Genomic insight for algicidal activity in Rhizobium strain AQ_MP

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Abstract
Occurrence of Harmful Algal Blooms (HABs) creates a threat to aquatic ecosystem affecting the existing flora and fauna. Hence, the mitigation of HABs through an eco-friendly approach remains a challenge for environmentalists. The present study provides the genomic insights of Rhizobium strain AQ_MP, an environmental isolate that showed the capability of degrading Microcystis aeruginosa (Cyanobacteria) through lytic mechanisms. Genome sequence analysis of Rhizobium strain AQ_MP unraveled the algal lytic features and toxin degradative pathways in it. Functional genes of CAZymes such as glycosyltransferases (GT), glycoside hydrolases (GH), polysaccharide lyases (PL) which supports algal polysaccharide degradation (lysis) were present in Rhizobium strain AQ_MP. Genome analysis also clarified the presence of the glutathione metabolic pathway, which is the biological detoxification pathway responsible for toxin degradation. The conserved region mlrC, a microcystin toxin-degrading gene was also annotated in the genome. The study illustrated that Rhizobium strain AQ_MP harbored a wide range of mechanisms for the lysis of Microcystis aeruginosa cells and its toxin degradation. In future, this study finds promiscuity for employing Rhizobium strain AQ_MP species for bioremediation, based on its physiological and genomic analysis.

Keywords Harmful algal blooms · Microcystis aeruginosa · Rhizobium sp. · Scanning Electron Microscopy (SEM) · Degradation · Microcystins (MCs)

Introduction
Cyanobacteria are the photosynthetic organisms found in both freshwater and marine environment (Pal et al. 2018), which can be pelagic and benthic (Wehr et al. 2015). Although cyanobacteria have few positive trends, such as fixing atmospheric nitrogen but on the other hand, few species release toxic secondary metabolites such as dermatoxins, hepatotoxins, cytotoxins, and neurotoxins (Carmichael 2001; Pearson et al. 2010; Schmidt et al. 2014). Cyanobacteria have another ability to avoid predation by grazers (Lampert 1987). They can form elongated shapes, colonies and release some toxic secondary metabolites. The increased anthropogenic activities these days have increased the concentration of nitrogen and phosphate in water bodies which in a major contributor to the proliferation of algal bloom (Beaver et al. 2014). Algal bloom affects the entire water body by hindering light penetration and toxin release (Paerl et al. 2011; Pal et al. 2020). The recreational activities and drinking of these water affected by algal blooms often disturb animal health (Pal et al. 2020). Some of these algal bloom species release lethal toxins. The Microcystis species, such as M. aeruginosa, M. fosaqua, M. wesenbergii, M. ichthyoblabe, and M. phertaare are the leading cause of almost 90% of the harmful algal blooms in freshwater. M. aeruginosa is the most commonly observed cyanobacterial species causing harmful algal blooms and releases toxin, mainly microcystins (MCs) (Kim et al. 2019), which are neurotoxin and hepatotoxins. To date, more than 90 types of microcystins are released. Still, microcystin-LR is the most abundant and highly toxic variant (Pal et al. 2020). Toxic mechanism of MCs is due to the disruption of cytoskeleton formation and inhibition of protein phosphatase. In humans, MCs can enter through toxin-contaminated water or diet; they can cause oxidative stress, leading to cell damage (Beaver et al. 2014). Microcystins are readily water-soluble because their log of
octanol and water distribution ratio is approximately -1 (pH 7). The essential binding sites of microcystins to the protein phosphatase are methyl-dehydroalanine (Mdlha), and 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid (ADDA) groups. Microcystin toxicity depends on the amino acid combination present at the two different positions of the peptide ring. Microcystin-LR has amino acid leucine and arginine at the variable peptide ring, and Microcystin-RR has arginine and arginine (Rinehart et al. 1994; Corbel et al. 2014).

