Degradation of Domoic Acid Under Common Storage Conditions

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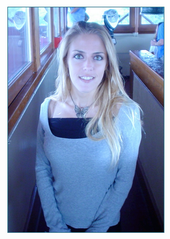
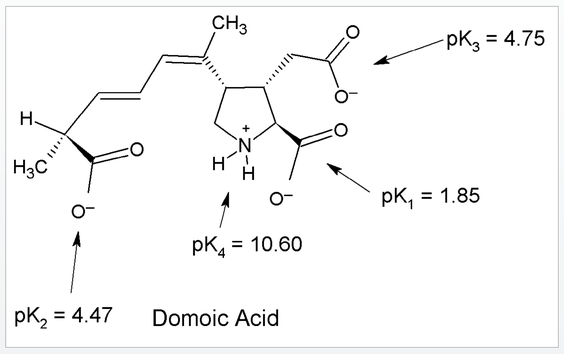
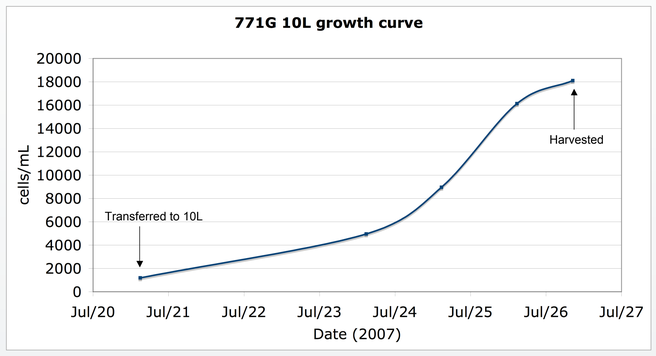


Questions:

1. **Does domoic acid degrade in particulate phytoplankton samples, when the samples are stored under common storage conditions (liquid nitrogen, -80ºC, -20ºC or 4ºC)?**
2. **Is one of these storage methods preferable in terms of degradation and reproducibility?**

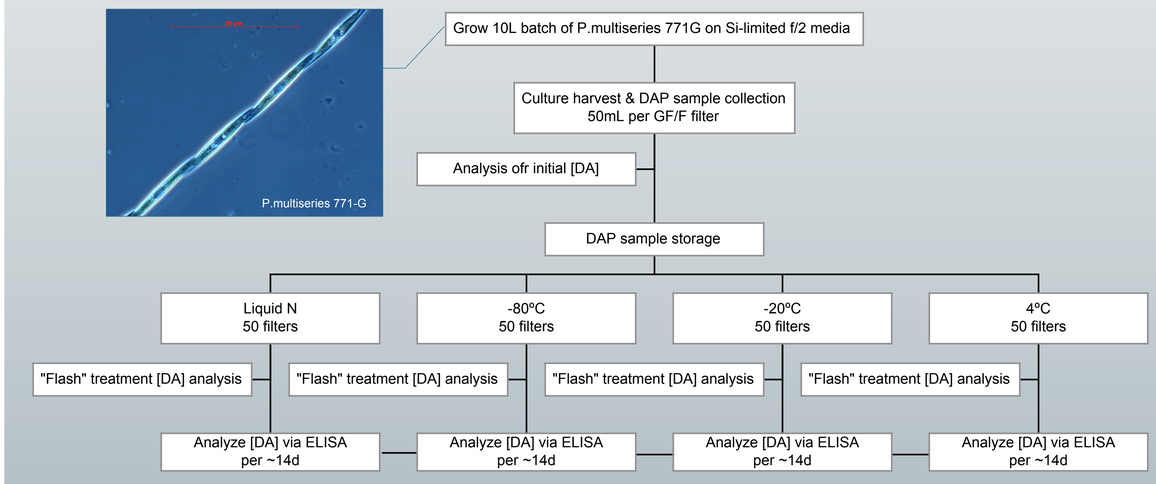




Abstract

Domoic acid (DA), a potent neurotoxin, is produced by various species of the diatom *Pseudo-nitzschia*. The study of *Pseudo-nitzschia* physiology and toxin production in both the field and in the laboratory commonly involves the collection and (preferably) short-term storage of filtered phytoplankton samples. Because it is generally not practical to process individual phytoplankton samples for DA immediately, the effects of storage on the DA content of the samples becomes an important question. The majority of literature addressing domoic acid degradation has focused on shellfish samples[1,2]. Degradation of DA in filtered phytoplankton samples has been addressed to a lesser extent, predominantly in the context of photodegradation[3,4]. One exception is a study on DA stability in field samples collected during a *Pseudo-nitzschia* bloom[5]. In this study, particulate DA (DAP) samples were sub-sampled, stored under various conditions (room temperature, -20°C, and 4°C), and measured over the course of 6 months. While this study offered recommendations to improve current DAP sample storage methods, it incited additional questions and concern regarding DA stability in DAP samples. Here, we address some of the still outstanding questions surrounding DA degradation through a study of DA degradation in DAP samples recovered from a single toxic uniclonal culture of *P. multiseries*. Recovered DAP samples were stored under a variety of conditions commonly implemented for DAP sample storage [liquid nitrogen (-196°C), -80°C, -20°C, 4°C]. To assess relative degradation rates between treatments, filters from each treatment were measured for DA in triplicate roughly every 2 weeks over a 3-month period.

