(E): Evaluation and Management of Environmental Risks: Rapid Detection and Identification of Pathogenic and Harmful Microorganisms Using a Novel Combination of Molecular Technologies

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PROBLEM

There has been a dramatic rise in the incidence of pathogenic bacteria and harmful algal blooms in coastal environments (Fig. 1). At the same time human exposure risk is rising due to recreational demands, aquaculture, and increased microbial growth due to global warming.

Current technologies do not adequately address these problems since they typically require large populations of pathogens and harmful algae for positive identification, and are inaccurate and labor intensive. This severely limits our response capabilities to containment measures when early and accurate recognition of small populations could have prevented economic damage or even loss of life.

New technologies are needed that allow accurate, high throughput and affordable monitoring of small populations of pathogenic bacteria and harmful algae. This would enable us to develop effective monitoring and early warning systems, and to learn crucial information about the ecology of pathogens and harmful algae with the ultimate goal to be able to predict conditions leading to outbreaks or blooms

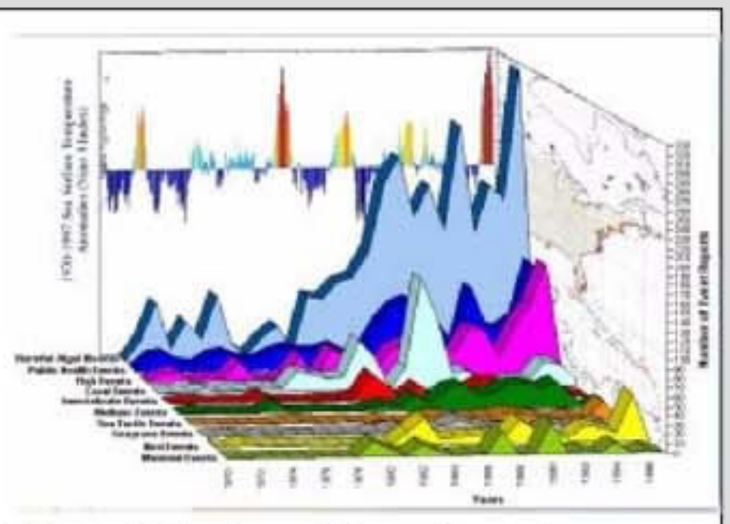

Figure 1. Rise in morbidity and mortality events in US coastal environments from 1970 to 1996. See http://www.heedmd.org/ for details.

APPROACH

We propose to develop and apply new molecular technology for the high resolution monitoring of pathogenic or harmful microorganisms. Genetic markers are increasingly used for detection of microorganisms. However, unambiguous identification remains laborious and expensive because sequence information is ultimately required. Our approach, using a combination of PCR and Constant Denaturant Capillary Electrophoresis (CDCE) represents an advantage over other techniques since a simple one-step analysis can be performed with little loss in accuracy (Fig. 2).

GOALS

- Develop PCR-CDCE as a new technology for rapid pathogen identification
- Explore accuracy and application of the method using environmental samples
- Test potential for automation of the analysis for rapid and efficient use in environmental monitoring facilities

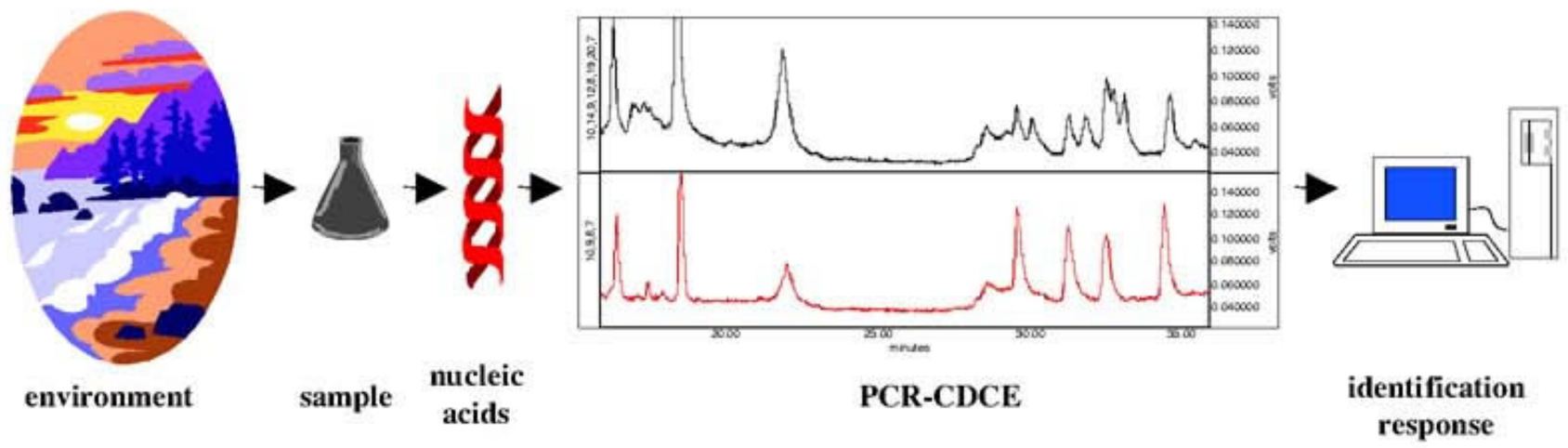

Figure 2. Schematic representation of PCR-CDCE analysis of pathogenic bacteria from coastal environments. The electropherogram shows an example of identification of different *Vibrio* species; each peak represents a co-occurring species (upper panel), which can be matched with separation patterns of pathogenic vibrios (lower panel).

Principle: CDCE can generate fast and high-resolution separation of DNA sequences differing in length or nucleotide composition by as little as one base pair. This is based on the principle that the electrophoretic mobility of a partially melted DNA molecule is sharply reduced. When specific genes are PCR amplified with primers of broad specificity, the amplification products can be identified and quantified. This is achieved by incorporation of a fluorescent molecule in one of the amplification primers that can then be quantified by a laser-photodetector system coupled to the CDCE. This technology was originally developed for analysis of mutations in human tissues but its sensitivity to detect low frequency genes ($1:10^6$) makes it an ideal technology to quantify harmful microorganisms in complex microbial communities.

Pathogenic Vibrio as a model: We will focus on marine bacteria of the *Vibrio* genus; however, the method will ultimately be broadly applicable. The genus Vibrio contains many pathogens, including *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*, that can cause severe problems when contaminated water or seafood are ingested, or when infections spread in aquaculture facilities.