A Rapid Method for Assaying Thiaminase I Activity in Diverse Biological Samples

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Abstract

Vitamin B₃ (thiamine) deficiencies can lead to neurological disorders, reproductive failure and death in wild and domestic animal populations. In some cases, disease is brought about by the consumption of foods high in thiaminase I activity. Levels of thiaminase activity in these foods are highly variable and the factors leading to production of this enzyme are poorly understood. Here we describe improvements in a spectrophotometric thiaminase I activity assay that measures the disappearance of 4-nitrothiophenol, a favored nucleophile co-substrate that replaces the thiazole portion of thiamine during the inactivation of thiamine by the enzyme. Scalable sample processing protocols and a 96-well microtiter plate format are presented that allow the rapid evaluation of multiple, replicated samples in the course of only a few hours. Observed levels of activity in bacterial culture supernatant, fish, ferns and molluscs using this colorimetric assay were similar to previously published reports that employed a radiometric method. Organisms devoid of thiaminase I, based upon previous work, showed no activity with this assay. In addition, activity was found in a variety of fishes and one fern species from which this enzyme had not previously been reported. Overall, we demonstrate the suitability of this technique for measuring thiaminase I activity within small amounts of tissue and environmental samples with replication levels that were heretofore prohibitive. The assay provides a considerable improvement in the ability to examine and understand the properties of an enzyme that has a substantial influence on organism and ecosystem health.

Introduction

The important role of thiamine (vitamin B₁) in human and animal health has been long-recognized, but a broad appreciation of its role in environmental health has developed more slowly [1]. In animal husbandry, instances of mortality from thiamine deficiency were first described in the 1940s in mink and foxes raised for fur production [2,3], then later in cattle, sheep and goats [4,5,6]. Japanese scientists pursued extensive studies during and following World War II linking thiamine deficiency in humans to thiamine decomposing bacteria [7]. Thiamine deficiencies in wild populations of predatory fish were first recognized in the 1990s as responsible for a widespread mortality syndrome observed for decades in valuable Baltic Sea and Laurentian Great Lakes fisheries [8].

One common dietary link in thiamine-deficiency mortality syndromes observed in commercial fisheries, animal husbandry and humans has been the consumption of food with high thiaminase activity. Two thiamine-degrading enzymes have been described: thiaminase I and thiaminase II or TenA [9]. Thiaminase I catalyzes the replacement of the thiazole moiety of thiamine with any of a variety of organic nucleophiles [10,11]. Thiaminase I is the only thiaminase linked to fish reproductive failure and animal mortality, consequently it has been the focus of studies investigating syndromes associated with enzymatic thiamine degradation [14,15,16]. Predatory salmonine fishes suffering from thiamine deficiency in North America and Europe prey upon clupeid fishes with high thiaminase I activity [17,18]. In addition, feeding experiments showed that mink and foxes died from thiamine deficiency when fed raw fish that contained thiaminase I [2,3]. Finally, domestic animals such as horses and cattle regularly die from thiamine deficiency after feeding on bracken fern that contains high thiaminase I activity [19].

In the 1950s, researchers isolated thiaminase I-producing bacteria from human feces [20,21]. One of these bacteria was subsequently found in rumen of ailing sheep with cerebrocortical necrosis, a disease associated with thiamine deficiency [22]. For decades the thiaminase literature has referred to non-microbial sources of thiaminase, such as plants, fish, shellfish (including mollusks), and crustaceans [23], but it is still unclear whether multi-cellular organisms produce a divergent thiaminase I or whether the enzyme activity within these organisms originates from microbial sources [18,24]. Recent genome sequencing efforts have helped identify additional putative bacterial genes that likely code for thiaminase I homologs while no clear homolog has been identified in sequenced genomes or transcriptomes of fish.

