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Enzyme Dynamic in Plant Nutrition Uptake and Plant Nutrition

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Abstract

Soil contains enzymes, constantly interacting with soil constituents, e.g. minerals, rhizosphere and numerous nutrients. Enzymes, in turn, catalyse important biochemical reactions for rhizobacteria and plants, stabilize the soil by degrading wastes and mediate nutrient recycling. The available enzymes inside soil could originate from plants, animals or microbes. The enzymes that are produced from these organism could exhibit intracellular activities, at the cell membrane, interacting therefore with soil and its constituents, or extracellularly (so freely available). Therefore, vis-à-vis to plant nutrition, the (extra or sub) cellular localization has a key role. Typical major enzymes available in soil can be listed as dehydrogenases, hydrogenases, oxidases, catalases, peroxidases, phenol o-hydroxylase, dextransucrase, aminotransferase, rhodanese, carboxylesterase, lipase, phosphatase, nuclease, phytase, arylsulphatase, amylase, cellulase, inulase, xylanase, dextranase, levanase, poly-galacturonase, glucosidase, galactosidase, invertase, peptidase, asparaginase, glutaminase, amidase, urease, aspartate decarboxylase, glutamate decarboxylase and aromatic amino acid decarboxylase. An interesting strategy for improving the nutritional quality of the soil would be to inoculate microorganism to soil while giving attention to mineral or other compounds that affect enzyme activity in soil. Since, some elements or compounds could show both activation and inhibitory effect, such as Fe, Na, etc. metals, the regulation of their bioavailability is crucial.

Keywords: plant growth promoting rhizobacteria, amino acid, organic acid, nutrient element, hormone, plant physiology

1. Introduction

Soil contains, among many others, enzymes that are constantly interacting (regulating, being regulated by) with soil constituents, for example, minerals, rhizosphere and numerous nutrients. Enzymes, in turn, catalyse important biochemical reactions for rhizobacteria and plants, stabilize the soil by degrading wastes and mediate nutrient recycling [1].

The available enzymes inside soil could originate from plants, animals or microbes (bacteria or fungi). The enzymes that are produced from these organism could exhibit activities intracellular of the source organism, at its cell membrane, interacting therefore with soil and its constituents, or extracellularly (so freely available). Therefore, vis-à-vis to plant nutrition, or bioavailability of the macro- or micro-nutrients, the (extra or sub) cellular localization has a key role. Typical major enzymes available in soil can be listed as dehydrogenases, hydrogenases, oxidases, chief among those being glucose, aldehyde, urate, catechol, p-diphenol, ascorbate oxidases, catalases, peroxidases, phenol o-hydroxylase, dextransucrase, levan sucrase, aminotransferase, rhodanese, carboxylesterase, arylesterase, lipase, phosphatase, nuclease, nucleotidase, phytase, arylsulphatase, amylase, cellulase, laminarinase, inulase, xylanase, dextranase, levanase, poly-galacturonase, glucosidase, galactosidase, galactosidase, invertase, proteinase, peptidase, asparaginase, glutaminase, amidase, urease, inorganic pyrophosphatase, polymetaphosphatase, adenosine triphosphatase, aspartate decarboxylase, glutamate decarboxylase and aromatic amino acid decarboxylase [1].

An interesting strategy for improving the nutritional quality of the soil would be either inoculating microorganism to soil while giving attention to mineral or other compounds that affect enzyme activity in soil. Since, some elements or compounds could show both activation and inhibitory effect, such as Fe, Na, etc., metals, the regulation of their bioavailability is crucial.

Measurement of soil enzyme activity is important to determine soil characteristics, for further studies, such as, improving soil composition for plant growth using enzymes. A simple example can be given for proteases. Soil, when supplemented with proteases, would degrade proteins, thereby, increasing the amount of available nitrogen, which in turn is expected to improve plant nutrition. Similarly, soil supplemented with urease would increase bioavailable nitrogen level, and as such, this enzyme can be seen as a 'knob' for nitrogen regulation in soil and indirectly in plants. Finally, the use of enzymes, typically from microorganisms as plant growth promoting rhizobacteria (PGPR), is important not only from an economical perspective (improved crop yield), but also environmental point-of-view (reduced use of chemical fertilizers).

