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Full Length Research Paper

Molecular Characterization of Lytic and Lysogenic Genes of Cyanophages Infecting *Microcystis* spp.

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Abstract
Microcystis spp. forms massive blooms in eutrophic freshwaters and cyanophages exist in large quantities in marine and freshwater ecosystems. Complete coding sequence of genes responsible for lytic and lysogenic production in cyanophages infecting Microcystis spp. was amplified and obtained. After the RNA was reversely transcribed into the cDNA, reverse transcribed total was then subjected for the amplification of the g91, g135 and g136 genes Sequence alignment analysis showed that the coding sequence of the isolated g91 gene was 99 % identical to the g91 gene of AB231700 and 95 % identical to the g91 gene of AB690499.Thegene g135 was 99 % identical with that of AB231700 and 98 % identical to KF356199. The gene g136 was 97% identical with that of AB231700 and 94 % identical to AB543094. The conclusion is that the presence of lysogenic associated genes within cyanophages might explain how these cyanophages can coexist with the Microcystis spp. in the aquatic streams. The dynamics between cyanophages and Microcystis spp. needs more investigation.

Keywords: *Microcystis, Cyanophages, g91, g135, g136, Lytic, Lysogenic.*

Introduction

Microcystis spp. forms massive blooms in eutrophic freshwaters, where it is constantly exposed to lytic cyanophages (Morimoto *et al.,* 2018). Cyanophages exist in large quantities in marine and freshwater ecosystems (Bergh *et al.,* 1989).They are typically small entities measuring in the range 20–200 nm, even though the discovered Mimivirus is estimated to be 400 nm in diameter (La Scola*et al.,* 2003). Cyanophages consist of nucleic acid - either DNA or RNA, single or double-strandedsurrounded by a capsid which is a protein. Some may also contain lipids (Alberts *et al.,* 2002).They infect living organisms to replicate, which ultimately results in cell lysis. Therefore, they play important roles in nutrient cycling and microbial community structuring (Fuhrman, 1999, 2000). Also cynaophages play important roles inregulating the abundance of host populations and catalyze themovement of nutrients and organic carbon from host cells to pools of dissolved and particulate organic matter a process referred to as a *"viral shunt"* (Suttle, 2007).

There are various ways of cyanophage infection. These include lytic infection, lysogeny, and pseudolysogeny (Dimmock *et al.,* 2001). The two main modes of infection are lysis and lysogeny. Phages may have a lytic or lysogenic cycle although a number of them are capable of carrying out both propagation strategies. Lytic phages such as T4 lyse bacterial cells causing death of the host, following viral replication and assembly of the virion progeny. Lytic infection occurs when the virus attaches to the host cell, injects its genetic material and uses the host replication machinery to produce progeny viruses. Lysogeny in phage occurs when, after injection, the phage nucleic acid forms a stable association and incorporates into the host genome (Ackermann and DuBow, 1987).

The phage genetic material is replicated along with that of the host. It has been theorized that lysogeny dominates when the host abundance is too low due to low resources or high viral lysis rates (Hennes *et al.,* 1995; Miller and Ripp, 1998, 2002; Weinbauer*et al.,* 2003). Phage can persist in this lysogenic state for several generations until an environmental or biochemical 'trigger' event occurs, which causes the phage to switch to the lytic pathway (Sode*et al.,* 1994; Brüssow*et al.,* 2004; Sime-Ngando and Colombet, 2009; Stough *et al.,* 2017).

The cyanophage, Ma-LMM01 genome encodes machinery necessary for lysogeny and induction, including one transposase (*g135*) and the recombinase (*g136*) making up a two gene mobile genetic element called IS607, originally identified in *Helicobacterpylori* (Thingstad and Lignell, 1997; Kersulyte *et al.,* 2000; Kuno *et al.,* 2010; Bobay *et al.,* 2013). The aim of our study was to amplify and obtain complete coding sequence of genes responsible for lytic and lysogenic production.

Material and Methods

Area of Study

Subsurface water sample was collected once from a small artificial lake in Giza Zoo located in Giza province, Egypt (30°01'15.1"N 31°12'47.4"E) in October 2017.