Analytical approaches to assess the thiaminase activity in plant or animal tissues and bacterial cultures have evolved since...
thiamine-degrading enzymes were described in the 1940s. Sealock et al. [23] first used the term “thiaminase” to describe thiamine-degrading enzymes in fish by noting that this substance could be regarded as a type of thiaminase only if its enzymatic nature was confirmed. Shortly thereafter, Krapfiz and Woolley [26] reported the distinguishing enzymatic action of a thiaminase (obtained from an aqueous extract of carp viscera) as its ability to split thiamine into its pyrimidine and thiazole moieties, after which Yudkin [27] firmly established use of the term thiaminase. In a review of studies of enzymes responsible for paralysis in animals feeding upon fish. It is interesting to note that this early report [26] of an apparent hydrolytic enzymatic cleavage by carp thiaminase of thiamine into its thiazole portion (isolated as 4-methyl-5-hydroxyethylthiazole) and a hydroxylated-pyrimidine portion (isolated as 2-methyl-4-amino-5-hydroxymethylpyrimidine) is characteristic of the type II thiaminase enzyme reaction. Such a finding is in conflict with subsequent studies reported by Fujita [23], Sato et al. [28], Bos and Koziak [29], and Wistbacka et al. [18] in which the action of carp thiaminase enzymes have been consistently characterized as a base-exchange, type I, thiaminase reaction. Early studies measured thiaminase activity by assaying the disappearance of thiamine in the presence of biological tissues, typically from fish [25,26,30]. Several alternative methods were developed [31,32] before Edwin and Jackman [4] laid the foundation for a radiometric thiaminase assay that was used extensively for the next 40 years [see [18,33,34] for subsequent modifications of this method]. The radiometric assay uses [14]C-thiamine as a substrate for thiaminase I, which releases thiazole - 2-[14]C that is then extracted and the associated radioactivity is measured. Unfortunately, requirements for handling radioactive materials and the expense and limited availability of radiolabeled thiamine have restricted the use of this assay to a small number of laboratories.

We found this limitation in conducting studies examining evidence that bacterial symbionts are the source of thiaminase within fishes and other aquatic organisms, which led us to develop a spectrophotometric thiaminase assay [35] to evaluate activity in bacterial cultures, environmental samples and animal tissues. This assay measures the consumption of 4-nitrothiophenol (4-NTP), a favored nucleophile co-substrate that replaces the thiazole portion of thiamine during the inactivation of thiamine by thiaminase I. A preliminary comparison of the radiometric and 4-NTP colorimetric methods was conducted that described refinements in the assay for measuring thiaminase I in samples of fish tissues [36], though this report raised some concern about differences in sensitivity between the two assays. Here we describe further modifications of the 4-NTP assay for high throughput use with a microtiter plate reader, as well as demonstrate its application to a broad variety of organisms previously described as containing thiaminase I. Specifically, we demonstrate the efficacy of the 4-NTP assay to measure thiaminase I in both bacterial cultures and tissues from plants, fish, crustaceans, and molluscs. We also report refinements that improve the ability to quantify high-activity samples and demonstrate that this more accessible assay is as effective as the radiometric assay for biological samples.

**Materials and Methods**

**Ethics statement**

All fish used in this study were collected in waters of New York State (Table 1) under the authority of a fish collection permit authorized by the Bureau of Fish, Wildlife and Marine Resources of the New York State Department of Environmental Conserva-
the volume of phosphate buffer was added to a tissue sample) and the extraction using a smaller amount of buffer (generally 1 times for small samples with low activity, we would occasionally repeat this mixture was clarified with centrifugation as above. Note that phosphate buffer and ground with a pestle (USA Scientific), then in a 1.5 ml microcentrifuge tube with 2.5 times volume of contents. Small and soft tissue samples (small mussels) were placed Alewife intestine samples were analyzed intact with their internal shell) were homogenized in buffer using a Tissuemiser (Fisher Scientific), then the homogenate fluid was removed from the shell) was transferred to a Pierce centrifuge column 6 microcentrifuge tube and centrifuged at 16,000 g for 10 min. Cloudy supernatant was transferred to a clean tube and stored for 1 min. Supernatant was transferred to a clean tube and stored for 10 min. Generally 3 μl of sample supernatant was used in each assay well. Smaller samples (fish muscle or gill tissue, quagga mussels removed from the shell) were homogenized in buffer using a Tissuemiser (Fisher Scientific), then the homogenate fluid was clarified by two rounds of centrifugation, as described above. Alewife intestine samples were analyzed intact with their internal contents. Small and soft tissue samples (small mussels) were placed in a 1.5 ml microcentrifuge tube with 2.5 times volume of phosphate buffer and ground with a pestle (USA Scientific), then this mixture was clarified with centrifugation as above. Note that for small samples with low activity, we would occasionally repeat the extraction using a smaller amount of buffer (generally 1 times the volume of phosphate buffer was added to a tissue sample) and rerun the assay. Small samples that were not easily homogenized with a plastic pestle (e.g. zooplankton) were placed in a 2 ml screw cap tube with an equal volume of zirconia/silica beads (BioSpec Products), then a small amount of buffer was added and the mixture was processed with a mini-beadbeater (BioSpec) for 3 minutes. Tubes were centrifuged as above.