Experimental design



Methods

Algal culture and batch growth

A uniclonal culture of Pseudo-nitzschia multiseries (771-G) was transferred to Guillards f/2 media with Si-limitation, and serially transferred up to 10L volume. The batch culture was incubated at 15±1 °C under ca. 100-200 µmol photons m^2 s^{-1} irradiance using standard (GE "soft white") fluorescence illumination on a 12:12 main light cycle and a 14:10 secondary light cycle (stepped increase/decrease in light).

Culture harvest

The culture was harvested just prior to the day 6 time-point, as the batch culture appeared to be entering into early stationary phase. Domoic acid particulate (DAP) samples were collected by gentle filtration (~100 mmHg) onto Whatman® GF/F glass-fiber filters. Fifty (50) mL of culture was filtered per GF/F, yielding a total harvest of 200 DAP filter samples. The DAP filters were stored individually in Nalgene® 2mL cryovials.

Sample storage

The samples were divided evenly among four common storage conditions. The storage conditions were:

- Dewar filled with liquid nitrogen--samples were kept in the vapor (not submerged) (-196ºC)
- Storage freezer, maintained at -80 ºC
- "Standard" frost-free freezer, maintained at -20 ºC
- Refrigerator, maintained at 4 ºC

Analysis scheme

All [DA] values represent triplicate sample analysis; 3 filters were pulled from each treatment per time-point. At the time of batch harvest, three (3) "fresh" (no storage treatment) DAP samples were analyzed to ascertain the initial domoic acid concentration ([DA] "time-zero" time-point, [T_0]). All remaining filters were evenly divided and placed in their respective storage treatments. Roughly 2 hours following initial storage, DAP samples were pulled from each storage condition and analyzed ("flash-treatment" time-point). After the day of harvest, samples from each storage condition were analyzed roughly every 14 days.

DAP analysis

DAP filters were extracted via sonication in 3mL 10% methanol, followed by syringe filtration (0.2µm). All sample extracts were analyzed for [DA] using Mercury Science® Enzyme-Linked ImmunoSorbant Assay (ELISA) domoic acid test kits according to the prescribed methods (available at http://www.mercuryscience.com/DA%20User%20Guide%202007A.pdf). A DA internal standard (pure DA diluted in aqueous buffer solution) was analyzed in tandem with the experimental samples.

Statistical analysis

Statistical analysis was through a 2-way ANOVA with replication, with factor 1 being Treatment (storage temperature) and Factor 2 being Time. Subsequent determination of the significance of the degradation through time was determined using ANCOVA on the linear regressions of each treatment.

References

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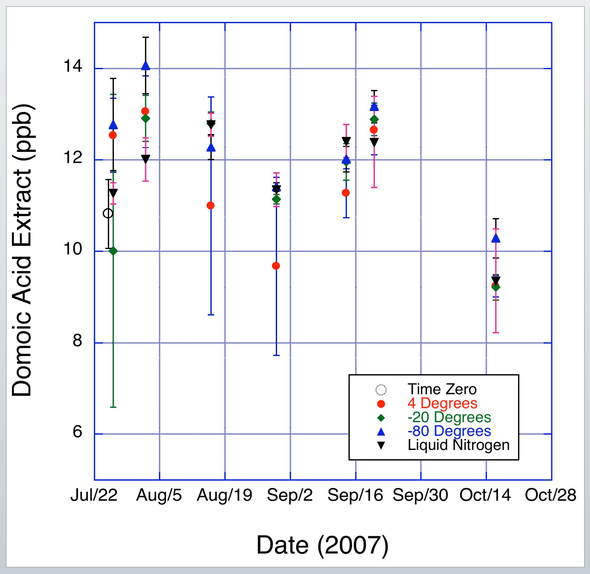
2) P. McCarron, S. Burrell, and P. Hess. Effect of addition of antibiotics and an antioxidant on the stability of tissue reference materials for domoic acid, the amnesic shellfish poison. Analytical and Bioanalytical Chemistry 387, 2495 (2007).
3) S.S. Bates, C. Léger, M.L. Wells, and K. Hardy, in Proceedings of the Eighth Canadian Workshop on Harmful Marine Algae, edited by S.S. Bates. Vol 2498 (2003).
4) R.C. Bouillon, T.L. Knierim, R.J. Kieber, S.A. Skrabal, and J.L.C. Wright. Photodegradation of the algal toxin domoic acid in natural water matrices. Limnology & Oceanography 51, 321 (2006).
5) K.A. Baugh, K. Lefebvre, J.C. Wekell, and V.L. Trainer, presented at the 3rd Symposium on Harmful Algae in the U.S., Asilomar, CA, 2005 (unpublished).