RNA Extraction

Genetic material was extracted from the filters according to Giovannoni *et al.,* 1990. Then centrifuged with a speed of 13,000×g.1.5 ml of Trizol reagent was added and incubated for 10 min at room temperature. $CHCl_3$ was then added to a final concentration of 0.2% (v/v) and the samples were shaken vigorously for 30 seconds before being incubated for a further 10 min at room temperature. After centrifugation at 15000×g for 10 min, the upper phase was precipitated by the addition of 0.5 volumes of isopropanol and left on ice for 12 min. The RNA pellet was recovered after centrifugation at 15000×g for 10 min. The pellet was washed with 1 ml of 75% (v/v) ethanol, and after a final centrifugation at 15000×g for 10 min the supernatant was removed and the pellet airdried for a maximum of 15 min. The RNA was dissolved in 90 µl of RNase-free water and 10 µl of 10× DNase buffer (Qiagen). Eight units of DNase I (Qiagen) were added to the samples which were incubated at 37°C for 20 min. The RNA was purified using an RNeasy Mini Kit (Qiagen).

Complementary DNA synthesis

For cDNA synthesis,400–1000 ng of RNA was used.Each reaction was in final volume 25µL containing total 1 µL RNA , RNase-free water, Nuclease-free water, 0.3 µMdNTPs and 50µMrandom hexamers, 100UReverse Transcriptase, 1X RT Bufferwerecombined for each reaction using master mixes and heated at 70 °C for5 min followed by 10 min at 4 °C using Thermocycler.

Amplification of the g91, g135, 136 Coding sequences

After the RNA was reversely transcribed into the cDNA, 25 ng reverse transcribed total RNA in 1 µl was then subjected for the amplification of the *g91*, *g135* and *g136* genes as follows: for the amplifications of the coding sequences of the studied genes the reaction was carried out in a solution volume of 25 µL containing 1 µL of Reverse transcribed reaction product, 0.3 µM of forward and reverse primers (Table 1), 2.5mM of dNTP, 1×PCR Buffer, 1 U Taq Polymerase. The optimal condition was as follows: Initial denaturation at 94 °C for 5 min followed by 30 cycle of denaturation at 94 °C for 30 seconds and 55 °C for 30 seconds and a final extension step at 72 °C for 5 min.PCR products were purified usingQIA quick PCR purification reagents (Qiagen, USA) and then sequenced with the Big Dye Terminater cycle sequencing kit (Applied Biosystems, Foster City, CA) in ABI Prism3700 sequencer (Perkin Elmer, Applied Biosystems, USA).

Table 1: PCR primers used in this study.

Targeting gene	Primers	Sequence (5 to 3)	Product size	Reference
***g91*(gene)**	Forward primer	AGCGTTCGTTTCGGTACTGT	2280 bp	**Present study**
	Reverse primer	AGCACGTTCGGCTTCAGTAA		
***g135*(gene)**	Forward primer	GCGGCTACCTGATGGATACC	1120 bp	**Present study**
	Reverse primer	TTCAAACGTCTCCCCTGTCG		
***g136* (gene)**	Forward primer	ACTGTTGGCTATTCCCGTGT	609 bp	**Present study**
	Reverse primer	ACTTGCCAACCGTTTTGAGC		

Database Searches

The coding sequences of *g91, g135* and*g136* amplified, were subjected to nucleotide sequence analysis to confirm the accuracy of the obtained products and wereexamined for homology with the use of the NCBI (nucleotide BLAST network service).

Statistical Analysis and phylogeny

Reference sequences from phage identified by sequencealignment were downloaded from NCBI. Sequences were aligned by CLUSTAL 2.1 andthis alignment was then used to generate a phylogenetic tree. Phylogenic treeswere constructed using the neighbor joining (NJ) method by MEGA 7.0.14 software (Saitou and Nei, 1987) with 1000 bootstrap replications to assess branching confidence.

Results and Discussion

Phylogenetic analysis

Neighbor joining method (NJ), with1000 bootstrap replications to assessbranching, was used to determine the evolutionaryrelatedness of *g91, g135* and *g136* to other*g91, g135* and *g136* genes isolated from other cyanophages. The *g91*gene sequence encoded a polypeptide which is responsible for the synthesis of phage tail. Sequence alignment with *g91* homologues from cyanophages revealed that the *g91*gene was conserved. The phylogenetic tree showed that the *g91*gene may have been introduced through horizontal transfer from a common ancestor (Figure 1). The transposase*g135*and *g136* genes belong to a potential family of mobile elements, IS607, which was originally identified in *Helicobacter pylori*. Sequence alignment with *g135* homologues from cyanophages revealed that the *g135* gene was conserved. The phylogenetic tree showed that the *g135* gene may have been introduced through horizontal transfer from a common ancestor (Figure 2).Sequence alignment with *g136* homologues from cyanophages revealed that the *g136* gene was

conserved. The phylogenetic tree showed that the *g136* gene may have been introduced through horizontal transfer from a common ancestor (Figure 3).

