Linking Population and Physiological Diversity in *Karenia brevis* from the Texas Coast

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Abstract

Blooms of the toxic dinoflagellate *Karenia brevis* along the Texas coast are increasing in frequency, yet the source population and specific factors influencing bloom initiation and intensity are poorly known. Significant differences in growth rates and toxin production were observed among clonal isolates from a 1999 bloom when grown under identical conditions. This may reflect genetic diversity that serves as a repository from which different subpopulations bloom in response to appropriate environmental conditions. To test this hypothesis, hypervariable genetic markers known as microsatellites (msats) are being developed to examine genetic diversity among populations of *K. brevis*. PCR amplification of two msat loci from genomic DNA of Texas *K. brevis* clones demonstrated different allele constitutions among these clones. In addition, msat markers appear to be useful fingerprinting tools for differentiating *K. brevis* and *K. mikimotoi*. The correlation between differences in microsatellite genotypes and growth rate and toxin production clearly demonstrates the utility of combining genetic and physiological approaches.

Introduction

The source population for *Karenia brevis* blooms and specific factors influencing bloom intensity are poorly understood in the western Gulf of Mexico. Previous results suggested a high level of genetic diversity exists within populations of *K. brevis* (Baden and Tomas, 1988; Magaña, 2001; Loret *et al.*, 2002). When maintained under identical conditions, the growth rate of *K. brevis* clone SP1 was twofold higher than SP2 and its cellular toxin concentration was threefold lower (Loret *et al.*, 2002). This genetic variability may serve as a repository from which different subpopulations bloom in response to appropriate environmental conditions. Such diversity emphasizes the need for new hypervariable genetic markers, such as microsatellites, which can be utilized in population- and species-level studies of harmful algal blooms. The objectives of our research were to: (1) establish clonal cultures of *Karenia* from bloom events along the Texas coast; (2) examine differences in growth rates among clonal isolates; and (3) identify microsatellite loci and develop PCR primers for genotyping analysis. Establishing links between allelic profiles and physiological properties will yield insight into population-level responses of *K. brevis* to changes in environmental variables. Ultimately, this information can be used to predict responses of *K. brevis* at the population level to environmental changes and to assess how these responses affect and influence bloom formation and dynamics.

Materials and Methods

Cultures Clonal cultures of *K. brevis* were established from samples collected during a fall 1999 bloom, and clones of *K. mikimotoi* were established from a winter 2002 bloom. Single cells were picked out using micropipettes under a stereomicroscope and transferred to wells in a microtiter plate containing "L1"/10 medium (Guillard and Hargraves, 1993). Once established, replicate clonal cultures were maintained in L1 medium made with either low salinity (29 PSU) or high salinity (36 PSU) filtered sea water and grown on a 12:12 light:dark cycle.

Growth Rates Experiments (performed in triplicate) were conducted with cultures acclimated to two light levels (71 and 100 $\mu E\ m^{-2}s^{-1}$), and for selected clones, at both 29 and 36 PSU, at 20°C. Growth rates were determined from daily measurements of *in vivo* fluorescence (Brand *et al.*, 1981). Fluorescence measurements were validated with cell counts.

Microsatellite Development Genomic DNA libraries of DNA fragments of *K. brevis* SP2 (300–500 base pairs in size) were constructed via an approach for producing "enriched"

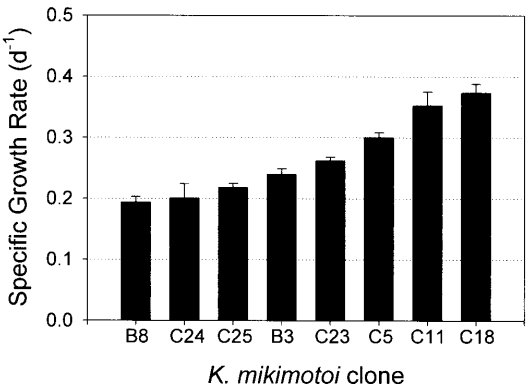

Figure 1 Specific growth rates of eight *K. mikimotoi* clones isolated from a coastal Texas bloom in 2002. A ~twofold range in growth rates was observed among the clones when grown at 20°C in L1 medium (29 PSU) under 71 $\mu Ein\ m^{-2}\ s^{-1}$. Growth rates of clones B8, C24, C25, and B3 are significantly different from C23, C5, C11 and C18 (t-test, $P < 0.05$). Error bars are ± 1 SD in all figures.

Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

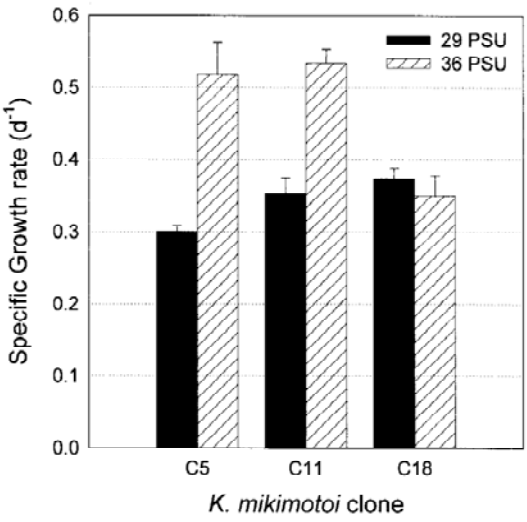

Figure 2 Comparison of specific growth rates among three *K. mikimotoi* clones grown at 20°C, 71 µEin $m^{-2} s^{-1}$ at low and high salinities. For C5 and C11, growth rates were significantly different at the two salinities (t-test, $P < 0.05$).

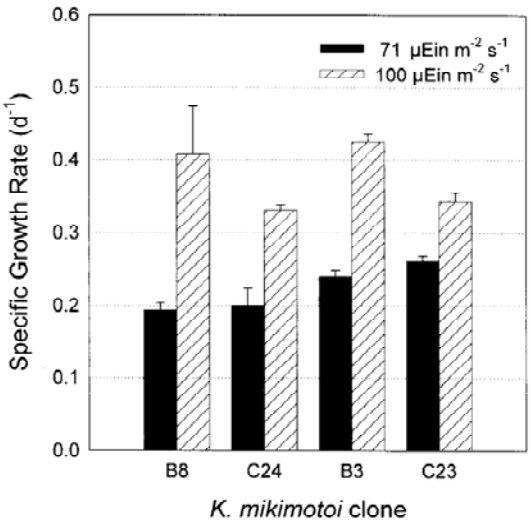

Figure 3 Variability in specific growth rates among *K. mikimotoi* clones grown at 20°C, in 29 PSU L1 medium at two light intensities.

msat libraries (Walbieser, 1994; Connell *et al.*, 1998; A. Pepper, pers. comm.). Biotinylated probes (containing desired repeat motifs [$(TA)_{30}$, $(CA)_{20}$, $(GA)_{20}$, $(TGA)_{15}$, $(ACA)_{15}$]) annealed to genomic fragments were bound to streptavidin-coated paramagnetic particles that held the complexes in place; non-bound genomic fragments that lacked repeat motifs were washed away. The bound fragments were then chemically released and cloned into "enriched" libraries. The enriched libraries contained a high percentage of msats, thereby increasing efficiency of screening. Approximately 200 clones from the library were sequenced. Primers were designed to genomic DNA flanking candidate msat loci using OLIGO™ (ver. 5.0, NBI, Plymouth, MN). PCR amplifications were performed in 10 µL reactions using ExTaq™