Zhang et al. (2017) reported an innate effective bacterium of *Sphingopyxis* sp. having microcystin-degrading capability. It contained enzymes of microcystin degradation, i.e., mlrA, mlrC and mlrD. The literature reported nearly 60 strains of Microcystin-degrading bacteria which were mainly categorized among 22 genera of three bacterial phylum (Actinobacteria, Proteobacteria, and Firmicutes), and widely belong to α- and β-Proteobacteria (Park et al. 2001; Zhang et al. 2011a, b; Bourne et al. 1996; Jiang et al. 2011; Rapala et al. 2005).

In addition, Pal et al. 2018 reported three bacteria showing algicidal activity, viz., *Rhizobium* sp., *Methylobacterium zanthini*, and *Sandaracinobactor sibiricus*, where *Rhizobium* sp. revealed both algicidal and microcystin degradation property. In Lake Taihu, *Stenotrophomonas* F6 showed intense algicidal activity contrary to prevailing cyanobacterial bloom species, mainly via; secretion of extracellular algicidal compounds (Lin et al. 2016). *Pedobactor* sp. showed algalytic activity against *Microcystis aeruginosa* by the secretion of mucous-like substance in a lake in Japan (Yang et al. 2012). Zhu et al. (2016) isolated *Rhizobium* sp. TH capable of degrading microcystin-LR under environmental condition. The associated gene cluster mlr, a microcystin-degrading gene, was cloned and verified by studying the heterologous expression. Mann et al. (2013) reported bacterium *Formosa agariphila* (KMM 3901T) has a broad potential of algal polysaccharide degradation. Similarly, in this study, bacteria was isolated which has algicidal and microcystin degradation property. *Rhizobium* showed algal lytic property as revealed via: interaction studies of *Rhizobium- Microcystis*; analyzed by SEM and the genome sequence analysis for genes encoding for microcystin degradative pathway and cyanobacterial polysaccharide lytic enzymes. *Rhizobium* genome sequence analysis confirmed the presence of pathways and genes responsible for algal lysis and also conserved region for microcystin degradation.

**Materials and methods**

**Isolation and cultivation of *M. aeruginosa* and *Rhizobium* strain AQ_MP used for interaction studies**

*Microcystis aeruginosa* was isolated from water samples of Ambazari Lake, Nagpur, Maharashtra. Culture was maintained by sub-culturing for 30–35 days as defined by Sangolkar et al. (2009). *M. aeruginosa* was poured first with the OD₆₇₈ 0.03, cultured in 250 mL of conical flasks using BG-11 medium under continuous cool fluorescent light (12:12 light and dark cycle, 3000 lx) at 25 ± 1 °C and mechanically shaken a day thrice. Different bacterial cultures were isolated, purified and characterized from the lake water sample published in previous studies (Pal et al. 2018). Fresh bacterial culture was prepared by inoculating *Rhizobium* strain AQ_MP into 250 ml conical flask containing 100 ml of Luria broth media and kept overnight in a shaking incubator (120 rpm, 30 °C) for 24 h. Growth curve of *Rhizobium* strain AQ_MP is shown in Supplementary Fig. 1.