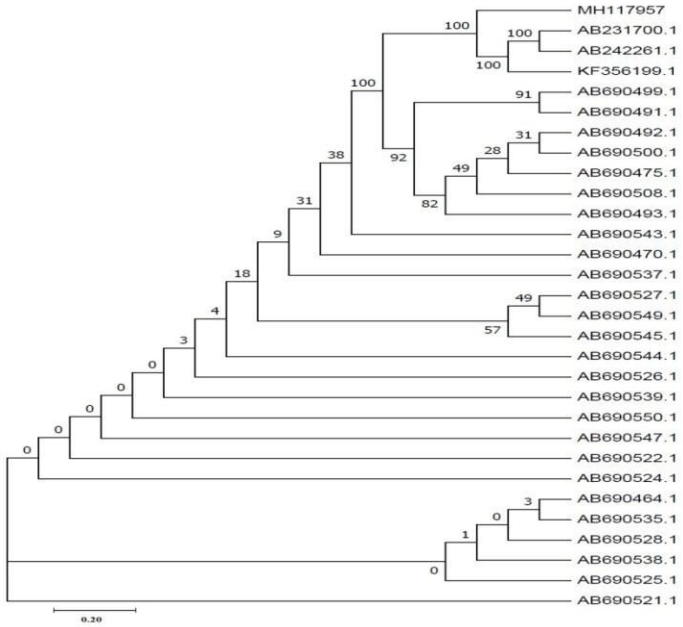

Fig 1: Neighbor joining phylogenetic tree generated on the deduced amino acid sequences of *g91* gene. A tree generated from the alignment of the nucleotide sequence of *g91*gene with those present at NCBI using CLUSTAL X and MEGA 7.The scale bar represents 0.2 amino acids per site.

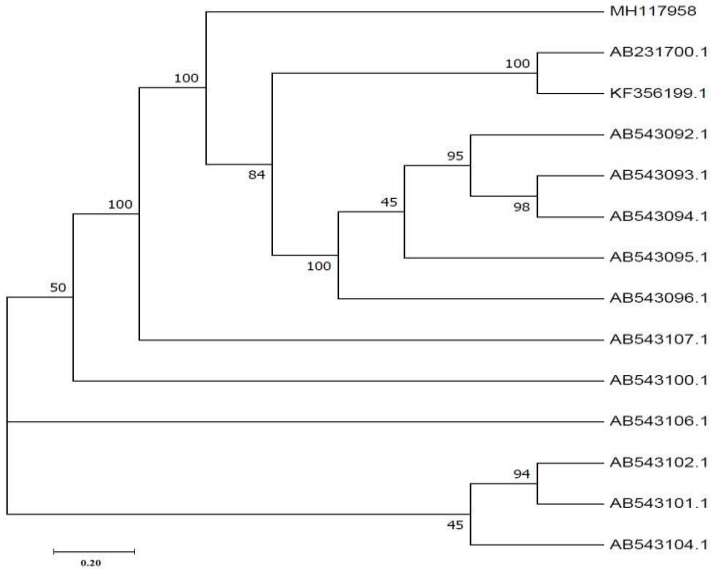

Fig 2: Neighbor joining phylogenetic tree generated on the deduced amino acid sequences of *g135* gene. A tree generated from the alignment of the nucleotide sequence of *g135* gene with those present at NCBI using CLUSTAL X and MEGA 7.The scale bar represents 0.2 amino acids per site.

In this molecular study the lysogeny of cyanophages was detected, similar results have been reported by Groth and Calos, (2004) who stated that in spite of *Microcystis* cyanophages are lytic, the cyanophages also has a number of genes not present in lytic phages, such as recombinase (*g135*) and a transposase (*g136*), which are used by temperate phages in the lysogeny process. However, Steffen *et al.,* (2015) stated thatin spite of the absence of lysogenic activity with *Microcystis* spp. observed in the laboratory. We analyzed a sample for lytic and lysogenic production by using Reverse Transcriptase PCR a technology by which RNA molecules are converted into their complementary DNA (cDNA) sequences by reverse transcriptase enzyme, followed by the amplification of the newly synthesized cDNA by usual PCR procedures using primers targeting the required genes (*g91, g135* and *g136*). This approach to study gene expression is universally known as RT-PCR, because of the role of reverse transcriptase (RT) in the synthesis of cDNA as stated by Robert, (2010).

The homology of cyanophages identified to phage genome sequences deposited in GenBank (NCBI, USA) was determined compared with *g91, g135 and g136* genes fragment analysis. The coding sequence of the isolated *g91* gene, is encoding a viral tail sheath

protein, with a predicted molecular mass of 83.6 kDa. Sequence alignment analysis showed that the gene was 99 % identical to the *g91* gene of AB231700 (Yoshida *et al.,* 2008) and 95 % identical to the *g91* gene of AB690499 (Kimura *et al.,* 2012).

