabalpur). A single colony was used to isolate DNA for PCR amplification of VCO1/ 139 genes. Separate PCR's were run for randomly picked colonies and were used for amplification of the above genes. The results of representative colonies as presented in Figure 4 highlight that in DNA preparations from colonies of Pariyat reservoir, Jabalpur, there was an amplification seen at a position of 741 bp, suggesting presence of VCO139 strains. The same colonies from the same lake also exhibited visualization of another prominent band at *ca*. 647 bp, indicative of presence of VCO1 strains. In the rest of the DNA samples of all the randomly picked colonies from lakes other than Pariyat reservoir, Jabalpur, amplification was seen only at *ca*. 647, bp representing that only VCO1 strain of *V. cholerae* was isolated.

Effect of a metabolite extracted from *A. fertilissima* on *V. cholerae* strains O1 and O139

While surveying for presence of VCO1 and VCO139 strains in the lakes, it was found that in three lakes in which *Anabaena* scum was prevalent throughout the year, neither VCO1 nor VCO139 was detected in amplification experiments of the corresponding genes (*cf.* Figure 2). A hexane-extractable metabolite from *A. fertilissima* was used to determine if the cyanobacterium exert any antagonistic effect on aforementioned strains of *Vibrio*. The metabolite was incubated with the two strains of *V. cholerae*, O1 and O139. A clear zone of inhibition (6-9 mm) was observed in both the strains. In negative control, zone of inhibition was not observed (Figure 5), whereas in azithromycin positive control, it was ~9 mm.

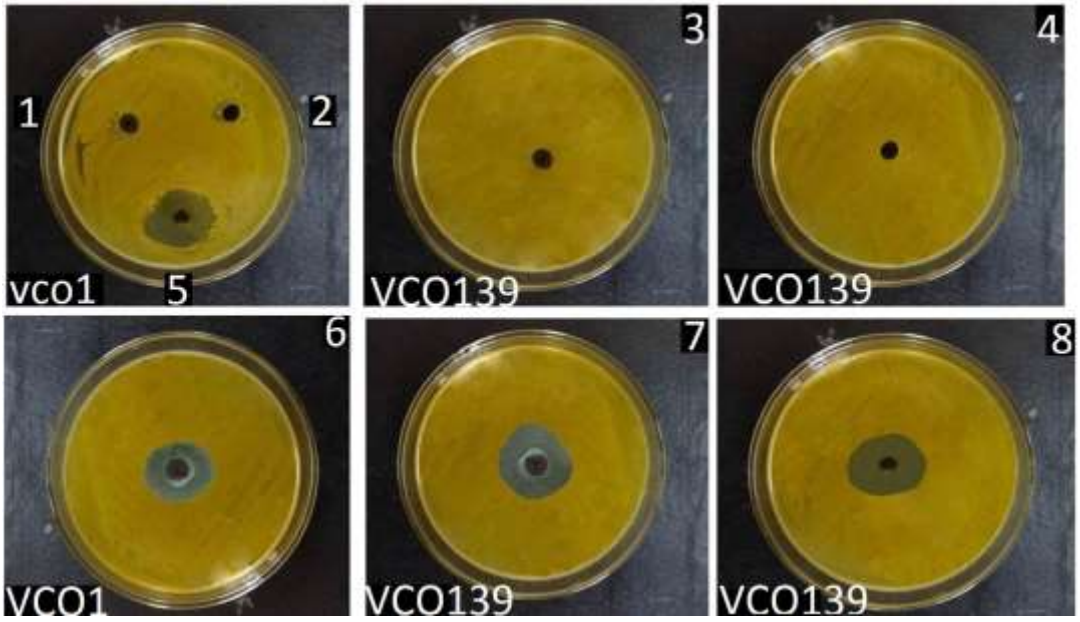

Figure 5. Effect of a hexane extracted metabolite of *A. fertilissima* on growth of *V. cholerae* O1 and O139 in bacterial lawns prepared on TCBS agar medium. Here well numbers 1, 2, 3 and 4 are the controls receiving solvent (10% methanol), 5 and 6 are the tests with cyanobacterial extracts in 10% methanol; and 7 and 8 are the positive control, with azithromycin in 10% methanol. The clearing zones are visible in positive control and with cyanobacterial extracts.