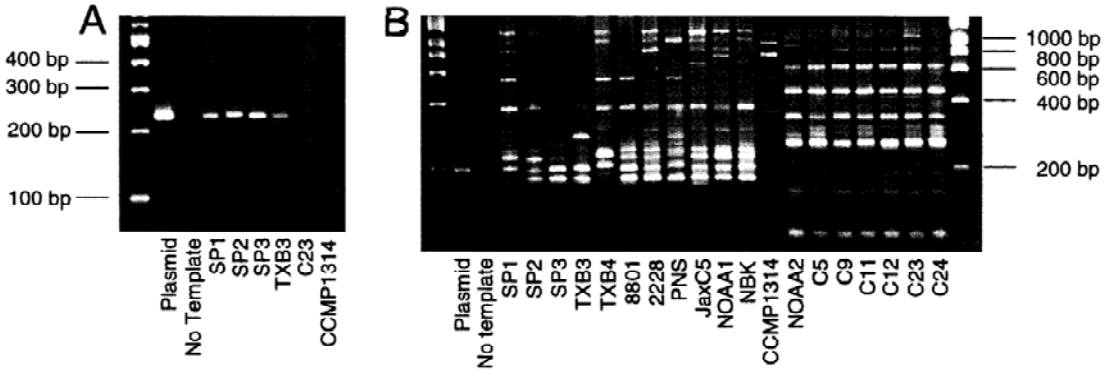

Figure 4 PCR-amplified products for msat loci *KB3* and *KB5*. **A** PCR amplification of msat *KB3* for Texas *K. brevis* clones from the 1999 bloom (SP1, SP2, SP3,TXB3), *K. mikimotoi* clone C23 from the 2002 bloom, and *Amphidinium carterae* (CCMP1314); *KB3* plasmid DNA from SP2 library as positive control, no template as a negative control; 100 bp ladder; *KB3*-forward primer: CGTGACTCAGAGTGGCAAATGG; *KB3* reverse primer: AACATGGCTGATCAACTCAACACC; 2% Metaphore agarose gel; **B** As in (A) for msat *KB5* with additional *K. brevis* 1999 clone (TXB4) and historical clones: 8801 (B. Richardson), NBK (C. Hyatt), CCMP2228, and PNS, JaxC5, NOAA1 (G. Doucette); *K. mikimotoi* clones NOAA2 (S. Morton) and C5–C24 (2002 TX bloom); *KB5* forward primer: ATCCAGGTCGTCCATTCAAGTC; *KB5* reverse primer: CGAAACGCCGGTTCTCTTC; 200 bp ladder.

polymerase and standard conditions as suggested by the manufacturer (Takara) and following a touchdown PCR protocol (Don *et al.*, 1991).

Results and Discussion

Five clonal cultures of *K. brevis* were established from a Texas coastal bloom off Brownsville in fall 1999: SP1, SP2, SP3, TXB3, TXB4 (Loret *et al.*, 2002; Magaña, 2001; B. Richardson, pers. comm.). Twenty-two clonal cultures of *K. mikimotoi* were established from a bloom in Corpus Christi Bay in January 2002. Internal transcribed spacer (ITS) and large subunit (LSU) rRNA sequences for the clonal isolates confirmed the species identifications.

Initial experiments with eight *K. mikimotoi* clones demonstrated significant differences among growth rates when cultures were grown under identical conditions (t-test; $P < 0.05$; Figs. 1–3). Observed variations in growth rates for strains grown at the different salinities and light levels suggest these are genetically distinct clones (Fig. 2 and 3). The almost-twofold range in growth rate among *K. mikimotoi* clones (Fig. 1) is similar to our previous findings for *K. brevis* clones (Loret *et al.*, 2002) and for other phytoplankton species (*e.g., Pseudo-nitzschia australis* and *P. multiseries;* Kudela *et al.*, this Proceedings).

Hypervariable molecular markers (msats) are being developed to determine profiles of genetic and ecological diversity of *Karenia* populations. Ten candidate msat loci have been identified. Examination of PCR products for two loci, *KB3* and *KB5,* demonstrated different sizes and banding patterns among the *K. brevis* clones from Texas and Florida (Fig. 4). Small size differences at locus *KB3* were noted among the *K. brevis* clones from the 1999 bloom (Fig. 4A), and sequencing results confirmed that the msat varied in number of GTT repeats among clones. Locus KB5 is a complex microsatellite and preliminary results indicate that this marker may be useful for fingerprinting (Fig. 4B). The capability to differentiate both between and among individual *K. brevis* and *K. mikimotoi* clones is a valuable tool. The correlation between differences in microsatellite genotype and physiology (*e.g.,* SP1 vs. SP2) demonstrates the utility of combining genetic and physiological approaches.

Further study of the extent of diversity in physiological properties and toxin production among *Karenia* isolates, together with genetic profiles, is necessary to develop realistic predictive models of bloom dynamics and to assess potential toxicity. By establishing links between allelic profiles and physiological properties, models can ultimately be employed to forecast the response of *K. brevis* at the population level to changes in environmental variables. With a better understanding of the responses of different genotypes, we can better predict variability in both spatial and temporal bloom dynamics.

Acknowledgements

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