**M. aeruginosa** cells lysis after exposure to *Rhizobium* strain AQ_MP

*Microcystis aeruginosa* culture was purified as axenic culture, by ultra-sonication followed by sequential antibiotic treatment, i.e., kanamycin (100 µg/ml), ampicillin (50 µg/ml), and imipenem (50 µg/ml), as per method reported by Pal et al 2018. Purified *M. aeruginosa* suspension (100 ml) was interacted with 10 ml of 1 OD₆₀₀ bacterial (Rhizobium) suspension. Two controls were set: one was *Microcystis* culture without Luria broth and another was *Microcystis* culture with Luria broth. The experiment was done for 10 days, where control and treated samples were collected every day. Samples (1 ml each) were centrifuged at 10,000 rpm for 10 min and fixed in 5% glutaraldehyde, and kept at 4 °C for Scanning Electron Microscopy (SEM) analysis. Cells (control and experimental) were centrifuged at 10,000 rpm and suspended and fixed with 5% v/v glutaraldehyde in 0.1 M phosphate buffer (30 min). Fixed control and experimental cells were centrifuged again, and the supernatant was discarded. Cell pellets were washed three times with 0.1 M phosphate buffer (15 min); dehydration was done with 35% ethanol (15 min), 50% ethanol (15 min), 75% ethanol (15 min), 95% ethanol (15 min), and two times with 100% ethanol (15 min). The gold coating of samples was done using Tescan SEM equipment. The cells were then examined in Vegag3 software, operating at 5.0 kV by a German scanning electron microscope (Kim et al. 2019). The total chlorophyll concentration was estimated for the algae-bacteria mixture for 10 days by a method described by (Gupta and Pawar 2018), wherein filtered dried biomass was extracted with 80% of ice-chilled acetone for estimation of chlorophyll using wavelength 663.2 nm and 646.8 nm. Cellulolytic activity of *Rhizobium* sp. using cellulose as substrate was determined by qualitative plate-zymography technique with Congo red staining (Bohra et al. 2019a, b), depicting cellulose as model substrate for Cyanobacterial polysaccharides (Supple Fig. 2).

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Whole-genome sequencing and annotation of functional genes: *Rhizobium* strain AQ_MP

The qualities and quantities of the extracted bacterial DNA were checked by resolving on 1% Agarose gel followed by NanoDrop. The gDNA was used as template for molecular identification by targeting the bacterial 16S rRNA gene using Sanger sequencing technique. PCR amplified fragment of the bacterial 16S rDNA with single distinct band of DNA was observed on the agarose gel and specific primers were used for sequencing reaction of 16S amplicons. For whole-genome sequencing, QC passed genomic DNA sample was used for the paired-end sequencing library preparation (after confirmation), using Illumina TruSeqNano DNA Library Prep Kit (Srinivasan and Rajamohan 2020; Tikaria et al. 2016). Gene prediction and functional annotation were done using Rapid Annotation using Subsystem Technology (RAST) server (Overbeek et al. 2014). CG Viewer server was also used to create a circular genome that allowed visualizing sequence feature information in sequence analysis outcomes. In this server only, PROKKA annotation was also done to merge CDS, tRNA, tmRNA and rRNA subunits. InterProScan database using Pfam system was used to check the presence of the genetic domain of microcystin protein family. dbCAN database was used to check the annotate CAZymes.

Result and discussion

Interaction study of *Microcystis aeruginosa* and *Rhizobium* strain AQ_MP

In this study, control and experimental *Microcystis aeruginosa* culture with *Rhizobium* strain AQ_MP culture were kept for 10 days to check the interaction and degradation of *M. aeruginosa* cells. After collecting samples, SEM analysis showed the contact between *M. aeruginosa* and *Rhizobium* strain AQ_MP cells. In 10 days of interaction, *M. aeruginosa* cells were lysed in flask experiments, as demonstrated in previous studies by Pal et al. (2018). It was observed that control *Microcystis* culture with and without Luria broth was not showing any degradation, also *Microcystis* cell numbers were increasing. But in the experimental setup, *Microcystis* cells were found to be lysed (Fig. 1). Many studies have already been suggested that *M. aeruginosa* have large mucilaginous aggregates comprised of a mucus substance called phycosphere. This mucous region typically comprises associated epiphytic bacteria (Kim et al. 2019). Zhang et al. (2011a), Gumbo and Cloete (2011) have already published some data on the interaction mechanism between bacteria and *M. aeruginosa*, depicting that the *M. aeruginosa* cell membrane gets damage, followed by the release of some extracellular substances. These extracellular substances serve as useful nutrients for bacteria growth. Such bacteria-cyanobacteria sometimes shows epiphytic relationships, where the dominant species flourish the most. Total Chlorophyll content was also estimated, which showed that the chlorophyll levels were decreased at the end of the 10th day (Supplementary Fig. 2).

