Diversity and abundance of inositol phosphate-utilizing bacteria in terrestrial and aquatic ecosystems on Cape Cod, Massachusetts USA

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Inositol phosphates constitute the majority of the organic P in most soils, and also occur in freshwater and marine sediments, produced *in situ* or delivered as sediments in runoff from terrestrial ecosystems. Inositol phosphates accumulate in soils because they sorb strongly to clays and form insoluble precipitates with metal ions. This limits their availability to plants and microbes, and the higher inositol phosphates are conventionally considered to be of low bioavailability. However, *myo*-inositol hexa*kis*phosphate can be bioavailable under certain circumstances and is apparently quickly mineralized in coastal and marine ecosystems, where it may contribute to eutrophication.

Many organisms including bacteria, fungi, animals and higher plants express the enzyme phytase that liberates ester-linked phosphates from the inositol molecule. Although some plants produce phytase, their ability to utilize inositol phosphates is greatly increased in the presence of soil bacteria, suggesting that the ability to use inositol phosphates is not a universal trait and that microorganisms may be largely responsible for inositol phosphate mineralization in most ecosystems. Moreover, there is evidence that the ability to mineralize inositol phosphates depends upon environmental conditions as well as the microbial community. For example, the dephosphorylation of inositol phosphates in marine sediments was reported to be twice as fast under anaerobic conditions compared to aerobic.

To date, there have been no comprehensive surveys of community-level diversity of microorganisms that use inositol phosphates, or studies of how diversity varies among habitats. Understanding the diversity of microorganisms that can use inositol phosphates and the conditions under which this is favorable is an important step for understanding the global P cycle. The objective of this study was to compare the abundance and taxonomic richness of bacteria that can utilize inositol phosphates as a sole P source in three different ecosystems on Cape Cod, Massachusetts, USA: an upland forest soil, a freshwater marsh, and a marine marsh. We expected that the three ecosystems would have opposing gradients of inositol phosphate stocks (forest soils > freshwater marshes > marine marshes) and capacity for inositol phosphate utilization as measured by abundance and diversity of microbial communities (forest soils < freshwater marshes < marine marshes).

To test this hypothesis, we collected environmental samples from these three ecosystem types and cultured bacteria on appropriate agar media containing *myo*-inositol hexa*kis*phosphate as the sole P source. To understand how variation in the abiotic environment might influence the abundances of inositol phosphate-utilizing microbes, samples from each ecosystem were cultured under different conditions. We assumed that the majority of the microbes in surface soils of forests may be cultured aerobically, but have

different pH ranges, so forest soil samples were incubated in media at pH 5.4 and 7.0. We further assumed that surface sediments from freshwater and marine marshes that experience fluctuating redox conditions, so samples from these habitats were incubated both aerobically and anaerobically in media at pH 7.0. Abundance of culturable inositol phosphate-utilizing bacteria was estimated via most probable number counts, and phylogenetic diversity was assessed by amplifying, cloning, and sequencing 16S rRNA genes from the communities of bacteria recovered on the plates.

The three ecosystems differed substantially in soil and sediment chemistry. The forest soils were significantly more acidic (mean pH 4.4), than the aquatic ecosystems (mean pH 6.5–6.7). The freshwater marsh had three times as much organic C (30.2%) compared to the forest soil (8.0%), and almost twenty times higher concentrations than the salt water marsh (1.5%). Total P concentrations were tenfold higher in the freshwater marsh compared to the forest soil and the marine marsh, but the forest soil and the marine marsh did not differ. Phosphate monoesters were the dominant organic phosphates, constituting between 40 and 54% of the extracted P. The presence of *myo*-inositol hexa*kis*phosphate was detected in the forest and freshwater marsh soils. However, inositol hexa*kis*phosphates were not detected in the salt marsh sediment, in which phosphate monoesters were mainly alkaline hydrolysis products of phospholipids and RNA. Phosphate diesters constituted between 6 and 10% of the extracted P in the freshwater marsh and forest soils, respectively, mainly in the form of DNA. However, only a trace of phosphate diesters was detected in the salt marsh sediment.

Differences in the abundance of inositol phosphate-utilizing bacteria paralleled the differences in total P among the ecosystems. Inositol phosphate-utilizing bacteria were most abundant in the freshwater sediments (2.2×10^6 colony forming units / g dry weight), and nearly an order of magnitude less abundant in forest soils and marine sediments (1.1×10^5, 2.3×10^5 CFU/g dry weight, respectively). Within ecosystems, there were no statistically significant differences in abundance between cultivation conditions, although the plates incubated anaerobically consistently yielded higher counts in both the marine and freshwater sediments.

A comparison of the sequenced 16S rRNA genes to a large database revealed that the majority of the sequences fell within the proteobacteria division, with 46% of clones representing gamma proteobacteria, and 16% falling within the beta proteobacteria. The bacterial communities differed among ecosystems. The forest soil community represented a distinct assemblage compared to the marine and freshwater marshes, whereas the marine community was a subset of the taxa found in the freshwater marsh.

Improving our understanding of P transformations within and among ecosystems will require a better understanding of microbial transformations of organic P compounds. Our studies suggest that the potential for mineralization of inositol phosphates varies among ecosystems. Freshwater marshes contain an abundant and diverse community of bacteria that can use one of the most important forms of organic P, and thus may serve as an important hotspot for P transformations at the landscape scale.