International Journal of Biotech Trends and Technology
ISSN: 2249 – 0183 / https://doi.org/10.14445/22490183/IJBTT-V12I3P603

Volume 12 Issue 3, 18-30, Sep-Dec 2022

Original Article

Microcystin-LR Exhibit Cytotoxicity in Myeloma Sp2/01 Cancer Cell Line and Emerging as a Potential Anticancer Therapeutics

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Received: 25 October 2022 Revised: 29 November 2022 Accepted: 16 December 2022 Published: 30 December 2022

Abstract *- Microcystis aeruginosa, widely distributed, produce the bioactive compound microcystin toxin, which induces liver cancer and poses many severe threats to human health. Microcystis aeruginosa produces secondary metabolites with broad pharmaceutical importance and biological activities in anticancer, antibacterial, antiviral and protease inhibition activities. New drug discovery is complicated, expensive, time-consuming and challenging for human life when treating disease, the new cutting-edge technology and the rapid growth of advanced computing bioinformatics tools for new drug identification and characterization, including anticancer therapies. In this work, the Microcystis aeruginosa strain was isolated from different regions of central India; the strain identification was based on morphological properties and mcyA, mcyB gene sequence analysis. The Cytotoxicity assayed against the myeloma Sp2/01 cancer cell line, 50 µl of Microcystin-LR (MC-LR) at 48 h; a marked cytotoxic effect was only detected after exposure to the highest toxin concentration (200 µl), and the MC-LR showed strong inhibition with an IC_{50} value of 29-39 µl. According to the findings, the MC-LR is a promising potential therapeutic anticancer compound, providing new leads for the characterization and development of a novel anticancer drug.*

Keywords *- Anticancer, Microcystis aeruginosa, Cyanotheraputics, Hepatic cancer, Therapeutics, Genetic diversity.*

1. Introduction

Cyanotoxins produced by cyanobacteria blooms occurring in continental aquatic ecosystems have increased in science last decades due to climate and anthropogenic change [7]. The problem of greatest concern is hepatotoxic cyanotoxins microcystins [11,10]. The effects of microcystin toxin on animals and plants have been extensively studied, and it has caused human poisoning worldwide [12]. The health problems are most likely related to chronic exposure to low concentrations of microcystin through consumption of contaminated water and food (agricultural products, fish, prawns, and mollusks), dermal exposure and inhalation, gastroenteritis and related diseases, allergic and irritation reactions, and some lesions can evolve. Therefore, the World Health Organization established a provisional reference value of 1 μgL^{-1} for microcystin-LR in drinking water [15]. Many jurisdictions have introduced specific water quality regulations to protect public health and safety [14].

Because strains or genotypes of the same species differ in their phenotypic responses to environmental cues (e.g., light, nutrients, colony formation), molecular methods such as nucleic acid sequence-based amplification of genes for organism detection, genotyping, and quantification have been studied for decades.[17]. The Microcystis aeruginosa mcy gene cluster consists of 10 genes, mcyA to mcyJ, nine of which encode catalytic domains for the production of microcystin [16]. The identification of the mcyA and mcyB genes, investigations of genetic diversity and phylogenetic relationships, and the function of these genes in the production of the microcystins toxin by Microcystis aeruginosa are all discussed in [17] and [18].

Microcystin has a 1000 Da chemical structure that includes seven amino acids in a ring formation with a unique -amino acid side chain (ADDA group). Cytotoxicity assays are a valuable tool in identifying compounds that might pose certain health risks in humans and developing new pharmaceutical products. The cells exposed to microcystins can react in various ways; they may lose membrane integrity and suffer cell lysis, stop growing and dividing, or suffer apoptosis. Microcystin-LR toxins in human liver cell lines show decreases in PP2A activity [19,1]. For the protein phosphatases of the Serine / Threonine (P.P.s) family, namely PP1 / PP2A, acting as inhibitors, these interactions are responsible for the cytotoxic effects of microcystins in animal cells [8,20,2]. Many studies [19, 3] reported microcystin-LR toxicity mechanisms on mammalian cells (rat hepatocytes or murine cell lines); additionally, Ikehara et al. [22] and Teneva et al. [21] discovered that the cytotoxicity response to microcystin-LR exposure was similar in human hepatocyte cell lines.

The most abundant bioactive metabolites produced by Microcystis aeruginosa are Microviridin toxin BE-4, which is used as an antibiotic and an anticancer [24]. Cyanotoxins are a rich source of natural cytotoxic compounds with the potential to target cancers by expressing specific uptake transporters. Their structure offers opportunities for combinatorial engineering to improve the therapeutic index and resolve organ-specific toxicity [25]. Microcystin's ability to inhibit the growth of cancer cell lines could lead to the development of effective anticancer drugs; the pharmacological and toxicological significance of the cyanobacterial genera Microcystis [23].

Bagchi [26] reported an increase in the incidence of cyanotoxins produced by the cyanobacteria Microcystis sp. and Arthrospira sp. in western Asia, including India. Many studies have reported Microcystis sp., Pharmidum sp., and Oscillatoria sp., in nutrients nitrogen (N) and phosphorus (P), rich in aquatics of various geographical regions, such as eastern and southern Asia and central India. Blooms pose an increasing threat to nutrient-rich lakes and fresh water, such as nitrogen (N) and phosphorus (P); resorcinol is widely used in drinking water and is required daily in central India [28, 28]. As a result, the central India region was chosen for the identification of cyanobacteria producing cyanotoxins and determination of cyanotoxins in different geographical areas such as Bhopal, Ujjain, Rewa, Gwalior, and Tikamgarh region of central India, the study of biodiversity of the occurrence of Microcystis aeruginosa in different aquatic habitats and bioactive metabolites microcystin toxins synthesis in different physico.

It is proposed that the current study provide necessary information on cyanobacterial genera producing cyanotoxins and the phylogenetic relationship of microcystin toxin-producing Microcystis aeruginosa in different geographical areas of central India, as well as study and characterize potentially toxic metabolites and describe their effects and modes of action.

