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Perspective

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Selection of Qualitative or Quantitative PCR Data for Detection of Toxic Cyanobacteria as an Indicator for Animal Poisoning by Cyanotoxins

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Perspective

The widespread photoautotrophic prokaryotes, cyanobacteria (aka blue-green algae), are an important constituent of freshwater microalgal communities. They often dominate the phytoplankton abundances and contribute to most primary productivity and thus occupy an important niche in ecosystems. However, they can proliferate explosively on the premise of excessive nutrients and optimum ambient temperature and irradiation. The overgrowing cyanobacteria finally aggregate into a visible 'bloom'-like assemblage within a short period of time. This phenomenon is named harmful algal bloom (HAB) in phycology and limnology, which obviously manifests its intrinsic characteristics. This harmful phenomenon can inhibit normal growth of other phytoplankton by unconstrained cyanobacterial assimilation of environmental nutrients and formation of a shading 'roof' at surface water to prevent solar irradiation from penetrating in deeper water columns. Moreover, bacterial degradation of tremendous numbers of cyanobacterial cells can result in widescale hypoxia making aquatic animals dead due to oxygen depletion. Nevertheless, to people's most concern should be the production of potent cyanotoxins that are lethal to animals and humans. These toxins can be enormously accumulated particularly when an HAB occurs.

Cyanotoxins can be categorized into hepatotoxins, neurotoxins, cytotoxins, etc. by toxicological impacts, or oligopeptides, alkaloids, etc. by chemical structures. They are synthesized by a group of enzymes translated from clustered toxin synthetase genes following a strict sequence of catalytic reactions. Amongst the most infamous toxins is microcystin, and it is a non-ribosomal heptapeptide and damages liver cells and kills animals at low doses (e.g., 4 ppb for a nursery pig) [1]. Animal poisoning cases by microcystin are always reported due to its prevalence and lethality. Microcystin is synthesized by 10 enzymes McyA thru McyJ which are the protein products of microcystin synthetase genes *mcyA* thru *mcyJ*. The fundamental structure of microcystin is formed by catalysis conducted by the 10 enzymes in spite of its over 100 functional isoforms by chemical modifications. Microcystin can be produced by many cyanobacterial species across different genera but principally by *Microcystis* spp. Therefore, most cyanotoxin poisoning incidents can be imputed to toxic *Microcystis* spp.

However, the identical morphology of toxic and non-toxic strains of a *Microcystis* species render the recognition of toxic cells not possible by observing their algal colonies with naked eyes or cellular traits under a microscope. Therefore, diagnostics of toxicity are usually implemented by direct testing of microcystin via ELISA, HPLC, LC-MS, etc. In addition, molecular technology provides useful tools to illustrate toxicity from the perspective of genes, that is to say, investigate the genetic fundaments for toxin synthesis. Many PCR/qPCR assays have been developed based on nearly all 10 mcy genes for detecting toxic *Microcystis* spp. Whilst they can manifest the possibility of toxicity, a correlation may not be taken for granted between gene and toxin in regard of presence or quantities due to latent regulatory mechanisms for toxin production.

Strangely, practical studies challenge or support the conclusion in both ways. A research was done in our lab to detect the toxic *Microcystis* spp. targeting the *mcyC* gene by conventional PCR in 60

water samples from four farm ponds serving as water sources for livestock facilities, and the positive results were comparable to those positive results by LC-MS (Table 1). A further statistical analysis by McNemar's test showed a strong dependency of the presence of microcystin on toxic *Microcystis spp*. ($p << 0.01$, Table 1), providing evidence for a close correlation between gene and toxin. Another research was conducted and conformed to the findings by PCR and LC-MS (data not shown), which did the same detection by qPCR and microcystin tests by ELISA on 100 samples from five ponds including the four above. However, quantitative determination of *mcyC* gene and microcystin did not follow a correlative relationship ($R^2 < 0.5$ in a linear regression by Pearson's r) and thus proved rational the regulation of toxin production. As a matter of fact, a correlation between gene abundance and toxin level is arguable because contradictory findings have been made so far.

Table 1: Detection of toxic *Microcystis* spp. by PCR and microcystin by LC-MS in 60 farm pond water samples.

		LC-MS[1]		Total
		0	1	
PCR[1]	0	23	3	26
	1	20	14	34
	total	43	17	60

1. Numbers 0 and 1 refer to the negative and positive detections, respectively.

According to the information above, it is safe and reliable to select PCR to detect the existence of *mcy* genes and indicate microcystin toxicity of samples. An answer of 'yes or no' is sufficient for making stringent strategies to prevent acute or chronic poisoning of implicated animals around causative waterbodies. Nevertheless, policy makers and stakeholders are likely to avert such actions to fully utilize natural resources, so a numeric result telling them whether a red flag is necessary is more valuable. But qPCR solely cannot address the issue, and a toxin test is absolutely a must. Therefore in conclusion, it is more reliable to measure the cyanotoxin levels for water toxicity and make decisions, and qPCR could be an auxiliary tool to disclose the abundance of toxic cyanobacteria, while it is advisable to choose the more cost-effective PCR method rather than an expensive toxin test by immunology or analytical chemistry to judge the cyanotoxin contamination in water.

References

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