Lysis of *M. aeruginosa* cells exposed to *Rhizobium* strain AQ_MP and its mechanism of action

Congo red assay showed a clear yellow zone indicating utilization of cellulose (Supplementary Fig. 3) by *Rhizobium* culture. It was further proved by the presence of CAZyme GH1 and GH5 (Supplementary Table 1), which remains solely responsible for cellulose degradation through Cellulase enzymes. Nobles, et al. 2001 reported the presence of cellulose in different Cyanobacteria as their cell wall building block component. In addition, Kim and Han (2003) studied cellulase as an algalytic enzyme. Thus, Congo red assay representatively verified the degradative potential of the isolated *Rhizobium* against Cyanobacterial polysaccharides. Further, SEM analyses showed the cellular interactions between the *Rhizobium* strain AQ_MP and *M. aeruginosa* cells. On the first day of experiment, *M. aeruginosa* was observed as a dominant organism. As the incubation continued, the *Rhizobium* strain AQ_MP cells emerged as a dominant organism indicating the decline of *M. aeruginosa*. Figure 1 shows a close interaction between the *Rhizobium* strain AQ_MP and *M. aeruginosa* cells from day second onwards. SEM image of day 5th and day 7th showed the ruptured *M. aeruginosa* cells. The ruptured cells release the intracellular component, which was utilized as a nutrient by *Rhizobium* strain AQ_MP. Control experiment showed the healthy and dominant *M. aeruginosa* cells after the 10th day. In supplementary Fig. 4, we hypothesized the actual mechanism of lysis between bacterial and *M. aeruginosa* cells. *M. aeruginosa* cells were surrounded and attached by *Rhizobium* strain AQ_MP cells, which destroyed the cell wall of the *M. aeruginosa*, resulting in cell lysis. Pal et al. (2018) had shown that *Rhizobium* strain AQ_MP cells utilized microcystin toxin as a carbon source. The present study advances the previous findings where *Microcystis* cell lysis occurred with increased bacterial numbers. Increased *Rhizobium* strain AQ_MP cells caused light hindrance, which affected the *M. aeruginosa* cell growth. The lytic enzymes (CAZymes) from *Rhizobium* strain AQ_MP cells targets the lysis of cell wall polysaccharides from *M. aeruginosa* after adhering on their surfaces. Cellulases (GH1 and GH5), Glucocidases (GH32), amylases (GH13), and polysaccharide lyases (PL1 and PL 5) are few bacterial extracellular enzymes that are reported as algal lytic enzymes (Supplementary Table 1) (Demuez et al. 2015). Lysed *M.
aeruginosa cells may serve as a nutrient for the growth of Rhizobium strain AQ_MP cells. The process thus continues further causing deterioration of M. aeruginosa cells in the presence of Rhizobium strain AQ_MP culture.