2. Material and Method

2.1. Isolation and Identification of the Microcystis Strain

The samples were collected from different regions of central India and cultured in 200ml of BG-12 medium: $NaNO_{3}$-100 mg^{-L}, K_2HPO_4 – 10 mg^{-L}, $MgSO_4$ $7H_2O$-75 mg^{-L}, $CaC1_2$ $2H_2O$-40 mg^{-L}, Na_2CO_{3-20} mg^{-L}, Ferric citrate-6, Disodium EDTA. $2H_2O$-1 mg^{-L} , Vitamin B12-0.1 mg^{-L}, pH-8.0, [63]. Microcystis aeruginosa strains from various regions of central India were harvested during the exponential growth phase using a centrifuge at 6000 rpm for 5 minutes. and inoculated in triplicates in 250 ml of liquid medium BG-12 at pH 7.9 in a conical flask, placed at 26 20 °C under illumination with a cool light fluorescence tube (intensity approximately 50 K.W./cm2), and maintained under photoautotrophic growth conditions until the ninth day [30]. The *Microcystis* strain was isolated using an agar plate procedure, according to Rippka et al. [30]. The Microcystis strain was identified under a Liaca microscope at 100X. The *Microcystis* biomass culture for anticancer activity assayed was carried out in a 500ml conical flask at 26C °C with illumination by tube light of 300–400 lux on a 10:14 h dark: light cycle.

2.2. Genomic DNA Extraction

The genomic DNA extraction and PCR-based amplification of 16 srRNA, *mcyA* and *mcyB* genes were performed according to the method described by (Sambrook *et al.,* 2001). The PCR amplification of 16sRNA, mcyA and mcyB genes of *Microcystis aeruginosa* using primer target genes: *Microcystis*16S rRNA- CYA106F-GGGGAATYTTCCGCAATGGG, CYA781R-GACTACWGGGGTATCTAATCCCWTT [48], mcyA(*Microcystis*) gene: mcyA tet F-AAAAGTGTTTTATTAGCGGCTCAT, mcyA tet R-AAAATTAAAAGCCGTATCAAA [49], mcyB(*Microcystis*): mcyB 2959F-TGGGAAGATSTTCTTCAGGTATCCAA, mcyB 3278R-AGAGTGGAAACAATATGATAAGCTAC (Nonneman and Zimba, 2002). PCR was run in a PTC-100 thermal cycler (M.J. Research, USA), according to, Quahid *et al.* [50]. The PCR product is directly preceded for sequencing. The obtained sequences were analyzed by the BLASTn tool to get the relative identification of *Microcystis aruginosa* species.

2.3. Preparation of Microcystis Extract

The *Microcystis aruginosa* sample was harvested in the exponential phase (around 8–10 days of culture) by continuous centrifugation at 10000 rpm at 4°C for 15 min. and extracted after 10 10ml of methanol/acetone (1:1, v/v). The extract was concentrated under vacuum to the residue and stored at -20 °C until analyzed according to the modified method of Chauhan *et al*. [64].

2.4. LC-MS Identification and Quantification

Following that, the HPTLC analysis for the presence of microcystin compound results revealed the presence of microcystin in only 9 out of 10 isolates. The identification and determination of the ratios of the amounts of microcystin in potent planktonic Microcystis aeruginosa isolated from different geographical locations in central India were carried out by LC-MS (Agilent 1100 Series G1956A LC/MS System with DAD detector).

2.5. Data Analysis

According to the data of LC-MS analysis, the microcystins-LR, R.R. variants in the 10 *Microcystis aeruginosa* isolates were determined by LC-MS prominent ions, m/z=1,038.57 and m/z=995.68 result characteristics of microcystins-RR and L.R. respectively, based on patterns of peak comparison with microcystins standard published data

[52]. The concentrations of microcystin were calculated using the molar extraction coefficients for MC-RR and MC-LR [32].

2.6. Cytotoxicity Assay

The Microcystis extract was tested for Cytotoxicity against the Sp2/01 cancer cell line (Job no. 2031-01/02/2019) obtained from NCCS, Pune, 41107, Maharashtra, India, using MTT assay viability carried out using a modified method of Mosmann [53]. The MTT assay measures cell growth by utilizing the ability of metabolically active cells to reduce the dye to formazan. The cells are seeded into 96-well tissue culture plates at a density of $1X10^6$ cells per well in a 200 µl aliquot of the medium after successful harvesting. The seeded plates were incubated for 24 hrs at 37 ^{0}C and 5% CO2 in the humified atmosphere. The Microcystis extract was applied at final concentrations of 128; 32; 8, 3; and 0.5 l/mL/culture, and the cultures were inoculated for three days at 37 degrees Celsius with 5% CO2. Control wells were prepared by adding 200 µl of culture medium. Following exposure, cells were incubated for 72 hours at 37 oC in a 5.0% CO2 incubator. At the end of the incubation period, 10 µL of MTT (5.0 mg/mL in PBS) was added into each well, and the cells were further incubated for 4 h. Then 50ul of MTT was prepared at the concentration of 1mg mL^{-1} in FBS and added to the microculture wells. After 4hrs incubation, 250 µl supernatant was removed from each well, and 100 µl of DMSO was added and mixed thoroughly. Absorbance was measured at 540nm by a spectrophotometer. The IC50 (29 l) value was calculated based on the growth percentage. The photomicrographs were taken for cell morphological analysis using an Olympus CK40 inverted microscope coupled with an Olympus PM-20 camera. Cell viability was estimated as the percentage absorbance of the sample relative to the control, and optical density was determined at 570nm using a microtiter ELISA plate reader. The percentages of cell viability were calculated by the following formula:

Cytotoxicity (%) = $1X10^6$ – cell viability

Inhibition (%) = O.D. $_{control}$ – O.D. $_{sample}$ / O.D. control X 100

As $1X10^6$ cells were used in each and every well, $1X10^6$ =100%

2.7. Statistical Analysis

All statistical analyses of data were performed with SYSTAT software package-5.0.

