TECHNICAL REPORT

Report Title: Lethality of *Pseudomonas fluorescens* Strain CL0145A to the 2 Zebra Mussel Species Present in North America

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ABSTRACT:

These experiments indicated that bacterial strain CL0145A of *Pseudomonas fluorescens* is equally lethal to the 2 zebra mussel species present in North America, *Dreissena polymorpha* and *Dreissena bugensis*. Thus, this bacterial strain should be equally effective at killing zebra mussels in power plant pipes, irrespective of which species is present.

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EXECUTIVE SUMMARY:

Subtask 2.1 of the Statement of Work (SOW) requires that the Contractor shall determine if bacterial strain CL0145A of *Pseudomonas fluorescens* is equally lethal to the 2 zebra mussel species present in North America, *Dreissena polymorpha* and *Dreissena bugensis*. To address this subtask, laboratory experiments were conducted with sympatric populations of these 2 species occurring both in Lake Erie and Lake Ontario. Test results indicated that both species are of near equal susceptibility. This is significant because this bacterial strain should be equally effective at killing zebra mussels in power plant pipes, irrespective of which species is present.

EXPERIMENTAL MATERIALS AND METHODS:**Production of Bacteria - Culturing of Strain CL0145A:**

Under static conditions, 10-ml tubes containing 6 ml of tryptic soy broth (TSB) (Difco) were each inoculated with 0.1 ml stock frozen bacterial culture. The tubes were then incubated at 26°C under static conditions for ca. 41 hr. Tubes were subsequently used to inoculate 250-ml flasks (1 tube per flask) containing 100-ml TSB and incubated at 26°C under static conditions for ca. 72 hr.

Obtaining the Cell Fraction (CF):

Final whole cultures were centrifuged (30 min at 827 x g) in 50-ml batches, supernatants were discarded, and cell pellets were resuspended in dilution water (80 ppm KH_2PO_4 , 405.5 ppm $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ in deionized water, pH adjusted to 7.2 with NaOH). The optical density of the CF inoculum was determined by taking 1 absorbance reading (spectrophotometer, $\lambda = 660 \text{ nm}$) from each of 3 separate samples of the CF. Based on an absorbance equation developed during previous trials (author, unpublished data), the optical density of the CF was then used to calculate the volume of CF inoculum required to treat the mussels at the desired target concentration of 200 ppm (200 mg dry bacterial mass per liter). Three 0.5 ml samples of the CF inoculum

were air dried in a dessicator then weighed a week later on a Cahn C-30 microbalance to determine actual treatment concentration.

Treatment of Zebra Mussels with Bacterial Cells:

To test the relative susceptibility of the 2 *Dreissena* spp., sympatric populations of the 2 species were field collected mussels from 2 locations in the Great Lakes were used in the experiments.

Lake Erie Populations: Intermixed populations of *Dreissena polymorpha* and *D. bugensis* were collected on May 25, 2001 at Presque Isle Bay, Pennsylvania. Mussels were held under flow-through conditions at ambient Lake Erie water temperatures at the Buffalo State College Great Lakes Center Laboratory (Buffalo, New York) until June 6 when they were transported under refrigeration to the New York State Museum's Field Research Laboratory (Cambridge, New York).

Lake Ontario Populations: Intermixed populations of *Dreissena polymorpha* and *D. bugensis* were collected on September 17, 2001 near Oswego harbor, Lake Ontario (New York), and mussels were transported the same day under refrigeration to the New York State Museum's Field Research Laboratory.

Standard Testing Methods: Upon arrival at the laboratory, mussels were brought up to 23°C over a 24 hr period in aquaria under aerated conditions, and then were separated in to "small" and "large" size classes (Table 1) following the sieving method of Biss et al. (1996 [5726]). One hundred mussels, either small or large and either *D. polymorpha* or *D. bugensis*, were then placed in a 4-L glass bottle containing 1.5 L of aerated water and allowed to acclimate for ca. 24 hr pretreatment. For both species and for both size classes, the experiment included 1 untreated bottle and 5 replicate treated bottles. Immediately before bacterial treatment, the volume within each aerated bottle was brought up to 2.0 L with additional water. Bottles were treated with bacteria at a target concentration of 200 ppm (bacterial mass/L) and then maintained under aerated conditions for 48 hr. Following bacterial exposure, small and large mussels were transferred to dishes containing, respectively, 70 ml and 250 ml of previously aerated water and held under static conditions for an additional 8 days. Water was changed and mortality was scored each day. All testing was conducted at 23°C ($\pm 1^\circ\text{C}$) using hard, synthetic, fresh water as defined in Peltier and Weber (1985 [4828]). All binomial mortality data were analyzed following angular transformation (Sokal and Rohlf (1995 [6655])).

Table 1. Size of mussels used in experiments.

	Mean ($\pm$ SD) mussel length (mm)			
	<i>D. polymorpha</i>		<i>D. bugensis</i>	
	Small	Large	Small	Large
Lake Erie mussels	8.1 (± 1.6)	12.1 (± 1.6)	7.4 (± 1.1)	13.6 (± 3.0)
Lake Ontario mussels	11.7 (± 2.3)	17.5 (± 1.9)	10.5 (± 1.8)	16.8 (± 2.5)

RESULTS AND DISCUSSION:

Subtask 2.1 of the Statement of Work (SOW) requires that the Contractor shall determine if bacterial strain CL0145A of *P. fluorescens* is equally lethal to the 2 zebra mussel species present in North America, *D. polymorpha* and *D. bugensis*, and these experiments addressed this question. Test results indicated that both species are of near equal susceptibility (Table 2).

Table 2. Mussel mortality recorded in experiments.

Mussel populations	Bacterial treatment concentration (ppm)**	Mean ($\pm$ SD) mussel mortality* (%)			
		<i>D. polymorpha</i>		<i>D. bugensis</i>	
		Small	Large	Small	Large
Lake Erie mussels	209	96.6 ($\pm$ 2.4)	93.4($\pm$ 3.2)	98.4 ($\pm$ 1.1)	91.4 ($\pm$ 2.7)
Lake Ontario mussels	177	84.8 ($\pm$ 3.7)	83.5 ($\pm$ 4.0)	88.4 ($\pm$ 3.8)	92.9 ($\pm$ 1.9)

* All control mortalities in untreated bottles <1%.

** Mean of 3 replicates of 0.5 ml of inoculum used to determine actual treatment concentration.

The mortality data from the Lake Erie populations indicated that strain CL0145A was equally lethal to both species; there was no statistical difference in mean mortality between small *D. polymorpha* (96.6%) and small *D. bugensis* (98.4%) or between large *D. polymorpha* (93.4.6%) and large *D. bugensis* (91.4%). Mortality data from the Lake Ontario populations, however, suggested that strain CL0145A might even be slightly more lethal to *D. bugensis*; although there was no statistical difference in mean mortality between small *D. polymorpha* (84.8%) and small *D. bugensis* (88.4%), there was a statistical difference ($P = 0.004$) between large *D. polymorpha* (83.5%) and large *D. bugensis* (92.9%). The higher mortality of large *D. bugensis* versus *D. polymorpha* from Lake Ontario, however, is likely an experimental aberration that would need to be reexamined by further testing before any solid conclusions could be drawn.

CONCLUSIONS:

Test results indicated that both species are of near equal susceptibility. This is significant because bacterial strain CL0145A of *P. fluorescens* should be equally effective at killing zebra mussels in power plant pipes, irrespective of which species is present.

REFERENCES:

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