Small Regulatory Ribonucleic Acids (srRNAs) for the Control of Harmful Algal Blooms

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Aquatic Plant Control Research Program

Problem One of the biggest challenges in battling harmful algal blooms (HABs) is how to suppress cyanobacterial growth and disrupt or destroy the bloom process in a species-specific manner, particularly to inhibit the growth of cyanotoxin-producing species.

Objective Develop an environmentally benign and species-specific novel bionanotechnology for efficient HAB management.

Approach An RNA interference (RNAi)-based gene silencing approach was employed to transiently inhibit essential biological processes such as photosynthesis and nitrogen assimilation without genetic alteration. Some gene silencing agents or GSAs (e.g., srRNAs) needed to be introduced intracellularly via a nanoparticle-mediated delivery system, whereas other modified GSAs (e.g., FANA-ASOs and LNAs) were capable of crossing cell membrane and internalizing without the need of any delivery vehicle.

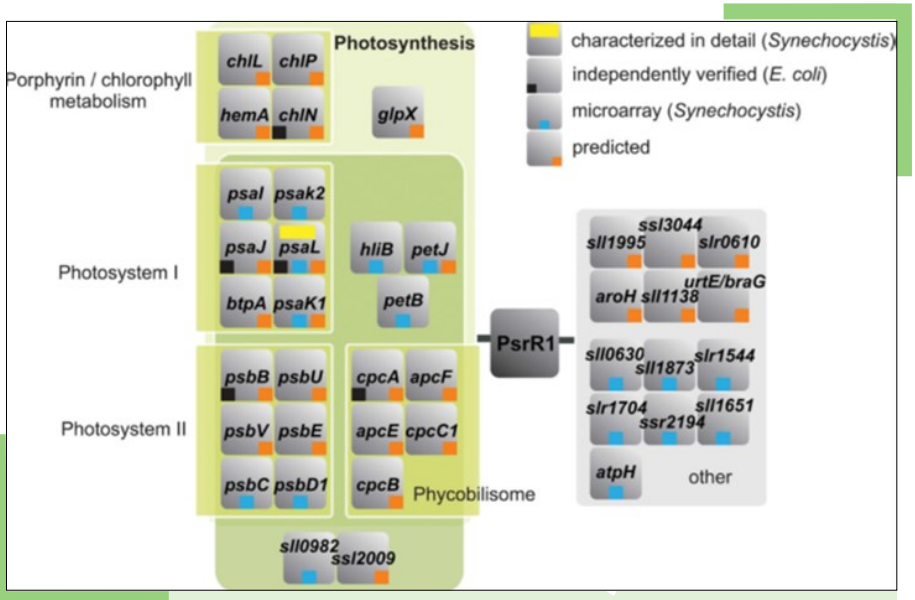

Figure 1: *An srRNA such as PsrR1 acts as a small interfering RNA (siRNA) to silence a suite of essential genes and pathways causing cyanobacteria mortality.*

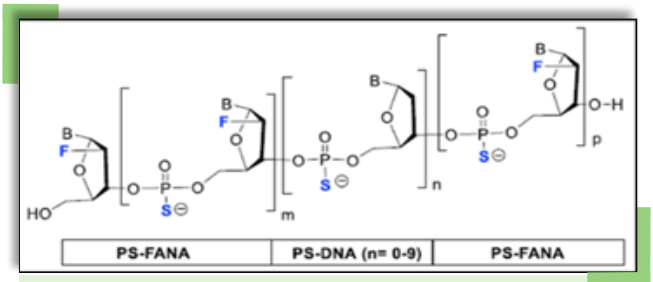

Figure 2: *FANA-PS modified DNA or ASO (anti-sense oligo)*
FANA = 2'-deoxy-2'-fluoro-β-D-arabinonucleic acid
PS = Phosphorothioate

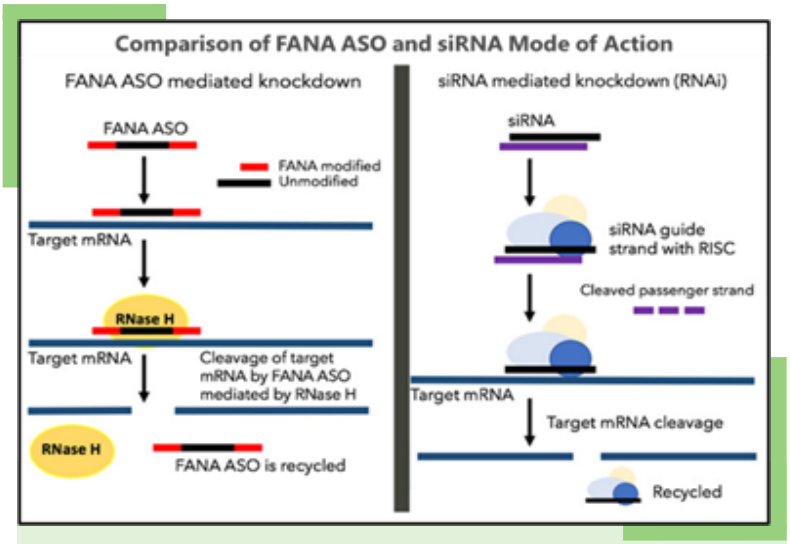

Figure 3: *Mechanisms for antisense oligo (ASO, shown as FANA-modified ASO) and small interfering RNA (siRNA, including srRNA, dsRNA, miRNA, etc) to suppress mRNA expression*