3. Results and Discussion

3.1. Isolation and Identification of Microcystis

During the microscopy examination of samples, Microcystis aeruginosa was taxonomically identified. In addition, all cells were ovoid and spherical in shape; the variation of Microcystis aeruginosa colonies was observed between 40 and 90 m; different subtropical climates, rainfall, daylight, and temperature had different effects on the morphological and physiological characteristics of *Microcystis aeruginosa*. Similar observations were also proposed in *Microcystis* morphospecies [33,34], and colonies size variation (90- 100 µm) geographical distribution and climate conditions of aquatic habitats [35,36] also reported geographical distribution and climate conditions of aquatic habitats [37].

3.2 Detection and Quantification of Microcystin from Isolates

3.2.1. Detection of Microcystin compound by HPTLC

High-performance thin-layer chromatography (HPTLC) is a rapid, robust, and inexpensive method for detecting low concentrations of the microcystin compound in all *Microcystis aeruginosa* isolates. The HPTLC analysis results clearly identified the microcystin compound and revealed that the methenolic extract of *Microcystis aeruginosa* isolates, which eluted at two different major peaks at retention times of R.T. = 4,6 and 5.3 min, which are characteristic of microcystins-RR and L.R. respectively), revealed the presence of microcystins compound in all study sites. A large number of other peaks could also be observed in the HPTLC profile, but the nature of this compound could not be ascertained. These results were obtained through HPTLC and reported a positive response due to the environmental conditions of the different geographical locations of the study sites. The results revealed the presence of microcystin compounds in the water bodies, with microcystins-RR detected in study sites LWL, SRL, and LPD, and microcystins-LR detected in study sites MSL, HST, and MPT, GVL, RNT, and TKL.

3.2.2. LC-MS Quantification of Microcystin

Subsequently, the HPTLC analysis for the presence of microcystin compound revealed the presence of microcystin, but HPTLC was not more efficient in determining compound concentration in a biological sample; therefore, further samples were sent for Liquid Chromatography Mass Spectrometry (LC-MS); an exceedingly sensitive and specific analytical technique, to determine the identities and concentration of compounds within a biological sample. The present study revealed that LC-MS analysis results of microcystin in the isolates compared to an authentic standard were used to establish its identity by LC-MS comparisons. The two prominent ions, m/z=1,038.57 and m/z=995.68, showed characteristics of microcystins-RR, and L.R., respectively. The m/z for the presence of microcystins was observed for the putative microcystin peaks of the chromatogram by L.C.–M.S. The microcystin variants MC-LR in study site MPT, GVL RNT and TKL showed variation in between the range of m/z= 450 - 995.68. The highest peak m/z=995.68 showed that microcystin variants MC-LR and only study sites MPT, GVL and TKL recorded

the highest percentage concentration of MC-LR. In study sites, MSL and RNT recorded comparatively lower concentrations of MC-LR whereas, in the study sites, HST showed the range of m/z= 450 - 1,038.57, the highest peak m/z=1,038.57 showed that microcystin variants MC-RR chromatogram shown in (Figure 1). The detection of an array of microcystin compounds obtained from HPTLC and LC-MS analysis results found that *Microcystis* isolates were positively shown to have microcystin characteristics with different concentrations due to the environmental conditions of the different geographical locations of the study sites in central India.

The LC-MS detection of microcystin results reveal the concentration of microcystin variants was comparatively higher in this study site (microcystin-LR 85%, 0.76 gL-1 in study site MPT), similarly the presence of microcystin concentration was comparatively higher (microcystin-LR study site 85%, 0.77 gL-1 in GVL, and TKL 69.5%, 0.68 gL^{-1} The study site measured MSL microcystin-RR 29.05% (0.033μgL^{-1}) and HST microcystin-LR 19.8% (0.076 μgL^{-1}) which had a comparatively lower concentration. The two microcystins (MC-LR and -R.R.) were detected in the bloom samples, where MC-LR was predominant. The microcystins were referred to as total microcystin for each variant. Samples spiked with standards, and microcystins were identified based on m/z match and retention time. The presence of total microcystin variants in different sampling stations MC-RR represented 49.85% (21 mgL^{-1}) followed by MC-LR 42.17% (37 mgL^{-1}).

Overall, LC-MS analysis results revealed that only at study sites MPT, MSL, HST, GVL, RNT, and TKL were quantifiable microcystin concentrations found, as shown graphically in Fig. 1a and 1b. Microcystin-LR concentrations are common globally but well below the WHO-recommended safety level of 1 g/L. The geographical location of Gwalior, Tikamgarh, and Rewa microcystin abundance in bloom samples throughout the year indicate moderate health effects. The microcystin concentrations reported here are below the average values reported in the majority of previous studies [38,40]. The sample collected from natural aquatic habitats (rivers, lakes, ponds, dams, pools, tanks, and waterfalls), temperature, rainfall, microcystin concentrations, nutrient concentrations, and wind conditions, expressed either in terms of total toxins per litre of water or total toxins per cell varies due to the different geographical locations of the study sites. The relative proportions of microcystin toxin concentration and variants differed greatly between sample sites, according to LC-MS results, quantify microcystin variants MC-LR and MC-RR from natural waters containing Microcystis aeruginosa from different geographical locations such as Gwalior, Tikamgarh, and Rewa.

Similarly, MC-LR and MC-RR present in strains of *Microcystis aeruginosa* in natural water from India were also identified [42,43]. Srivastava *et al.* and Singh et al. [40,41] found concentrations of microcystins reaching a maximum of 1μgL^{-1} in lakes, ponds, and rivers of different geographical locations in central India, with reports from Germany and Portugal showing maximum toxin concentrations of 3μgL^{-1} [44] and water samples from Japan reaching 1μgL^{-1} in a number of different studies [45]. Studies report LC-MS for cyanotoxins in natural waters across Europe, including France, Italy, Ireland, and Germany, and describe microcystin concentrations <3 μgL^{-1} in tropic environmental conditions [47]. Similar studies reported that the variation in microcystin concentration depends on different geographical locations and is associated with high air temperatures and higher-than-expected rainfall and sunshine hours [46]. LC-MS data showed the variation in microcystin concentration with different geographical locations in central India, which is linked to temperature and meteorological parameters in study sites.