Whole-genome sequence statistics: Rhizobium strain AQ_MP

The filtered high-quality PE reads of the bacterial samples were assembled into scaffolds using SPAdes assembler (v-3.13.0). Nanodrop reading was observed as 152 ng/µl. Total data were 616 Mb; the total number of bases was 615,855,661, total number of reads was observed 2,069,397, total number of scaffolds was 122. The average scaffold size was (bp) 43,485. Max and min scaffold size was 620,217 bp and 200 bp. Whole-genome was submitted to NCBI/Genbank under the accession number JACJVI01000000 as Rhizobium strain AQ_MP. The degree of genomic similarity of the strain with closely related species was calculated using OrthoANI software, where average nucleotide identity values between closely related species were found ranged from 73.86 to 84.51%, which is lower than that of standard ANI cut-off for a new species is 95–96% (Lee et al. 2016; Yoon et al. 2017). It was found that the genome of Rhizobium rosettiformans have 84.51% relatedness to Rhizobium strain AQ_MP genome (Fig. 2). Two most closely related type strains with Rhizobium strain AQ_MP (Accession No. MF185100) include Rhizobium rosettiformans (Accession No. CP032405.1) and Rhizobium sp. (Accession No. CP058350.1). Using NCBI genome data, proteins/enzymes present in the genome were downloaded from NCBI. RNA from genomic FASTA (fna) file was downloaded from NCBI website and checked for the 16S rRNA gene relatedness with the old 16S rRNA partial sequence submitted to NCBI with the accession number MF185100 (Pal et al. 2018). It was found that Rhizobium strain AQ_MP genome has three 16S rRNA sequences. One sequence was used to prepare a phylogenetic tree with Rhizobium sp. (Acc. No. MF185100) partial sequence, and other related sequences showed the best match in BLAST search (Supplementary Fig. 5). The 16S rRNA sequence present in the genome was closely associated with the 16S rRNA partial sequence submitted by Pal et al. 2018. FASTA file downloaded from NCBI was then uploaded into the RAST server to check the M. aeruginosa lytic and microcystin degradative pathways. CG viewer server database results showed a circular representation of the Rhizobium strain AQ_MP genome. From outward to inward: ORF (circle 1), CDS (circles 2 and 3), GC skew (circle 4), GC content (circle 5), and ORF (circle 6) are shown in Fig. 3. RAST server data results depicted the presence of
mlrC gene sequence from 184,197 to 185,678 in scaffold 2. Size of the sequence was found 1482 bp and 494 aa (Fig. 4). Some mlr (microcystin-degrading gene) genes from NCBI were compared with conserved regions of *Rhizobium* strain AQ_MP, and match was observed and checked in Pfam and InterProScan (Mitchell et al. 2019; Finn et al. 2017). The result of InterProScan for the *Rhizobium* strain AQ_MP conserved genome sequence is represented in Fig. 4. At domain level, target conserved genome sequence were classified under the protein family IPR009197 and domain IPR015995, IPR010799. This protein family was nearly related to mlrC domain. This family signifies the C-terminus of a bacterial gene cluster product associated with the degradation of the toxin microcystin and is encoded in the mlr gene cluster. Phylogenetic relationship of selected mlrC sequence (from NCBI) was compared with mlrC sequences of *Rhizobium* strain AQ_MP (Fig. 4 C). Glycosyltransferase gene was also compared as outer protein family. It was seen that *Rhizobium* strain AQ_MP mlrC conserved region was related to *Sphingopyxis* sp. and *Sphingomonas* mlrC genes.

**Microcystin degradative pathway**

Analysis of the whole-genome sequences depicted that *Rhizobium* strain AQ_MP followed a Glutathione metabolic pathway (Sies et al. 1980) for the degradation of microcystins, in which glutathione-S-transferase (gst) (MBC2773493.1) and gamma-glutamyltransferase (tgm) (MBC2775265.1) enzymes were present. Microcystin has ADDA and Mdha site in which in the Mdha site, glutathione was attached and formed Microcystin-RR-glutathione (Beaver et al. 2014). Due to the presence of Gamma-glutamyltransferase enzyme Microcystin-RR-glutathione, get cleaved, and gamma glutamic acid was released, which leads to the formation of Microcystin-RR-cysteine-Glycine (Wang et al. 2018; Lance et al. 2014). Cyc-gly Dipeptidase (dug) was used to cleave the gamma-glutamylecysteine intermediate’s glycine to get the cysteine-conjugated product, as a result, oxidized by acetyl transferase-acetyl co-A (acat) to form the mercapturic acid metabolite (Manahan 2003), which is Microcystin-RR-Mercapturic acid (Fig. 5). Another pathway was found to degrade microcystin, wherein *Rhizobium* strain AQ_MP, three enzymes jointly denoted as microcystinase operate in a sequential pathway to degrade MC. The first enzyme Microcystinase C precursor (mlrC)
linearizes microcystin through the cleavage of the peptide ring at the ADDA-arginine bond. The second enzyme, linearized microcystinase (mlrB) cleaves this linear intermediate at the alanine-leucine bond, yielding a peptide intermediate of ADDA-Glu-Mdha-Ala (Goldberg et al. 1995). The final enzyme degrades the products formed by the first two enzymes and releases ADDA from the tetrapeptide intermediate (Fig. 5). Genes denoting linearized microcystinase (mlrB) was the conserved region of beta-lactamase (scaffold 22, sequence = 33,495–35,177), was annotated via RAST, InterProScan, and checked via Uniprot identity (supplementary Fig. 6).