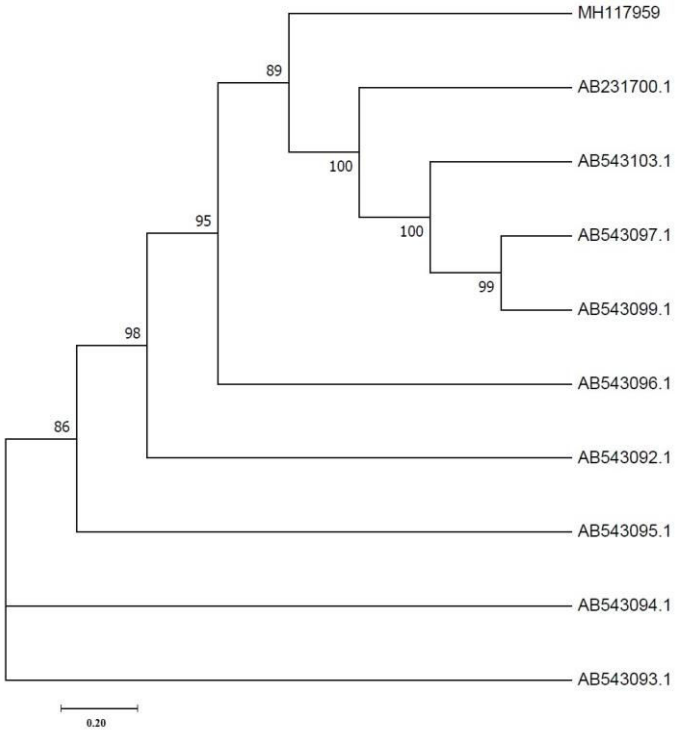

Fig 3: Neighbor joining phylogenetic tree generated on the deduced amino acid sequences of *g136* gene. A tree generated from the alignment of the nucleotide sequence of *g136* gene with those present at NCBI using CLUSTAL X and MEGA 7.The scale bar represents 0.2 amino acids per site.

Beyond an ability to infect and lyse *Microcystis* spp., the studied cyanophages encode machinery necessary for lysogeny and induction, one transposase (g*135*) and the recombinase (*g136*) make up a two gene mobile genetic element called IS607, originally identified in *Helicobacter pylori*. Sequence alignment analysis showed that the gene *g135* was 99 % identical with that of AB231700 (Yoshida *et al.,* 2008) and 98 % identical to KF356199 (Ou *et al.,* 2013). Sequence alignment analysis showed that the gene *g136* was 97 % identical with that of AB231700 (Yoshida *et al.,* 2008) and 94 % identical to AB543094 (Kuno *et al.,* 2010). Complete coding DNA sequences for *g91*, *g135* and *g136* were successfully obtained. The NCBI GenBank accession numbers for the sequences reported hereare MH117957, MH117958 and MH117959 respectively. All genes showed a distinct clade.

Conclusion

We concluded that beyond an ability to infect and lyse *Microcystis* spp., the studied cyanophages encode machinery necessary for lysogeny and the presence of lysogenic associated genes within the investigated cyanophages in this study means that these cyanophages can coexist with the *Microcystis* spp. in the aquatic streams.

References

Ackermann, H.W., & Du Bow, M.S. (1987).Viruses of Prokaryotes.In *General Properties of Bacteriophages*. Boca Raton: CRC Press, p. 202.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (eds) (2002).*Molecular biology of the cell*. New York: Garland Science.

Bergh, O., Borsheim,K..Y., Bratbak,G., &Heldal, M. (1989). High abundance of viruses found in aquatic environments. *Nature*,340, 467–468.

Bobay, L.M., Rocha, E.C., &Touchon, M.(2013).The adaptation of temperate bacteriophages to their host genomes.*Molecular Biology Evolution*,30, 737-751.

Brüssow, H., Canchaya, C., & Hardt, W.D. (2004). Phages and the evolution ofbacterial pathogens: from genomic rearrangements to lysogenic conversion.*Microbiology Molecular BiologyReviews*, 68, 560- 602.

Dimmock, N.J., Easton, A., & Leppard, K. (2001).*Introduction to Modern Virology*: Wiley Blackwell.

Fuhrman, J. (1999). Marine viruses and their biogeochemical and ecological effects.*Nature*,399, 541- 548.

Fuhrman, J. (2000). Impact of viruses on bacterial processes, p. 327–350. In D. L. Kirchman (ed.), Microbial ecology of the oceans .John Wiley and Sons Inc., New York, N.Y.