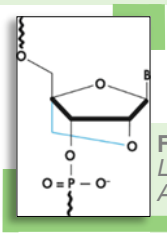

Figure 4: *A Locked Nucleic Acid (LNA)*

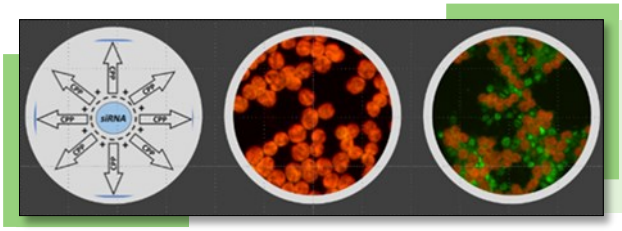

Figure 5: *Nano-carries (e.g., cell-penetrating peptides or CPPs) serve as the vehicle for intracellular delivery of GSAs (e.g., srRNA/siRNA labelled with a fluorescence dye for visualization) into a toxin-producing strain Microcystis aeruginosa.*

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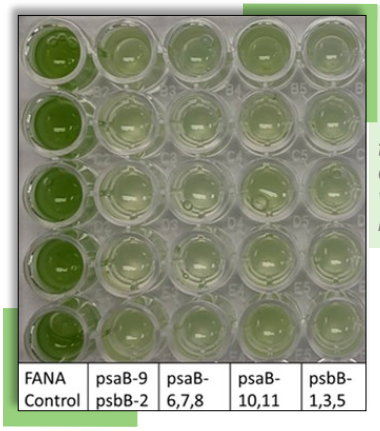

Figure 6: *Cyanobacteria (M. aeruginosa LE3) treated for 7 days with FANA-scramble oligo (control) or FANA-oligos targeting genes MapsaB or MapsbB showing visible, significant growth inhibition.*

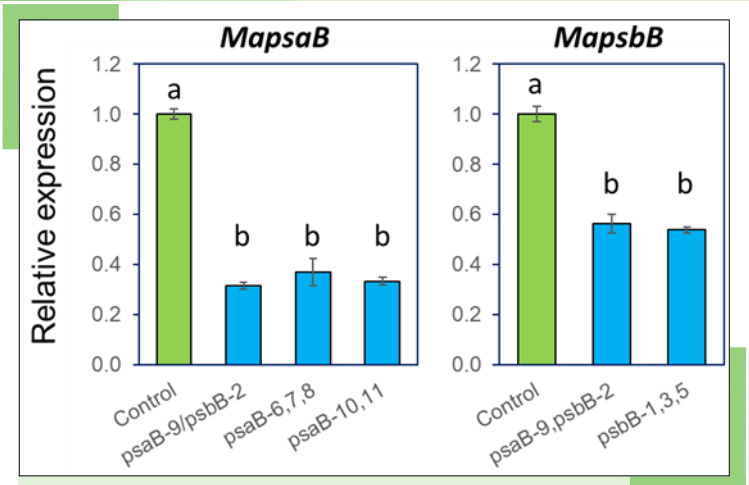

Figure 7: *Target genes MapsaB and MapsbB were both significantly suppressed by FANA-ASOs in M. aeruginosa LE3 after 7-d treatment. Physiologica endpoints (OD680 – chlorophyll a and OD750 – cell density, data not shown) correlated with gene expression and visual endpoints.*

Major Milestones

Date	Milestone
FY21, Q1	Design and construction of plasmids expressing GFP and srRNAs in cyanos
FY21, Q2	Testing self-expressing plasmids in *Microcystis* and *Synechococcus*
FY21, Q2	Curated database of 1934 cyano 16S rRNA gene sequences
FY21, Q3	Synthesis & testing of carbon nano dot (CND) and carbon nano tube (CNT)
FY21, Q3	Mesocosm studies using Microcystis, self-expressing plasmids & CND/CNT
FY21, Q4	Data analysis and reporting
FY22, Q1	Redesign and test GSA-SECs
FY22, Q2	Species selectivity of CPP and CND cytotoxicity
FY22, Q3	16S rRNA & srRNA PCR assay development & validation
FY22, Q3	FANA ASO design and testing on *Microcystis*
FY22, Q4	Microcosm studies
FY22, Q4	Manuscript: CPP and CND cytotoxicity to cyanobacteria
Costs	**FY21:**$337K **FY22:**$240K **TOTAL:** $577K

Partnership/Leveraging Opportunities

This work leveraged multiple collaborations and other work units including: "Development of a Near Real-Time Field Test Kit for the Rapid, Simultaneous Detection and Quantitation of High Priority Toxic Cyanobacteria" and "Rapid, portable and multiplexed detection of freshwater harmful algal bloom-forming genera".

Value to USACE Mission

This project offers a species-specific and environmentally benign solution for USACE and other stakeholders to combat HABs. This technology is particularly useful for mitigating cyanotoxin-producing cyanobacteria that often dominate HAB events.