3.3. Molecular Identification of Toxigenic Cyanobacteria

It is incumbent upon scientists to raise policymakers' awareness concerning the increased abundance of toxigenic *Microcystis aeruginosa* cyanobacteria. Molecular methods help identify toxigenic cyanobacteria; they are reliable, cost-effective tools available for tracking toxigenic cyanobacteria in water blooms worldwide. Subsequently, the LC-MS analysis for detecting and quantifying microcystin in different isolates revealed the presence of microcystin in six of nine isolates. Therefore, these isolates (GVL, RNT, TKL, HST, MSL and MPT) were further processed for molecular identification and quantification of toxigenic *Microcystis aeruginosa*.

3.3.1. Quality and Quantity of Genomic DNA

The quality and quantity of isolated genomic DNA were evaluated by horizontal Agarose gel electrophoresis (1% of Agarose gel) and measuring absorbance at 260 and 280 nm in a spectrophotometer. Isolated DNA showed a distinct band of genomic DNA under U.V. transilluminator, indicating good quality of the isolated DNA of 1000 kb from *Microcystin aeruginosa* isolates (GVL, RNT, TKL, HST, MSL and MPT) shown in (Figure 2).

3.3.2 Molecular screening of toxigenic Microcystis aeruginosa

The all-potent *Microcystin aeruginosa* isolates (GVL, RNT, TKL, HST, MSL and MPT) showed excellent amplification of the 16S rRNA gene of 640 kb in all *Microcystis aeruginosa* isolates shown in (Figure 3. The 16S rRNA gene is the most frequently used molecular marker in microbial ecology studies to determine the total cyanobacterial community members or the members of a particular genus or species.

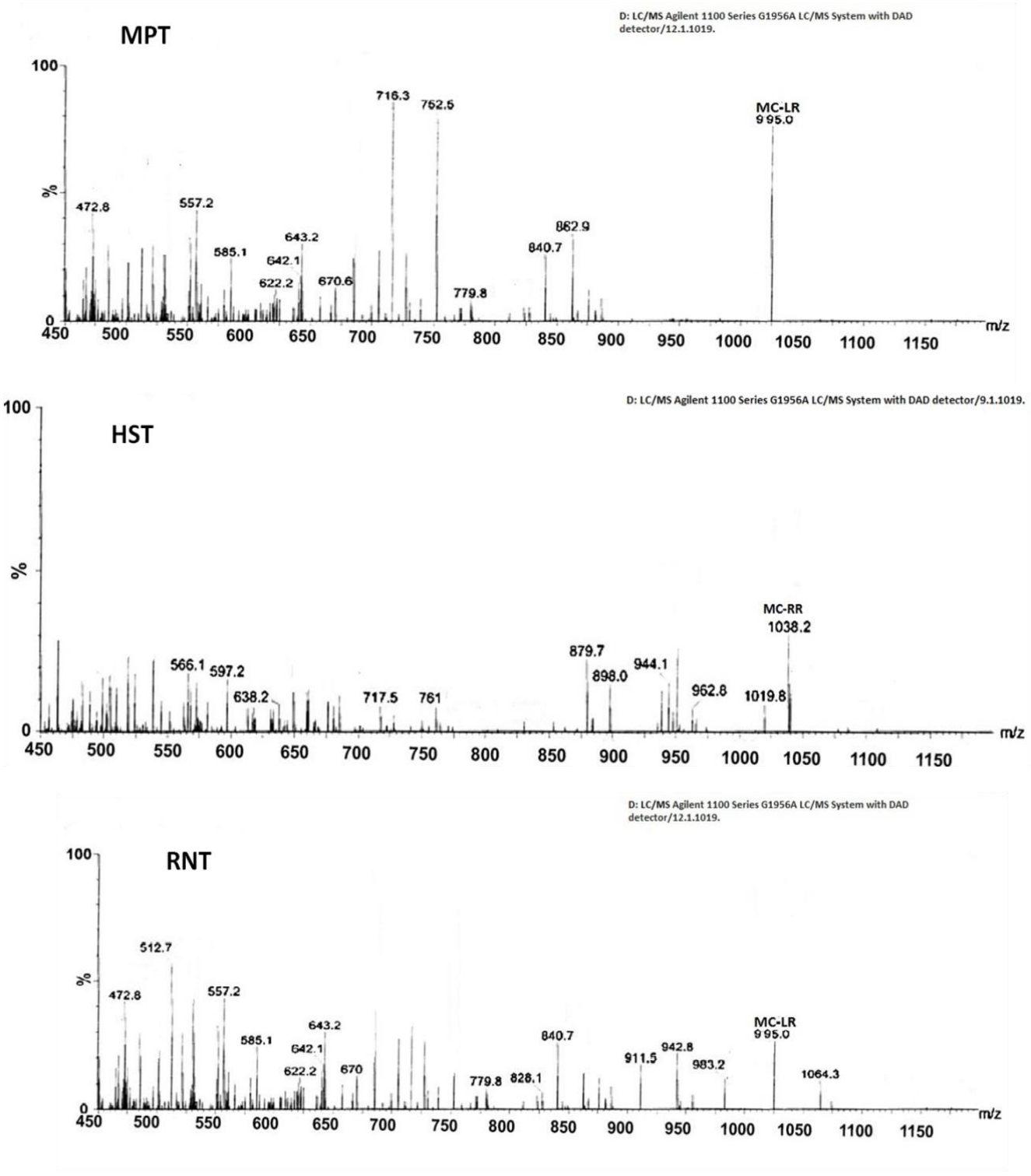

Fig. 1a LC-MS chromatogram of M. aeruginosa (MPT, HST and RNT).