Giovannoni, S.J., DeLong, E.F., Schmidt, T.M., & Pace, N.R. (1990).Tangential flow filtration and preliminary phylogenetic analysis of marine picoplankton. *Applied Environmental Microbiology*,56,2572–75.

Groth, A., & Calos, M. (2004).Phage integrases: biology and applications. *Journal of Molecular Biology*,335, 667–678.

Hennes, K.P., Suttle, C.A., & Chan, A.M. (1995). Fluorescently labeled virus probes show that natural virus populations can control the structure of marine microbial communities. *Applied & Environmental Microbiology*,61, 3623-3627.
Kersulyte, D., Mukhopadhyay, A.K., Shirai, M., Nakazawa, T., Berg, D.E. (2000). Functional organization & insertion specificity of IS607, a chimeric element of *Helicobacter pylori*.*Journal of Bacteriology*, 182, 5300-5308.
Kimura, S., Yoshida, T., Hosoda, N., Honda, T., Kuno, S., Kamiji, R., Hashimoto, R., Sako, Y. (2012).Diurnal infection patterns and impact of *Microcystis* cyanophages in a Japanese pond.*Applied Environmental Microbiology*,78, 5805–5811.
Kuno, S., Yoshida, T., Kamikawa, R., Hosoda, N., & Sako, Y.(2010).The distribution of a phage-related insertion sequence element in the cyanobacterium, *Microcystis aeruginosa*. *Microbes Environmental*,25, 295- 301.
La Scola, B., Audic, S., Robert, C., Jungang, L., de Lamballerie, X., Drancourt, M., Birtles, R., Claverie, J.M., &Raoult, D. (2003). A giant virus in amoebae. *Science*,299, 2033-2033.
Miller, R.V., & Ripp, S. (1998).The importance of pseudolysogeny to *in situ* bacteriophage-host interactions. In *Horizontal Gene Transfer*. Syvanen, M., and Kado,C.C. (eds). San Diego, CA: Academic Press, pp. 81-91.
Morimoto, D., Kimura, S., Sako, Y., & Yoshida, T.(2018). Transcriptome Analysis of a Bloom-Forming Cyanobacterium *Microcystis aeruginosa* during Ma-LMM01 Phage Infection.*Frontier Microbiology,*19, 9:2.
Ou, T., Li, S., Liao, X., &Zhang, Q. (2013). Cultivation and characterization of the MaMV-DC cyanophage that infects bloom-forming cyanobacterium *Microcystis aeruginosa*. *VirologicaSinica*,28, 266–271.
Robert, E. Farrell (2010).Ph.D, in RNA Methodologies (Fourth Edition).
Saitou, N., & Nei, M. (1987).The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology Evolution*, 4(4),406-25.
Sime-Ngando, T., &Colombet, J. (2009).Viruses and prophages in aquatic ecosystems. *Canadian Journal of Microbiology*,55, 95-109.
Sode, K., Oozeki, M., Asakawa, K., Burgess, J.G., & Matsunaga, T. (1994). Isolation of a marine cyanophage infecting the marine unicellular cyanobacterium, *Synechococcus*sp. NKBG 042902.*Journal of Marine Biotechnology*,1, 189-192.
Steffen, M.M., Belisle, B.S., Watson, S.B., Boyer, G.L., Bourbonniere, R.A., & Wilhelm, S.W. (2015). Metatranscriptomic evidence for co-occurring top-down and bottom-up controls on toxic cyanobacterial communities. *Applied Environmental Microbiology,*81: 3268-3276.
Stough, J.A., Tang, X., Krausfeldt, L.E., Steffen, M.M., Gao, G., Boyer, G.L. (2017). Molecular prediction of lytic vs lysogenic states for *Microcystis* phage: Metatranscriptomic evidence of lysogeny during large bloom events. *PLoS ONE 12* (9), e0184146.
Suttle, C. (2007). Marine viruses—major players in the global ecosystem. *Nature Reviews Microbiology*, 5, 801-812.
Thingstad, T.F. & Lignell, R. (1997). Theoretical models for thecontrol of bacterial growth rate, abundance, diversity andcarbon demand. *Aquatic Microbiological Ecology*,13, 19- 27.
Yoshida, T., Nagasaki, K., Takashima, Y., Shirai, Y., Tomaru, Y., Takao, Y., Sakamoto, S., Hiroishi, S., & Ogata, H. (2008).Ma-LMM01 infecting toxic *Microcystis aeruginosa* illuminates diverse cyanophage genome strategies.*Journal of Bacteriology*,190, 1762-1772.