**Polysaccharides degradative enzymes**

CAZymes are essential enzymes for polysaccharide degradation (Srivastava et al. 2020). dbCAN meta server data showed the presence of many CAZyme in the Rhizobium strain AQ_MP genome such as 147 Glycoside hydrolases (GH), 140 Glycosyltransferases (GT), 15 carboxylesterase (CE), 13 carbohydrate-binding modules, and four polysaccharide lyases (PL). Supplementary Table 1 is showing different CAZymes present in the Rhizobium strain AQ_MP genome. It showed distinct specificity for polysaccharides (Xing et al. 2015), glycoproteins, and proteins' degradation, which are required for the growth of cyanobacteria. For the degradation of the algal polysaccharides, PLs and GHSs are anticipated, including chitin, agarose, fucoside (N-linked glycan), fucoidan (fucose-containing sulfated polysaccharides), rhamnogalacturonan, homogalacturonan, starch (glucan), and xylan. Among these, agarose is a unique cell wall polysaccharide; rhamnogalacturonan and homogalacturonan are the pectic compounds generally present in the red seaweed cell walls; likewise, xylan is present in the green algae, red algae, and plant cell walls. The presence of these CAZymes in the Rhizobium strain AQ_MP suggested the better degradation potential of cyanobacterial polysaccharides. PL0 and PL 5, alginate lyases indicated that bacteria could cut down alginate into different oligosaccharides or monosaccharides (Zhu et al. 2016). The annotated agarase (GH16) and galactosidase (GH2) likely degrade agarose (Hehemann et al. 2010). Endo-1,4-glucanase (GH5) and Glucosidase (GH32) are cellulose degraders (Taylor et al. 2006). Chitin can be hydrolyzed by Rhizobium strain AQ_MP with hexosaminidase (GH19) and chitinase (GH16). The annotated L-fucosidases (GH65 and GH33) can degrade fucoidans and fucosides (Ale et al. 2011). Homogalacturonan could be degraded by polygalacturonase (GH28). Two bi-functional enzymes, i.e., xylosidase/L-arabinofuranosidase (GH3), are expected to degrade arabinoxylan hemicelluloses (Lee et al. 2003). The unsaturated rhamnogalacturonyl hydrolase (GH105), L-arabinofuranosidase (GH3), and L-rhamnosidase (GH78) can be
degrade rhamnogalacturonan (Weiner et al. 2008). Starch can be hydrolyzed with glucosidases (GH36) and amylase (GH13). The putative xylosidase (GH38 and GH43), endo-1,4-xylanase (GH43), and L-arabinofuranosidase (GH3) can degrade xylan. 540 transporters were also found, of which 353 were ABC transporter. Transporter proteins are also responsible for the degradation of microcystins. PhzF family phenazine biosynthesis protein (MBC2772314.1) was also found in the genome related to algal biodegradation (Dakhama et al. 1993). These loci play a pivotal role to degrade algal polysaccharides and microcystins. In most bacterial genomes, CAZymes typically account for 2% of the genes and occasionally exceed 5% for those bacteria which are specifically responsible for carbohydrate degradation. This requires the niche-adapted microbial experts for the rigorous breakdown of composite polysaccharide mixtures present in nature. In bacterial systems, polysaccharide degradation genes are organized in larger regulon and operon structures, termed polysaccharide utilization loci (PULs). PULs encode many transcriptional regulators, sulphatases, transporters, and carbohydrate-active enzymes (CAZymes). CAZymes are enzymes for deterioration and synthesis/modification, polysaccharide binding/recognition in glycoside hydrolases (GHs), carbohydrate-binding modules (CBMs), carbohydrate esterases (CEs), polysaccharide lyases (PLs), and glycosyltransferases (GTs) (Srivastava et al. 2020). Above-mentioned description suggested that these loci play a crucial role in polysaccharide bio-deterioration. The Rhizobium strain AQ_MP genome revealed all the features to reduce a wide range of cyanobacterial polysaccharides.