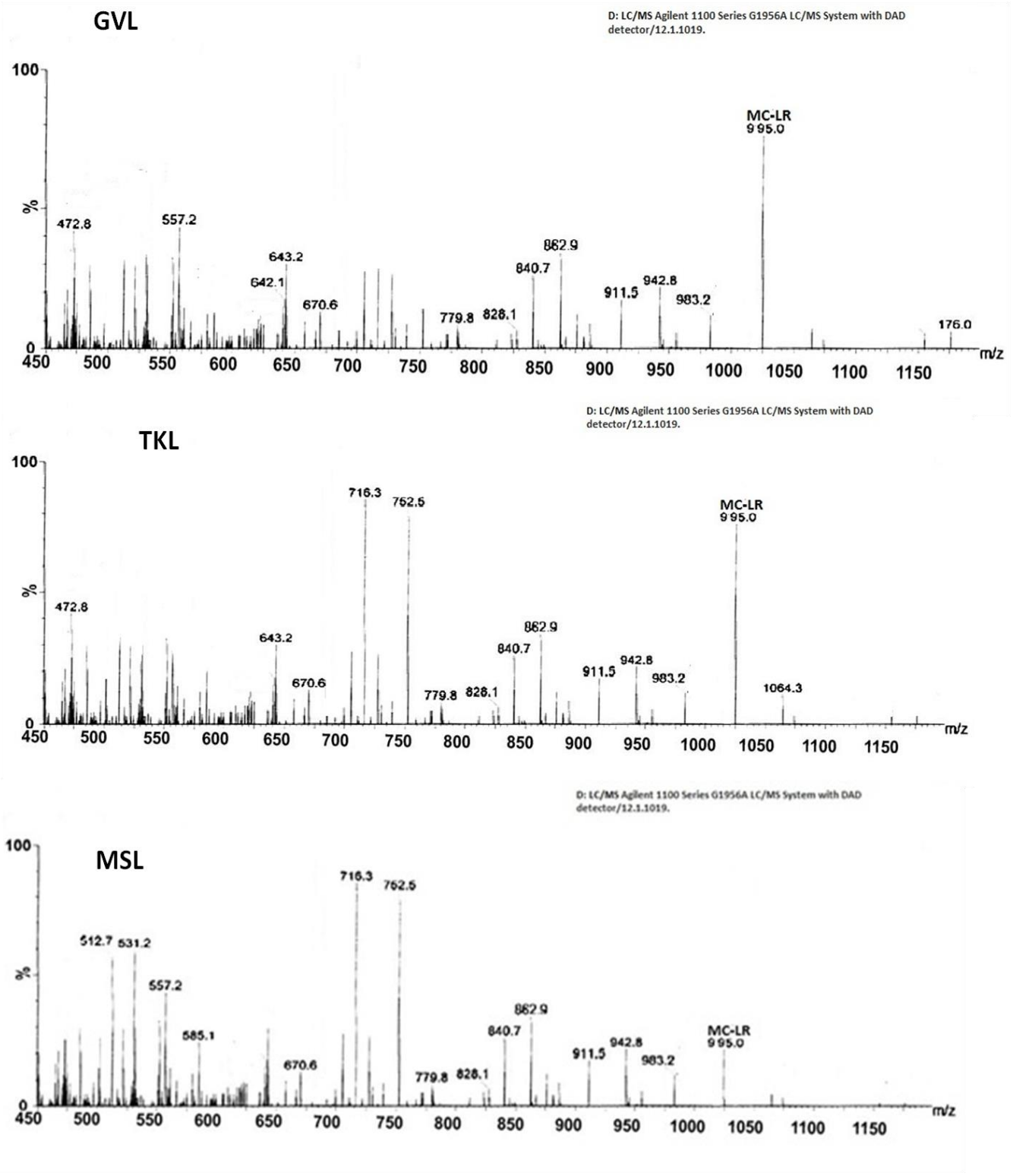

Fig. 1b LC-MS chromatogram of M. aeruginosa (GVL, TKL and MSL).

In fact, a phylogenetic study of the 16S rRNA gene of the *Microcystis aeruginosa* morphospecies led to the conclusion that *Microcystis aeruginosa* is phylogenetically closer to *Microcystis aeruginosa.* Similar studies on the phylogenetic relationship of *Microcystis aeruginosa* [40],[41].

The *mcyA* and *mcyB* gene sizes of various *mcy*A 230 kb and *mcy*B 240kb gene amplicons matched the sizes reported for other *Microcystis* sp. contain closely related strains of *Microcystis as was* evident from the molecular and morphological characters of the colonies. PCR was carried out to screen for the presence of the microcystin-producing genes m*cy A* and *mcy B* in *Microcystis aeruginosa* with gene-specific primers, which showed that all isolates had positive PCR bands (Figures 4 and 5). Positive PCR bands indicate the presence of the respective gene in the samples. Many studies reported PCR method is a fast way of detecting gene clusters and identifying structural variants in toxigenic *Microcystin aeruginosa* [54], [55], [56].

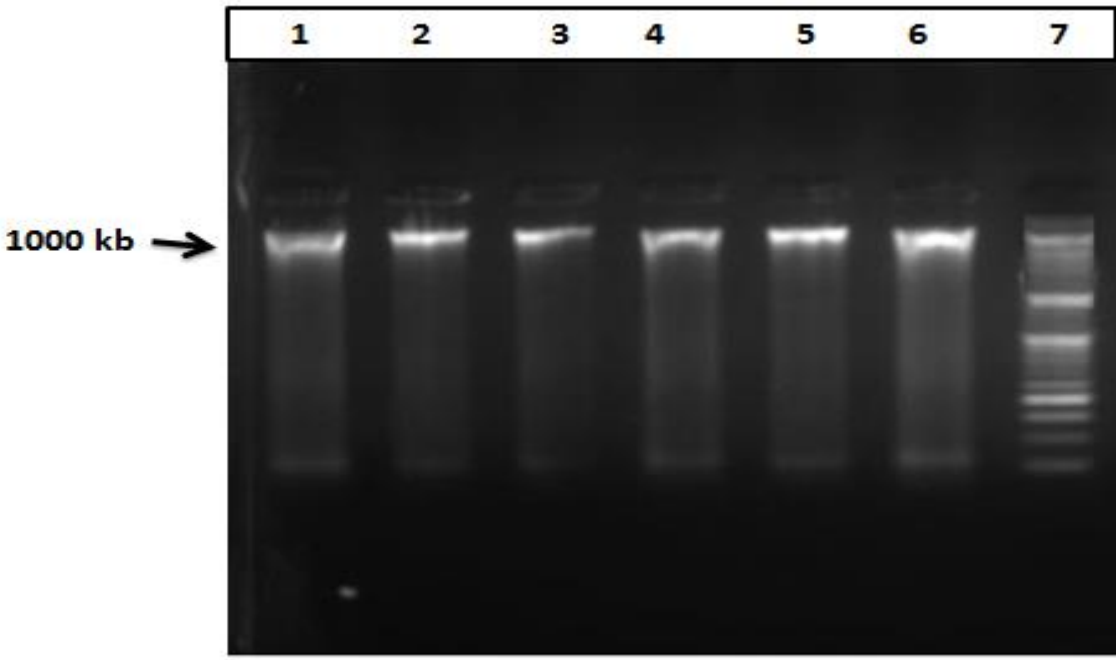

Fig. 2 Electrogrm of genomic DNA of isolated Microcystis aeruginosa strain, 1-GVL, 2-RNT, 3-TKL, 4-HST, 5-MSL, 6-MPT 7-Marker1.5kb.