Culture supernatant was used in bacterial thiaminase activity assays. A 0.5 ml aliquot of the culture was centrifuged at 16,000 × g for 1 min. Supernatant was transferred to a clean tube and stored at −80°C until assayed. Generally, 3 μl of supernatant was used in each assay well. The lower detection limit of the thiaminase assay was evaluated by testing nine 1:1 serial dilutions (with buffer) from each assay well. The lower detection limit of the thiaminase assay was evaluated by testing nine 1:1 serial dilutions (with buffer) from each assay well.

100 mM phosphate buffer, pH 6.5. After vortexing, and allowing large pieces to settle, the supernatant was transferred to a 1.5 ml microcentrifuge tube and centrifuged at 16,000 × g for 10 min. Cloudy supernatant was transferred to a Pierce centrifuge column (39868) and centrifuged again. Generally 3 μl of sample supernatant was used in each assay well.

| Organism              | Common name | Length ± standard deviation | Location collected | Date       |
|-----------------------|-------------|----------------------------|--------------------|------------|
| Alosa pseudoharengus  | Alewife     | 15.2 ±1.2 cm               | 43.28 'N 76.33' W  | 6/12/2010  |
| Osmerus mordax        | Rainbow smelt | 13.1 ±2.1 cm               | 43.41 'N 74.53' W  | 5/12/2011  |
| Alosa mediocris       | Hickory shad | 30.5 ±2.2 cm               | 39.39 'N 76.10' W  | 4/21/2011  |
| Fundulus diaphanus    | Banded killfish | 4.5 ±0.7 cm               | 42.27 'N 76.27' W  | 11/14/2011 |
| Rhinichthys atratulus | Blacknose dace | 4.8 ±0.9 cm               | 42.27 'N 76.27' W  | 11/14/2011 |
| Camostoma anomatum    | Central stone roller | 7.8 ±0.3 cm               | 42.27 'N 76.27' W  | 11/14/2011 |
| Luxilus cornutus      | Common shiner | 6.2 ±0.5 cm               | 42.27 'N 76.27' W  | 11/14/2011 |
| Semotilus atromaculatus | Creek chub    | 7.6 ±0.5 cm               | 42.27 'N 76.27' W  | 11/14/2011 |
| Exoglossum maculina   | Cutlips minnow | 7.0 ±2.3 cm               | 42.27 'N 76.27' W  | 11/14/2011 |
| Semotilus corporalis  | Fallfish     | 15.4 ±0.1 cm               | 42.27 'N 76.27' W  | 11/14/2011 |
| Etheostoma flaviblare | Fantail darter | 5.0 ±1.5 cm               | 42.27 'N 76.27' W  | 11/14/2011 |
| Rhinichthys cataractae | Longnose dace | 5.3 ±0.5 cm               | 42.27 'N 76.27' W  | 11/14/2011 |
| Etheostoma olmstedi   | Tesselated darter | 6.2 ±0.8 cm               | 42.27 'N 76.27' W  | 11/14/2011 |
| Catostomus commersonii | White sucker  | 13.2 ±3.1 cm               | 42.27 'N 76.27' W  | 11/14/2011 |
| Dreissena rotiformis bugensis | Quagga mussel | 1.5 ±0.8 cm               | 42.35 ‘N 76.37’ W  | 7/4/2011   |

**Table 1.** Fish and mussel samples evaluated in this study.

![Table 1](https://doi.org/10.1371/journal.pone.0092688.t001)

4-NTP thiaminase I assay

This assay relies upon measuring the disappearance of the yellow co-substrate 4-NTP, which is used by thiaminase I to...
inactivate thiamine by replacing the thiazole portion of thiamine with a favored nucleophile co-substrate [35]. All chemicals were purchased from Sigma-Aldrich except Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), which was purchased from Solutec Ventures. Reagents were prepared as described previously with modifications [36]. TCEP buffer (100 mM NaCl, 50 mM phosphate buffer, pH 6.9; 10 mM TCEP) was placed on ice and bubbled for 15 min with O2-free N2 (which was passed over heated copper fillings to remove residual O2 [38]) then used immediately. A stock solution of 4-nitrothiophenol (4-NTP), 3 mg/ml dissolved in dimethyl sulfoxide, was diluted to a final concentration of 200 μM 4-NTP in TCEP buffer or TCEP buffer plus 400 μM thiamine HCl. The 4-NTP-TCEP solution with thiamine is referred to as the “experimental” solution and the solution with no added thiamine is the “control”.