RAST server data exhibited the presence of siderophores in the Rhizobium strain AQ_MP genome. Iron is a crucial element essential for key biotic processes. The common bacterial groups necessitate iron for existence and progression. Bioavailability of Iron is limited, which is a persistent source of pressure in many biological structures. There is accruing indication that Fe restricts phytoplankton biomass in the equatorial Pacific (Wells et al. 1994), the North Pacific gyre (Martin et al. 1989; Liu et al. 2014), and the Southern Ocean (Helbling et al. 1991). For microorganisms, obtaining iron is a significant challenge: capturing and integrating iron governs their existence. Cyanobacteria and algae, particularly responsible for biomass’s primary production, require ten times higher iron content than non-photosynthetic prokaryotes (Brand 1991). Bacteria, fungi, microalgae, and many higher plants have established specific approaches for low iron bioavailability. Siderophores secretion is one of the approaches among them. Siderophores are the molecules that chelate iron with high affinity (Guerinot et al. 2008).
Siderophores extracellularly solubilize the iron from minerals of organic substances and transport them into cells when there is iron deprivation. Photosynthesis and capturing light energy are closely related to photosynthetic pigments. In photosynthesis, iron plays a crucial role in chlorophyll-a production (Imai et al. 1999). Studies say cyanobacteria require higher iron uptake than other algae (Brand 1991). Liu et al. (2014) have suggested that due to the presence of siderophores, photosynthetic pigment synthesis was inhibited in M. aeruginosa. In this study, Rhizobium strain AQ_MP is connected to siderophore release, which indicated the inhibition of photosynthetic pigment synthesis. It could be responsible for inhibiting M. aeruginosa growth due to the low bioavailability of iron (Martin et al. 1989). In previous studies, it was observed that bacteria and cyanobacteria tend to compete in freshwater for the low bioavailability of iron (Liu et al. 2014). Protein FASTA sequences of these loci were downloaded from NCBI and were submitted to Phyre software, and Pymol generates the structures of different siderophore and CAZymes (Supplementary Fig. 7). To control algal blooms, significant removal of nitrogen sources through denitrification pathways have been highlighted and reported by Jiang et al. (2020). Rhizobium strain AQ_MP harbored denitrification pathway genes (Supplementary Fig. 8) illustrating their role in removal of nitrogenous sources and thereby inhibit cyanobacterial growth, as
nitrogen acts a good source for the growth of cyanobacteria. Thus functional denitrifying genes existed in the genome, which includes nar, nir, nos and nor, where nir gene is responsible for converting nitrite to ammonia (Tikariha and Purohit 2019).

**Conclusion**

*Rhizobium* strain AQ_MP was isolated from the lake water, which showed the lysis of harmful Cyanobacterial species *Microcystis aeruginosa*. Scanning electron microscopy (SEM) and chlorophyll estimation revealed the algicidal property of *Rhizobium* strain AQ_MP. Genome analysis predicted that *Rhizobium* strain AQ_MP have secretion ability for extracellular substances like CAZymes responsible for algal polysaccharide degradation. The SEM analysis, presence of glutathione metabolic genes, toxin (microcystin) degradative pathways and functional gene clusters for polysaccharide degradation (CAZymes) ensured the algal lytic characteristics of *Rhizobium* strain AQ_MP.

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**Declarations**

**Conflict of interest** All the authors have mutually agreed to submit the manuscript to this journal and declare no conflict of interest.

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