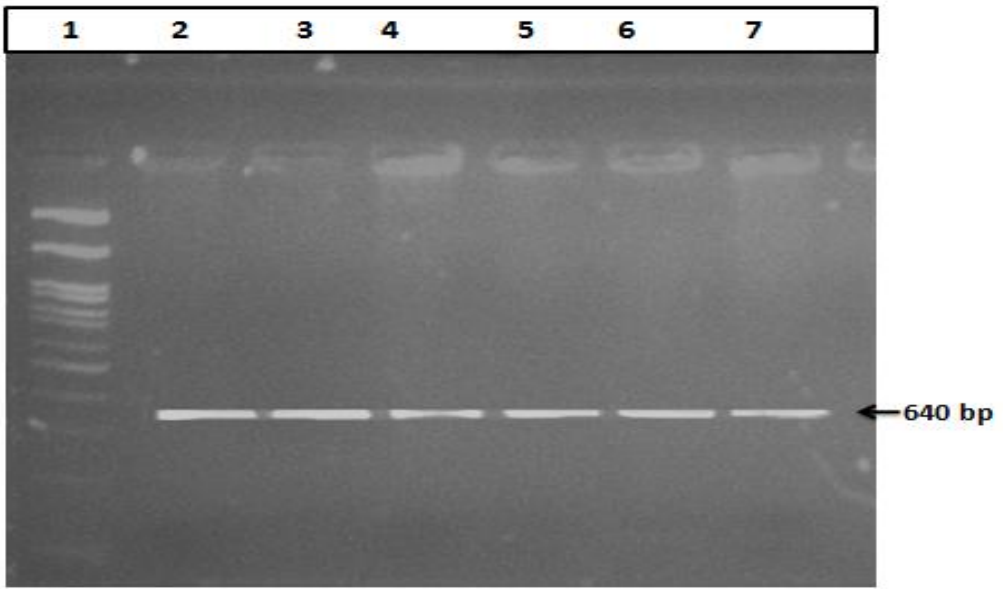

Fig. 3 Agarose gel profile of amplified 16 sRNA gene in Microcystis aeruginosa isolates, 1- Marker1.0kb, 2-GVL, 3-RNT, 4-TKL, 5-HST, 6-MSL, 7-MPT.

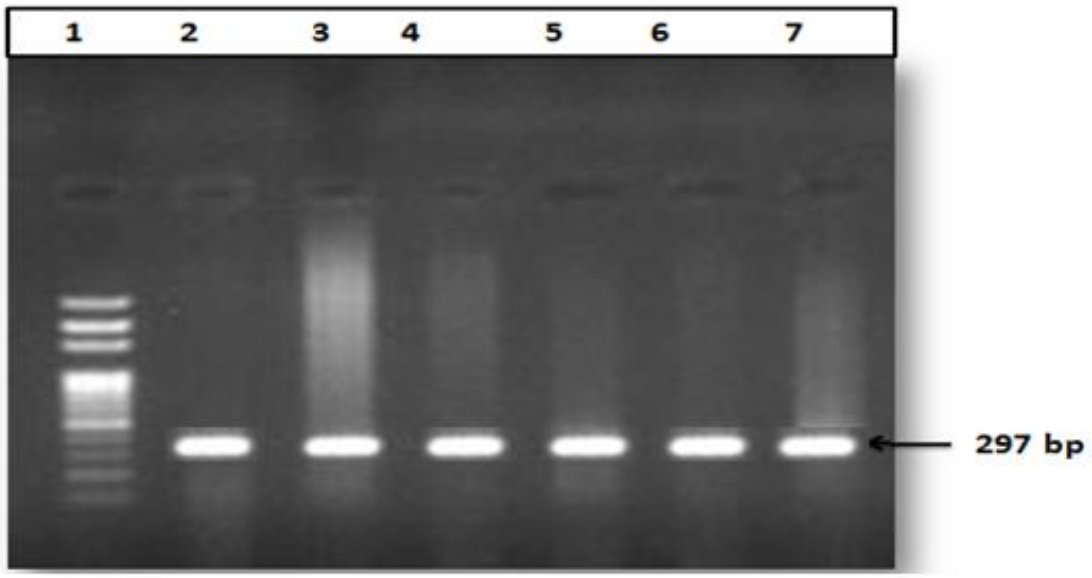

Fig. 4 Agarose gel profile of mcyA gene in Microcystis aeruginosa isolates, 1- Marker1.0kb, 2-GVL, 3-RNT, 4-TKL, 5-HST, 6-MSL, 7-MPT.