A 96-well microtiter plate reader was used to assay the disappearance of 4-NTP, using either a 12-sample or 24-sample format. The 12-sample format provided 4 replicates of experimental and control wells from a tissue extract or culture type; the 24-sample format provided duplicates of each. All wells of the microtiter plate were filled with 97 μl of solution using a multi-channel pipette; half of these wells being filled with the experimental solution and half with the control. Each well also received 3 μl of sample extract or culture supernatant before being placed in Powerwave XS2 plate reader (BioTek) that was preheated and maintained at 37°C. Readings of absorbance at 411 nm were taken every minute over the course of 1 hour to record the disappearance of the yellow 4-NTP. A path-length correction feature of the plate reader was used to standardize absorbance to a 1 cm path length.

Data analysis

The plate reader Gen5 software (BioTek) was used to determine the maximum velocity of 4-NTP degradation by comparing rates of change in optical density over all of the possible 10-min intervals. The maximum values were averaged for replicate wells. To correct for non-enzymatic degradation of 4-NTP, the mean maximum velocity of the control wells (without thiamine) for each sample was subtracted from the maximum velocity of the experimental wells. Change in absorbance was converted into a rate of 4-NTP degradation using its extinction coefficient in TCEP: 13,650 M⁻¹·cm⁻¹ [35]. The 4-NTP cosubstrate concentration (mols/L) was converted to moles by multiplying the reaction volume (10⁻¹ L). For samples with a low raw activity (< 25 pmol 4-NTP degraded·min⁻¹) but greater than a minimum detectable raw activity value (4.8 pmol 4-NTP degraded·min⁻¹), the significance of observed differences in the maximum velocities of control wells and experimental wells was evaluated using an unpaired t-test. If a significant difference was found (P-value < 0.05), the presence of thiaminase I activity was considered to have been substantiated. 4-NTP degradation rates were converted to thiaminase activity, assuming a 1:1 degradation of thiamine:4-NTP. For all calculations, we used 1 ml = 1 g. Measures for tissue extracts were converted to mass-specific rates of thiamine degradation (nmol thiamine degraded·min⁻¹·g⁻¹) as follows. The raw thiaminase activity value was divided by the combined weight of the buffer and the sample multiplied by the amount of prepared sample added to the assay. Typically, the raw value was converted by multiplying it by the factor (1 g/3.5 g) · 0.003 g⁻¹. For bacterial culture supernatant assays, the measured activity was standardized to activity per ml of culture, and converted using the factor (0.003 ml⁻¹).

Results

In agreement with previous reports [38], high thiaminase I activity was found in the supernatant of one-day-old cultures of C. sporogenes and P. thiaminolyticus, with greater activity observed in cultures of P. thiaminolyticus (Figure 1). No thiaminase I activity was detected with the 4-NTP assay in culture supernatants or cell lysates of A. aneurinilyticus or B. subtilis, bacteria with thiaminase II activity [12,23].

The dilution series of P. thiaminolyticus supernatant exhibited a proportional decline in thiaminase I activity down to a dilution level of 1/256 (Figure 2). This represents a raw activity value of approximately 4.8 pmol thiamine degraded·min⁻¹ or an equivalent activity of about 1.6 nmol thiamine degraded·min⁻¹·ml⁻¹. We therefore consider this assay to have sensitivity down to that value (i.e. below which the assay failed to distinguish a further decrease in thiaminase I activity). Measured values below that level were considered to have an activity of zero. The difference in absorbance at 411 nm between control and experimental wells for a sample with the minimum detectable raw activity value of 4.8 pmol 4-NTP degraded·min⁻¹ was 6.6 mOD units.

Substantial thiaminase I activity was found in whole alewife and rainbow smelt (Figure 3), with greater levels of activity localized to specific tissues within alewife, most notably the intestine (Figure 4). High levels of thiaminase I activity were found in bracken fern rhizomes, and lower levels of activity were found in Christmas fern rhizomes and fine roots, but no activity was detected in rhizomes or fine roots from ten other ferns common to upstate New York (Figure 5). The greatest activity was found in the rhizomes or roots of bracken or Christmas ferns, by comparison with the stipe and fronds of the same plant (bracken fern stipe and fronds: 0 and 29 nmol thiamine degraded·min⁻¹·g⁻¹, respectively; Christmas fern stipe and fronds: no activity was found).

