New Method for Measuring Phytase Activity Could Help Characterize Nutritive Status in Surface Water

Phytase presumably plays an important role in converting phytic acid to nutrient P (i.e., orthophosphate) in aquatic ecosystems. Unfortunately, there is no information available on measurements of phytase activity in surface water, making the assessment of the role of phytate-degrading enzymes in the aquatic P cycle problematic.

To address this issue, Virginia Tech researchers have developed a reliable method, employing a substrate analog of phytic acid, tetrachlorofluorescein (TET) tethered (T) InsP₅, for measuring phytase activity. Phytases sequentially remove phosphate groups from TET TInsP₅, producing dephosphorylated probe species that are readily separated by high-performance liquid chromatography. By design, the TET group is retained by all dephosphorylated probe species, allowing highly sensitive quantification with fluorescence detection. This new method, employing this innovative probe, affords researchers the means to unambiguously quantify an extremely small amount of phytase-generated dephosphorylated product(s), enabling the measurement of enzyme activity over a reasonably short time duration in an environmental sample containing low concentrations of phytate-degrading enzyme.

In the January–February 2013 issue of the Journal of Environmental Quality, researchers at Virginia Tech report successfully employing this new method to measure phytase activity in eutrophic pond water. Interestingly, the results revealed that phytase activity associated with the particulate plus water-soluble fraction was greater than that observed for the water-soluble fraction alone. The research findings also suggest that organisms other than bacterioplankton were actively involved in producing phytate-degrading enzymes in pond water, giving rise to the possibility that a heretofore-unrecognized group of microorganisms are involved in phytic acid mineralization. Based on the results of this investigation, one could reasonably conclude that refractory organic P in the form of phytic acid was contributing to eutrophication of a freshwater pond.

“We are now positioned to develop a methodological approach for assessing nutritive status (i.e., availability/biodegradability) of particulate-associated organic P pools in aquatic ecosystems,” notes D.F. Berry, co-author of the study. Nutrients from exogenous sources perpetuate eutrophic conditions and high-biomass algal blooms in freshwater ecosystems. Soil-borne particulate P serves as a continuous source of exogenous P. Assessing the contribution of soil-borne particulate organic P in promoting/supporting eutrophication is vital, Berry points out, because recent climate models project an escalation in the number and intensity of such rainfall events in many regions of the United States, which will undoubtedly result in increased overland transport of soil-borne particulate P to surface waters. Much of this P is refractory organic P and includes phytic acid. Berry contends that future research efforts should be driven by the guiding principle that enzyme production signals cellular intention, communicating the collective bio-catalytic response of the aquatic microbial community to nutritive status.