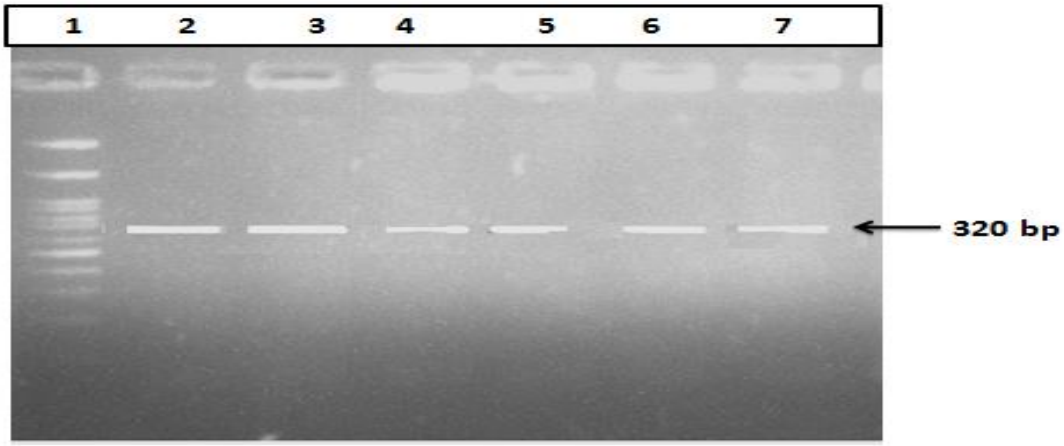

Fig. 5 Agarose gel profile of mcyB gene in Microcystis aeruginosa isolates, 1- Marker1.0kb, 2-GVL, 3-RNT, 4-TKL, 5-HST, 6-MSL, 7-MPT.

3.3.3. Homology analysis of 16s rRNA Gene

According to the BLAST results, *Microcystis aeruginosa* isolates (TKL, HST, MSL and MPT) sequences aligned with *Microcystis aeruginosa* strain N1ES2549 l6s RNA gene with 91% query coverage and 91% identities. *Microcystis aeruginosa* isolates (GVL and RNT) sequences aligned with *Microcystis aeruginosa* strain N1ES843, *Microcystis aeruginosa* strain PCC7806 N1ES84316S rRNA gene with 91 % query coverage and 97% identities. However, in the present study, Microcystis aeruginosa isolates (GVL, RNT, TKL, HST, MSL and MPT) have been aligned with available *Microcystis* strains with 98% query coverage and 71% identities. Sequence data from 16S rRNA sequence *Microcystin aeruginosa* has been submitted to Gene Bank, DDBJ accession numbers for 16S rRNA as GVL (LC473426), RNT (LC473427), TKL (LC473428), HST (MK894896), MSL (MK894897) and MPT (MK894898), respectively.

3.3.4. Relationships in the 16s rRNA Gene's Phylogenetic Tree

Sequences of the 16S rRNA gene from *Microcystis aeruginosa* Near-complete 16S rRNA gene sequences of the *P. aeruginosa* strains (LC473426, LC473427, LC473428), (MK894896, (MK894897), and (MK894898) were identified. These strains were received from isolates. The phylogenetic tree was recreated using an N.J. analysis based on matching all sequences with Microcystis sp. Clade I and clade II are distinct from one another in the corrected sequence alignment, which forms the basis of the

phylogenetic studies; Clade I contained all *Microcystis* species. All *Microcystis* species were found in Clade II and its members. The phylogenetic tree's bootstrap value for the similarity of the 16S rRNA gene to the Clade I and Clade II clusters was 60%. Due to this, the 16S rRNA [57,58]. Because of their shared genus (CPO1104 *Microcystis aeruginosa* NIES-2549, CPO12375 *Microcystis aeruginosa* NIES-2481, CPO20771 Microcystis aeruginosa PCC-7806), the similarity of the 16S rRNA genes of GVL, and RNT isolate between the *Microcystis sp*., Clade I and Clade II are distinct from one another. was almost 78%. The similarity of the 16S rRNA genes of TKL, HST, and MSL was about 98%. Still, Clade I and clade II are distinct from one another because they belong to the same genus CPO1104 Microcystis aeruginosa NIES-2549, CPO12375 *Microcystis aeruginosa* NIES-2481, CP009552 *Microcystis aeruginosa* NIES-843. [57,59].

3.4. Cytotoxicity activity of Microcystis Aeruginosa Crude Extracts when Exposed to the Mouse Myeloma Cancer Cell line Sp2/01.

Morphological changes of the mouse myeloma Sp2/01 cancer cell line after exposure to an extract having microcystin (LC473426) variants microcystin-RR, (LC473427) microcystin-LR, (LC473428) microcystin-LR, (MK894896) microcystin-LR, (MK894897) microcystin-RR, (MK894898) microcystin-LR. After 48 hours of incubation, the myloma Sp2/01 cancer cell line showed progressive damage; essentially, all myloma Sp2/01 cancer cell lines appeared to have some surface blebs that appeared irregular, demonstrated plasma membrane disruption, and were detached from the Matrigel-coated substrate; they were irregular, disrupted, and flattened (Figure 7a, b, c). Most of the control myeloma Sp2/01 cancer cell line (not exposed to the toxins) remained round and intact after 72 h incubation (Figure 7 a, b, c). Cells which are exposed to the extract (LC473426, LC473428 and MK894898) were found to be more effective for cell lines due to the presence of a higher concentration of microcystin, whereas *Microcystis aeruginosa* extracts (LC473427, MK894896 and MK894897) was responsible for causing damage to cell lines due to a lower concentration of microcystin. The effects of inhibition values near the standard value were found according to previous studies [60,61].

The current statistics demonstrate Cytotoxicity in terms of fatality percentages and support. When 200 ul of crude extract was applied to cancer cell lines, the aeruginosa strains (LC473426) 47%, (LC473427) 61%, (LC473428) 51%, (MK894896) 2.3%, (MK894897) 1.7%, and (MK894898) 1.2% were toxic. Microcystin-LR concentrations and exposure times in experiments significantly (p 0.001) reduced the effects of extracts from Microcystis aeruginosa. Although a significant reduction in cell viability was seen above 50 l of Microcystin-LR at 48 h, a marked cytotoxic effect was only detected after exposure to the highest toxin concentration (200 l). The lowest toxin concentration was required to induce a significant (p 0.05) and marked cytotoxic response (cell viability 50%) by the MTT assay. The cytotoxicity assessment of the cyanotoxins reported found an EC50 of 250 µl morphologically after exposure to microcystin-LR, and microcystin-LR was more sensitive than continuous cell lines [60], [62]. The results of the present study confirm that cyanotoxins are responsible for cell mortality at higher doses of exposure, i.e., 200 µl $1X10^6$ cells. Similarly, In the presence of NAFLD, MC-LR metabolism and detoxification are severely compromised; however, tailored antioxidant therapy may be able to restore these pathways[4].