Thiaminase I activity was detected in 9 of 11 species of fish collected from a tributary to Cayuga Lake (Fall Creek) and one
species collected from the Susquehanna River (Figure 6). Thiaminase I activity of Cayuga Lake quagga mussels was 60 nmol thiamine degraded\(\min^{-1}\cdot g^{-1}\) (± se 22). No thiaminase I activity was found in 14 samples of *D. pulicaria* that were assayed.

**Discussion**

Thiaminase I activity levels determined by the 4-NTP assay were consistent with previous observations of activity in bacteria and fish, ferns, molluscs, and specific tissues within these organisms. A key comparison showed that high levels of thiaminase I were found in cultures of two bacteria from which this enzyme was first identified in the 1950s: *C. sporogenes* (renamed from *Clostridium thiaminolyticum* and *P. thiaminolyticus* [16,21].

Another important comparison showed that substantial thiaminase I activity was found in two species of Laurentian Great Lakes forage fish, alewife and smelt, regularly reported to contain this enzyme. For example, Tillitt *et al.* [39] reported mean levels of thiaminase I activity in Lake Michigan alewife and rainbow smelt as 4.4 and 2.6 nmol\(\cdot g^{-1}\cdot \min^{-1}\), respectively, by comparison with our mean values of 100 nmol\(\cdot g^{-1}\cdot \min^{-1}\) for alewife collected from Lake Ontario and 129 nmol\(\cdot g^{-1}\cdot \min^{-1}\) for smelt from an Adirondack lake. However, these are general comparisons from fish captured in different circumstances, and substantial variability has been evident in previously published estimates of thiaminase I activity in different individual fishes [39]. The 4-NTP assay verified much greater activity in alewife intestinal tissues (462 nmol\(\cdot g^{-1}\cdot \min^{-1}\)) when compared with gill (45 nmol\(\cdot g^{-1}\cdot \min^{-1}\)) and muscle (7 nmol\(\cdot g^{-1}\cdot \min^{-1}\)) tissue; these values are greater than the only previous report of thiaminase I activity in intestinal tissue from a single Lake Michigan alewife (33 nmol\(\cdot g^{-1}\cdot \min^{-1}\); [34]).

4-NTP assay results in this study were consistent with numerous observations of high thiaminase activity in bracken fern and specific tissues within these plants, with the enzyme most abundant in the rhizomes compared to the stipe and fronds [19]. Evans reported thiaminase I levels in bracken fern rhizomes ranging from approximately 80 to 480 nmol\(\cdot g^{-1}\cdot \min^{-1}\) (estimated from Figure 1 in [19]), comparable to our mean of 126 nmol\(\cdot g^{-1}\cdot \min^{-1}\) in rhizomes. Furthermore, we found one additional species...
of fern (*P. acrostichoides*) with thiaminase I activity that belongs to the same genus as a British fern (*Polytetraphyllium setiferum*) previously reported to contain thiaminase I [40]. Our assay measurements resulted in a mean value of 34 nmol g⁻¹ min⁻¹ in rhizomes from *P. acrostichoides*, by comparison with reported activity in *P. setiferum* ranging from 8 to 16 nmol g⁻¹ min⁻¹ [40]. Activity in quagga mussels assessed with the 4-NTP assay was within the range reported by Tillitt et al. [41]: 19.5 to 223.8 nmol g⁻¹ min⁻¹.

In contrast to previous assays, the 4-NTP assay as described here provides the ability to analyze large numbers of samples at a minimal expense, without compromising detection of biologically relevant levels of thiaminase activity. Notably, the materials, equipment, technician time and training required to run the assay – as well as the ability to conduct the assay in a lab that does not require special permits or procedures for using radio-labeled thiamine – are minimal by comparison with the radiometric assay. A 96-well microtiter reader provides the capacity for 12, 16 or 24 samples allowing for 4, 3 or 2 replicates, simultaneously. In previous experiments, the use of the radiometric assay restricted the number of samples that we could analyze given the fixed budget constraints of a research grant [42]. We also suspect that expense constrained the use of replicates in thiaminase studies conducted decades ago [19,40]. The limitations of running the radiometric assay also seemed apparent in recent studies, such as an analysis of the distribution of thiaminase I in alewife tissues conducted using a single fish [34] or an analysis of thiaminase I in dreissenid mussels that was restricted to three samples for each collection event [41].