Results

DA in DAP samples

Question #1: Is there DA degradation in DAP samples? Answer: YES.



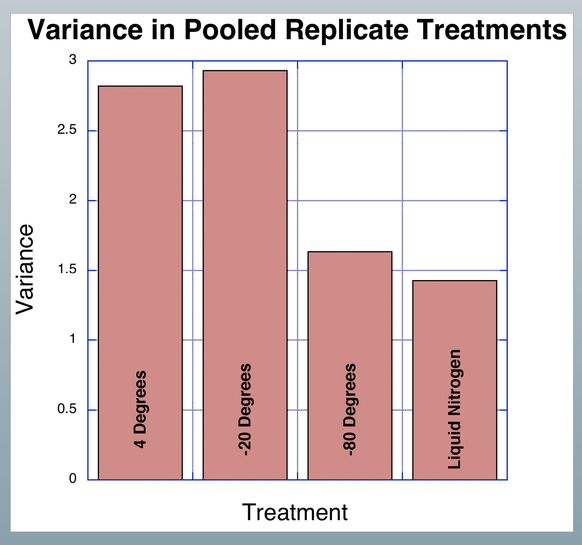
Two-way ANOVA

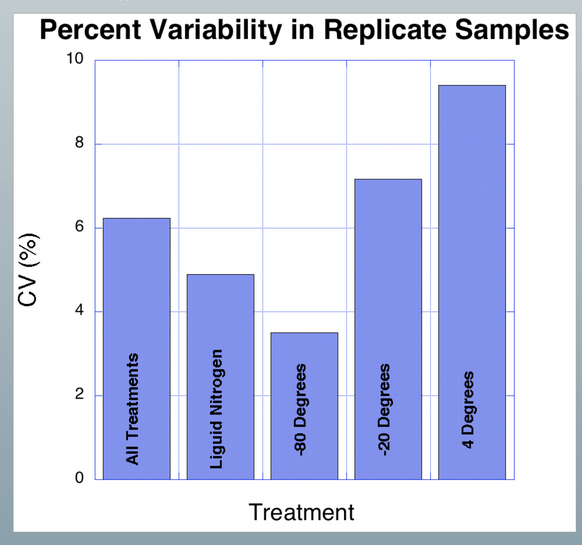
Source of variation	% of total variation	P value	Significant?
Interaction	14.37	0.1045	No
Temperature	5.16	**0.0250**	**Yes**
Time	51.78	**<0.0001**	**Yes**

Linear regression of degradation

	4 ºC	**-20 ºC**	**-80 ºC**	**Liquid N_2**
Slope (best-fit)	-0.031 ± 0.013	-0.015 ± 0.015	-0.029 ± 0.008	-0.018 ± 0.009
r^2	0.233	0.0543	0.412	0.173
P value	**0.0267**	0.3094	**0.0017**	0.0608
Is the slope significant?	**Yes**	No	**Yes**	**Moderately**

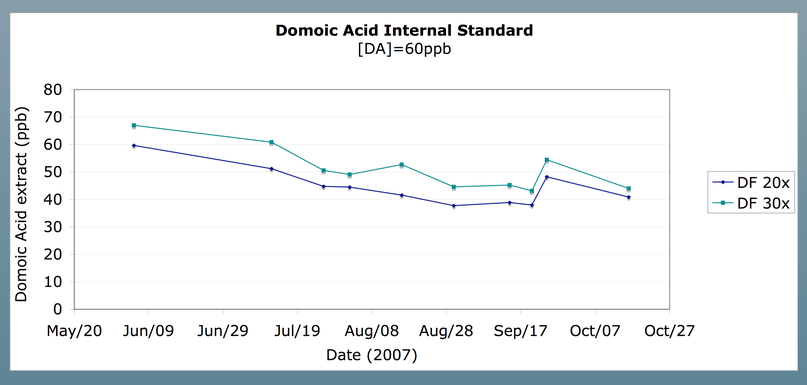
Question #2: Is there a difference between storage methods? Answer: YES.





What about that Internal DA standard?

The internal DA standard suggests degradation as well. The DA standard was kept in sterile buffer solution at 4°C (in the dark) for the duration of the experiment. Differences between dilution factors were accounted for in the methods.



Answers:

1. **There is statistical evidence for degradation of particulate phytoplankton domoic acid in samples under common storage conditions. Based on our methods, degradation would exceed measurement error after about 2 months of storage, and could result in substantial loss after about 1 year in storage assuming a constant degradation rate.**
2. **There is a statistical difference in storage methods. Increasing temperatures resulted in increasing variability of replicates; however, these differences are minor over short (< 1 month) periods compared to the analytical variability in [DA] measurement.**

Acknowledgements

This work was supported by a CDELSI graduate student fellowship and by MERHAB grant NA04NOS4780239, and is presented with the support of a NOAA CSCOR travel award. We would also like to thank Margaret Hughes, Meredith Howard, Peter Miller and Wayne Litaker.