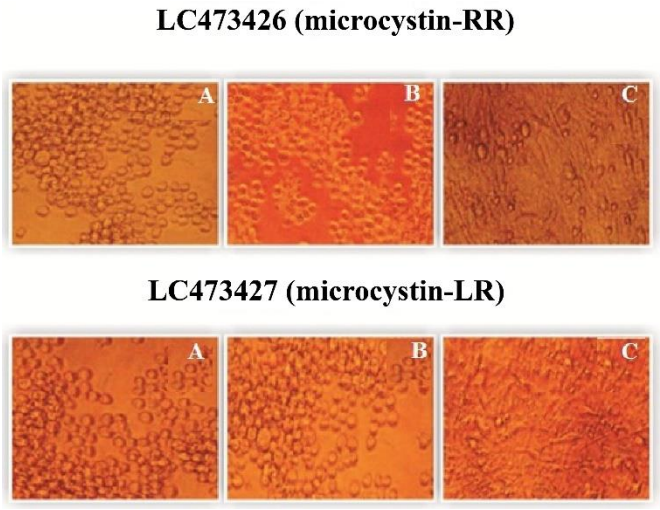

Fig. 7a Photomicrographs showing morphological changes as shown phase-contrast microscopy examinations of Sp2/01 mouse myloma cell line after exposure at 48 hrs of microcystin-RR, microcystin-LR, A – Control, B- 100 µl^{-ml}, (C) 200 µl^{-ml}, under 100X magnification.

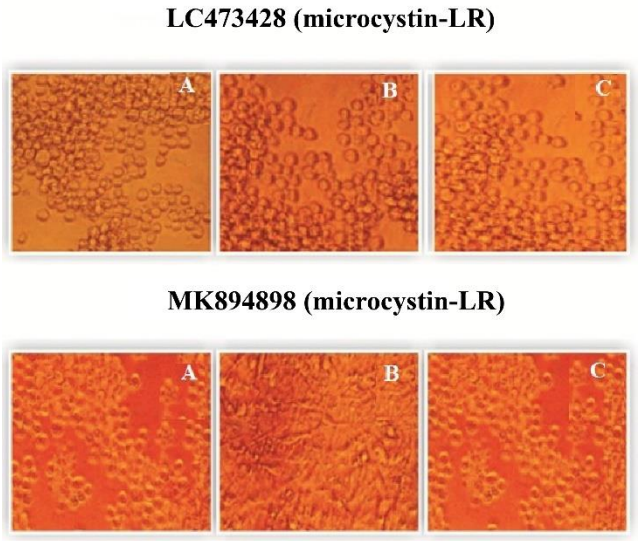

Fig. 7b Photomicrographs showing morphological changes as shown phase-contrast microscopy examinations of Sp2/01 mouse myloma cell line after exposure at 48 hrs of microcystin-LR, A –Control, B- 100 µl^{-ml}, C 200 µl^{-ml}, under 100X magnification.

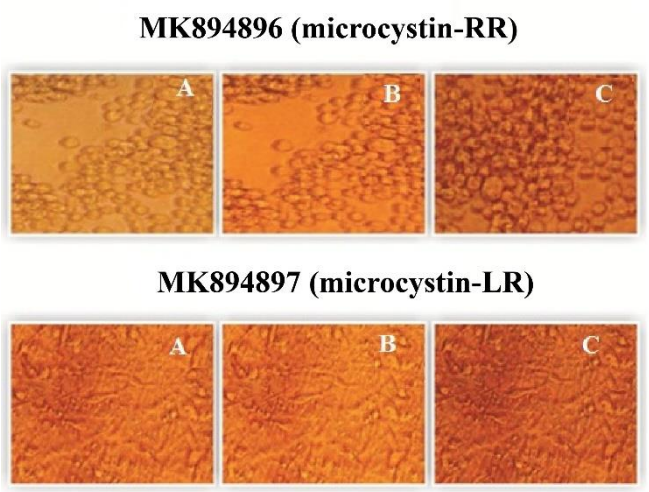

Fig. 7c Photomicrographs showing morphological changes as shown phase-contrast microscopy examinations of Sp2/01 mouse myeloma cell line after exposure at 48 hrs microcystin-RR and microcystin-LR, A – Control, B 100 µl^{-ml}, C- 200 µl^{-ml}, under 100X magnification.

Scientific interest in toxigenic Microcystis aeruginosa found in water produces toxins, and the production of extracellular toxin to aquatic environment lack sense because of difficult diffusion through the ecosystem and whether Microcystis aeruginosa from specific habitats possess the ability to produce microcystin toxin. Consequently, the high frequency of Microcystis aeruginosa with the intracellular toxin, microcystin toxins cytotoxicity response against myloma cancer cells was a more frequently observed feature; therefore, we suggest that Microcystin-LR can be exploited as a novel drug against cancer which can further be explored for its potency against various other cell lines which are fetal to humankind.

4. Conclusion

The results obtained during the experimentation have been presented, discussed and interpreted for the occurrence and abundance of microcystin toxins produced by *Microcystis aeruginosa* cyanobacteria and the quantification of microcystin toxins from different aquatics of different geographical locations of central India. Microcystin toxin concentrations in all study sites are comparatively not higher than the guideline the World Health Organization recommended, i.e. 1µg^{-L}. However, because backpacking and camping are common in the study areas from central India's different geographical locations, lakes could be used as drinking-water sources. Molecular genetics study results revealed that phylogenetic relationship analysis by using 16s rRNA of isolates results revealed that *M. aeruginosa* strain (LC473426), (LC473427), (LC473428), (MK894896), (MK894897) and (MK894898). The present study affirmed that only the *M. aeruginosa* strain (LC473426, LC473428 and MK894898) have microcystin-LR found to cause the toxic effect to myeloma Sp2/01 cell line. The impacts of cyanotoxins microcystin-LR few reported current information that is characterized as potentially therapeutic their effects and toxic mode of action in myeloma cancer cell line. Therefore it is suggested that microcystin compounds from isolates are novel targeted drugs for cancer research and pharmacological evaluation found, which is significant.

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