We consider it important to highlight several choices made in calculating enzyme activity from our assay procedure. First, in comparing activity measures from the 4-NTP assay within biological tissues to previously reported values determined using different assays, we considered our samples to have been assayed in units of wet weight instead of dry weight because these samples were not dried during sample preparation. By contrast, thiaminase I activity levels determined using a radiometric method applied to ferns have been reported in units of dry weight [19]. Second, for bacterial cultures we chose to report enzyme activity values in units that are not mass-specific (nmol thiamine degraded min⁻¹ ml⁻¹) because no consistent meaningful mass-specific unit conversion could be readily determined for bacterial culture supernatant. We considered using total protein for this conversion but reasoned that because thiaminase activity increases in culture supernatants over the course of 3 days (data not shown) that the concentration of secreted thiaminase I relative to total protein in the supernatant would vary with culture age in an unpredictable manner.

Use of a microtitre plate reader to take spectrophotometric measurements over a 60-min period facilitated determining the maximum activity (slope) of the thiaminase I enzyme within a given sample. We chose a 10-min window over which to determine maximum enzyme activity from trial assessments of activity in bacterial culture samples with different levels of activity. The greatest rates were typically seen early in the hour-long time course and exceptions to this only occurred when the rate was steady throughout the measurement period. We also note that samples with high thiaminase activity required dilution to ensure that sufficient 4-NTP remained throughout the duration of the assay.

We do not suggest a minimum biological sample size at which the 4-NTP assay can be used because detectable activity would depend in part on the level of activity in the material. However, the smallest amount of tissue from which we detected activity was 10 mg from quagga mussels. By comparison, Zajicek et al. [34] reported typically analyzing 1.0 g samples when using the radiometric assay, but smaller samples (50.0–250.0 mg) were also assayed. In that study, a 50 mg pellet of lyophilized *P. thiaminolyticus* was processed and activity measured in subsequent
dilutions, though they did not use these results to evaluate the detection limit of the radiometric assay.

Our fish and fern tissue analysis results are consistent with the hypothesis that microbial sources are responsible for the presence of thiaminase I in fishes, as well as other studies of the distribution of this enzyme in a variety of fish. Early work by Fujita [43] reported high thiaminase activity in specific fish organs, including the spleen and kidney of gray mullet (*Mugil cephalus*), the liver and gills of yellowfin goby (*Acanthogobius flamin cus*), and the kidney, intestine, gills, ovaries and liver of carp (both *Cassarasses auratus* and *Cyprinus carpio*). In making these observations Fujita used the disappearance of thiamine as a measure of “thiaminase” activity, as did several subsequent studies that measured thiamine-degrading activity and reported it as thiaminase I activity. For example, Sato et al. [29] reported thiaminase I as concentrated in the kidney and spleen of carp (*Cyprinus carpio*), and Arsan and Malyarevskaya [44] reported high activity in the liver and intestines of silver carp (*Hypophthalmichthys molitrix*). Some of these tissues (e.g. gastrointestinal tracts) contain abundant bacteria, and it is notable that we found very low levels of thiaminase in muscle tissue where bacteria are unlikely to occur. A similar pattern was reported by Fujita [43] in an overview of thiaminase in 17 shellfish species and seven crustaceans, in which muscle levels of thiaminase were zero or barely detectable when tested separately from viscera or other tissues from individuals that had high “total organism” levels of thiaminase. The greater levels of thiaminase I observed in bracken fern rhizomes provide similar evidence in support of the hypothesis that bacteria are the source of thiaminase I in these plants, given observations of the commonly observed localized distribution of bacteria in plant roots by contrast with other tissues [45].

We have demonstrated the availability of a high throughput assay that can rapidly test for thiaminase I activity in bacterial cultures, environmental samples and animal tissue. We expect that use of this assay will provide a valuable method to further foster an understanding of the importance of thiaminase I in nature by facilitating the application of controlled and replicated experiments as well as fine-scale analysis of environmental samples.

Acknowledgments

We thank Dale Honeyfield for his gift of *P. thiaminolyticus* 8103 as well as his advice in running the assay, comments on our calculations of thiaminase activity and comments on a manuscript draft. We also thank Jim Zacijek and an anonymous reviewer for helpful comments on the manuscript. We are grateful to Jennifer Sun and Rania Alou Kanzid for technical assistance with the thiaminase assay and Nelson Hairston Jr., Colleen Kearns and Lindsay Schaffner for assistance in raising *Daphnia*.

Author Contributions

Conceived and designed the experiments: CEK ERLG ERA. Performed the experiments: ERLG. Analyzed the data: CEK ERLG. Contributed reagents/materials/analysis tools: ERA. Wrote the paper: CEK ERLG ERA.

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