Effect of *A. fertilissima* cell mass on fate of *V. cholerae* counts in the "closed water system"

A simulated "closed water system" was designed in which lake water was poured, and to this added was a thick cell mass of *A. fertilissima* culture. Upon addition of *A. fertilissima* cell mass, there was a steady and significant reduction observed in the viable *V. cholerae* counts in the aliquots taken from the water system (Figure 6). Interestingly, within the same time period of experimentation, upon omission of *A. fertilissima*, the *Vibrio* counts remained steady and did not change significantly from what was the original value at start of the experiment (Figure 6).

DISCUSSION

In this study, data on a chemical-based antagonistic interaction between choleragenic *V. cholerae* strains and a natural isolate of a cyanobacterium from the same region was presented. Usually water blooms/scum provide ample substrate and nutrients for promoting bacterial growth but in certain situations they also exhibit deterrent effects as well. One such situation could be found in which in all those water resources e.g., Salondi lake (Jabalpur), Mahegong lake (Jabalpur), and Samnapur lake (Jabalpur) harboring *Anabaena* spp., there was no trace of culturable and non-culturable *V. cholerae* cells, as detected by (a) absence of colonies on TCBS medium and (b) negative amplification reaction for VCO1 and VCO139 genes. While, in the other water bodies, such as Khairy lake (Jabalpur), Shahpura lake (Dindori), Gangasagar lake (Jabalpur), Pariyat reservoir (Jabalpur) and Bahela lake (Jabalpur), with different cyanobacteria in their phytoplankton composition, both *V. cholerae* and *V. parahaemolyticus* was observed to thrive. The O1 and O139 antigens of *V. cholerae* are the etiological agents for epidemic and pandemic disease in this region (Sack et al., 2004) and therefore, it became important to examine as to whether *A. fertilissima*, an isolate from this region, can actually reduce the population size of this dreaded bacterium. A "closed water system" experiment gave reasonable proof that a lab-grown *A. fertilissima* cell mass indeed negatively affected the *V. cholerae* natural population size. A standard method was followed for bioassay-guided extraction of antibacterial metabolites from *A. fertilissima*. A hexane-extractable fraction showed strong antagonistic effect on the two pathogenic strains of *V. cholerae*. Although the nature of chemical-based interaction between the organisms is unknown as the putative antibacterial compound is yet to be purified, it can be predicted that one such candidate could be the cyclic peptide, called microginin previously isolated from lab-grown cultures of *A. fertilissima* along with a desmethyl-variant of microginin (Bagchi et al., 2016). Among other functions, microginins are also known for their

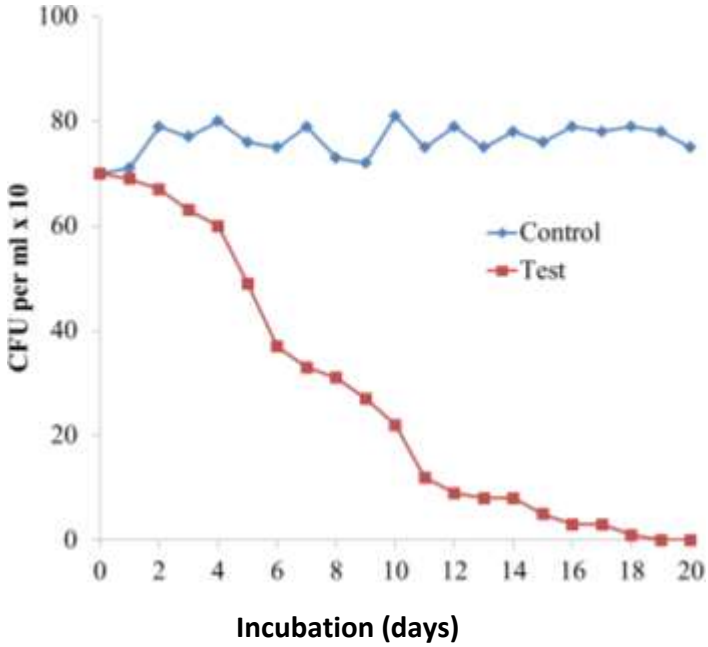

Figure 6. Effect of incubation with cell mass of *A. fertilissima* for 20 days on viable counts of *V. cholerae* in lake water in a "closed water system". Control panel, without and TEST panel, with cell mass suspended in the lake water. Periodically water aliquots were diluted and plated on TCBS agar to count the colonies that represent *V. cholerae*.

antibacterial effects (Silva-Stenico et al., 2010). Notwithstanding, compound(s) other than microginin could be responsible for the antibacterial effect. Future investigation is aimed at finding the exact chemical nature of the anti-*Vibrio* compound(s).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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