LETTER

A proposal for isolating and testing phosphate-solubilizing bacteria that enhance plant growth

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Apart from the basic need for phosphorus in plant nutrition, there are three reasons why phosphate fertilization is a major agricultural research topic: (1) the price of fertilizers skyrocketed in recent times, making P fertilizers beyond the reach of many farmers in developing countries; (2) competition for high quality rock phosphate in other industries providing costlier products, such as food preservatives, anti-corrosion agents, cosmetics, fungicides, ceramics, water treatment, and metallurgy; and (3) sources of high quality phosphates are rapidly depleted and expected to be exhausted in less than 100 years. Consequently, agricultural research focused on: (1) low-grade rock phosphate (9-11 % P₂O₅ or less) as a source of fertilizer in the future because low-grade ore is available worldwide in large quantities, and (2) other sources of phosphate, such as struvite derived from wastewater treatment. This trend especially happens in developing countries where lack of available P for crops is more acute. Where rock phosphate used as fertilizer has insoluble phosphate as the main component, which is almost unavailable for plant growth, phosphate-solubilizing microorganisms are used to transform

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Laboratory of Biochemistry, Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, 13 Prosp. Entuziastov, 410049 Saratov, Russia insoluble phosphate into available, soluble phosphate (Antoun 2012). This is the most common and logical approach.

Review of the literature and chemical considerations related to phosphate solubilization by microorganisms (Bashan et al. 2012) have shown that the commonly used selection factor for this trait, tricalcium phosphate (TCP), is relatively weak and unreliable as a universal selection factor for isolating and testing phosphate-solubilizing bacteria (PSB) for enhancing plant growth. Most publications describing isolation of PSB employed TCP. The use of TCP usually yields many (up to several thousand per study) isolates of "supposed" PSB. When these isolates are further tested for direct contribution of phosphorus to the plants, only a very few are true PSB. Other compounds are also tested, but on a very small scale. These phosphates (P), mainly Fe-P, Al-P, and several Ca-P, are even less soluble than TCP in water. Because soils greatly vary in pH and several chemical properties, it appears that there is no metal-P compound that can serve as the universal selection factor for PSB.

Because current biological and chemical knowledge indicate that a universal selection factor for biological phosphate solubilization does not exist, the following conclusions and guidelines can be drawn.

- TCP, as a universal factor for isolating and evaluating PSB, is not a good selector according to much literature concerning failure with inoculated plants when using this compound for selection of PSB. Consequently, its use as a sole selector should be abolished and the general technique should be complemented.
- There are several other common, insoluble metal-P compounds, some less soluble than TCP, but none can replace it reliably for a universal selection factor because of possible chemical interactions.
- A combination of two or three metal-P compounds, when used together or in a tandem, should replace the sole TCP as an initial selection factor. These combinations may or may not include TCP in the mix in alkaline soils.

- The selection of the metal-P candidates for potential PSB will depend on the type of soil (alkaline, acidic, or organic-rich) where the PSB will be used. Adding Ca-P compounds (including rock phosphates) for alkaline soils, Fe-P and Al-P compounds for acidic soils, and phytates for soils rich in organic P is suggested.
- Production of a halo on a solid agar medium should not be considered the sole test for P solubilization. When colonies grow without a halo after several replacements of the medium, an additional test in liquid media to assay P dissolution should be performed.
- The few bacterial isolates that are obtained after such rigorous selection should be further tested for abundant production of organic acids.
- Isolates complying with the above criteria should be tested on a model plant as *the ultimate test* for potential P solubilization.

- Parameters related to P nutrition in plants should be tested, not growth promotion in general because promotion of growth, even by PSB, can be the outcome of other mechanisms.
- Consequently, we propose that new manuscripts that report initial isolation of "potential PSB" should not be considered for publication without exhaustive testing.

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