Short communication

Rapid assay for amidohydrolase (urease) activity in environmental samples

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Abstract

The use of microplate technology for enzyme assays has made it economical to measure a wide range of activities in environmental samples. Urease is one of the most widely measured soil enzyme activities, but current methods are cumbersome. We have developed a rapid, safe and sensitive assay that can be performed on microplates.

Keywords: Amidohydrolase; Urease; Soil enzyme assay; Assay method; Microplate assay

Microbial ecology advances through technological improvements. For two decades, the commercial availability of chromogenic and fluorogenic substrates has facilitated research on enzyme activities in natural environments. Microplate technology has reduced the cost of enzyme assays, making it possible to assay up to 30 enzymes on a single plate (Foreman et al., 1998). Amidohydrolase (EC 3.5.1.5), commonly known as urease, is among the most commonly assayed soil enzymes. Interest in this enzyme stems from its role in the decomposition of urea fertilizer in agricultural systems (Bremner and Mulvaney, 1978; Xiaobin et al., 1995). More generally, it is considered an important agent for N mineralization in terrestrial and aquatic systems (Leftly and Syrett, 1973; Collier et al., 1999; Kandeler et al., 1999).

Urease decomposes urea into carbon dioxide and ammonium (Pettit et al., 1976). Activity is generally assayed by measuring the rate of NH₄ production (Tabatabai and Bremner, 1972; Kandeler and Gerber, 1988). A number of assay procedures that variously require long incubations, large samples, specialized equipment, or hazardous chemicals have been published (Tabatabai and Bremner, 1972; Holland and Polacco, 1992; Klose and Tabatabai, 1999). We have developed a urease assay that is fast, sensitive, safe and can be performed on 96-well microplates.

A sample suspension is created by homogenizing 1.0 g soil (or 0.5 g litter) in 100 ml of 50 mM, pH 5.0 acetate buffer, using a Brinkmann Polytron. More concentrated suspensions have more activity but they generate a higher absorbance background. We use pH 5 buffer to approximate the pH of bulk soil, but other buffers may be used to optimize sensitivity. Another option is to use purified water and rely on the native buffering capacity of the soil. The sample suspension is poured into a wide mouth dish and placed on a magnetic stirrer. While stirring vigorously, 200 µl aliquots are withdrawn and dispensed into the microplate wells using an 8-channel pipetter with wide orifice tips. We use three microplate columns (24 wells) per sample. Aliquots (10 µl) of substrate solution (400 mM urea in deionized water), are dispensed into the wells of two columns (16 wells) yielding a final urea concentration of ~20 mM. While this concentration is well above reported Kᵢ values (Bremner and Mulvaney, 1978), substrate saturation should be tested when applying this method to new soil types. The third column of wells acts as negative controls; each well receives 10 µl of water. Each microplate also contains a substrate control: a column of wells that each contain 200 µl of buffer + 10 µl of substrate solution.

Standards, prepared from ammonium chloride, can be added to the microplate. Standards ranging from 1–10 µM (54–540 µg NH₄Cl l⁻¹, 18–80 µg NH₄⁺ l⁻¹) are good for short incubations or samples with low activity. Wells in standard columns contain 200 µl of standard and 10 µl of water. The wells containing standards constitute the positive controls for the assay. Standards can also be prepared using sample suspensions which allows for the potential adsorption of NH₄ to particles. In this case, 10 µl aliquots of
standard, ranging from 20–200 μM, are added to wells containing 200 μl of sample suspension.

We incubate the microplates at 20°C for up to 18 h. Higher temperatures will increase sensitivity. Ammonium concentration in the wells is quantified using reagent packets from Hach (Loveland, CO 80539, USA) which are marketed for water testing kits (salicylate reagent — catalog no. 23952-66; cyanurate reagent — catalog no. 23954-66). The contents of the salicylate reagent packets are dissolved in deionized water (one packet per ml) and 40 μl of the salicylate reagent is added to each well, including controls and standards. After a 3 min reaction period, the contents of the cyanurate reagent packets are dissolved in deionized water (one packet per ml) and 40 μl of cyanurate reagent is dispensed into each well. Color development is complete in 20 min. The absorbance (ABS) of each well is then read at 610 nm using a spectrophotometric plate reader.

Urease activity is expressed as mmol NH₄⁺ released g dry soil⁻¹ h⁻¹. Net ABS = (mean ABS of assay wells) - (mean ABS of control samples). Activity (mmol NH₄ g⁻¹ h⁻¹) = (Net ABS) × V/E × T × M/A.

where V is the volume of the sample suspension (100 ml), E the extinction coefficient, ΔABS/nmmol NH₄, the slope of ABS vs. [NH₄] (for our machine E is about 25 pmol⁻¹), T the incubation time, h, M the mass of soil or litter used to make the sample suspension, A the volume of sample aliquot assayed (0.2 ml).

It is worth noting that analytical variation among replicate wells can be considerable. In our work, coefficients of variation range from 5 to 20% depending on site and sample type. Well-to-well variation can be minimized by adequate homogenization, vigorous mixing of samples while dispensing, and good pipetting technique. By using 16 replicate wells, the standard error of the mean is <5%.

We have used this assay to measure urease activities at the sites of three long-term research experiments. The Duke Forest site, near Durham, NC, is a Pinus taeda (loblolly pine) plantation, planted in 1983, which is currently subject to a Free Air Carbon Enrichment (FACE) experiment (DeLucia et al., 1999). The soil at Duke has a high clay content and is classified as an Ultic Alfisol, pH 5, with an organic matter content of 0.5% (Allen et al., 2000). The Oak Ridge, TN, site, also used for a FACE experiment, is a Liquidambar styraciflua (sweet gum) plantation, planted in 1989. Soil at the Oak Ridge site is classified as a Typic Dystrochrept with a shaly silt loam A horizon. The third site near Pellston, MI, is a 90 y old Acer saccharum (sugar maple) stand with soil classified as sandy, mixed, Frigid Typic and Alfic Haplorthods and an organic matter content of 3.5%. Since 1987, parts of it have been amended with 30 kg N ha⁻¹ y⁻¹.

The Duke and Oak Ridge sites were sampled in May, July and September 1999. On each date, twelve 2 x 15 cm² cores were collected in each of six plots. At the Duke site, litter samples were also collected from several locations in each plot. Composite samples from each plot were assayed for urease activity on the day of collection. The Pellston site was sampled on a single date in September 1999. Composite soil samples from three control and three N-amended plots were assayed.

At the Duke Forest, activity varied from undetectable to 0.73 μmol g⁻¹ h⁻¹ (mean = 0.32, n = 18) in soil and from 0.11 to 5.37 μmol g⁻¹ h⁻¹ (mean = 1.43, n = 18) in litter. Activity was lower in Oak Ridge soil, ranging from undetectable to 0.71 μmol g⁻¹ h⁻¹ (mean = 0.21, n = 18). At the Pellston site, activity in control plots averaged 1.63 μmol g⁻¹ h⁻¹ compared to 0.98 μmol g⁻¹ h⁻¹ in the N-treated plots.

These values fall in the range of published reports for forest systems. The availability of a microplate assay for urease facilitates long term monitoring and makes it easier to include urease in profiles of extracellular enzyme activity.

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References


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