Enzymes are, at industrial scale, typically produced using either fungi or bacteria, either technology having advantages and disadvantages. While cultivation of bacteria is easier to handle (from both process and genetics perspective) and to scale up, fungi has typically larger portfolio of enzymes and the latter is more resilient to stress conditions, a characteristic of the production and application conditions.

Vis-à-vis plant nutrition, enzymes have crucial roles, tightly coupled to soil remediation as soil contains impurities in the form of heavy metals as well as polymers, for example, starch and cellulose residues, polyphosphate rocks, urea from N-cycle, oils and fats from either plants or animals that cannot be readily used by plants, in particular for nutrition. Enzymes

are then responsible to break these residues into forms that renders them bioavailable to plants. The application depends on the soil type, the content of the above-listed polymers or substances. The conventional approach is to use directly plant growth promoting rhizobacteria (PGPR) to improve growth and yield. The mechanism of action of those is actually the use of key enzymes (not limited to the five enzymes listed here) for plant growth promoting effect, making nutrient-rich materials bioavailable, shortening composting time yielding highly rich soil, improving thereby plant nutrition and allowing soil remediation.

Taken together, the use of some key enzymes are promising for soil conditioning and plant nutrition. As a follow-up, to gather better soil environment for plants, information on both organisms and especially the enzymes that are produced is of great value. This chapter focuses on this idea and provides key properties for a handful of enzymes, relevant to plant nutrition. The focus is on amylase, cellulase, lipase, phosphatase, phytase and urease, some key properties thereof and list of applications relevant to plant nutrition.

2. Amylase

Amylases are enzymes hydrolyzing glycosidic bonds of polysaccharides. Usually these are classified into three sub-classes as α -amylase (E.C. 3.2.1.1), β -amylase (E.C. 3.2.1.2) and γ -amylase (E.C. 3.2.1.3). α -Amylase is responsible in endo-hydrolysis of (1-4)- α -D-glucosidic linkages, while β -amylase is responsible in the hydrolysis of (1-4)- α -D-glucosidic linkages in polysaccharides to remove maltose units from non-reducing ends. γ -Amylase, in contrast, is responsible in the hydrolysis of terminal (1-4)- α -D-glucose residues from non-reducing ends of the chains for releasing of β -D-glucose.

All the three versions of this enzyme are produced by bacteria and fungi. α -amylase have been reported by *Acinetobacter* spp., *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus subtilis* and some thermophilic actinomycetes organisms as well, for example, *Thermomonospora curvata* and *Thermomonospora vulgaris* [2, 3], while β -amylase have been reported to be produced by *Bacillus cereus*, *Bacillus circulans*, *B. megaterium* and *Paenibacillus polymyxa* [4, 5]. Lastly, for γ -amylase, in addition to the *Bacillus* species, halophilic *Halolactibacillus* sp. and thermophilic organisms, for example, *Thermoactinomyces vulgaris* have been reported to produce this enzyme [6–8].

Amylases are reported to be active in a broad range of pH 1–13 [9, 10], yet β - and γ -amylases have narrower ranges. The optimum working pH range is reported to be from 2 [2] to 10.5 [11] for α -amylase, the other two being in a narrower range. As for the temperature, again α -amylases are active in a broad range or temperature from 20 [12] to 145°C [13]. Lastly, molecular weights range between 10 [14] and 240 kDa [15].

Despite the broad range of pH and temperature where the amylases are active, there is fairly long list of inhibitors for the microbiologically produced amylases: Ag^+ , Ba^{2+} , Ca^{2+} , Co^{2+} , Cu^{2+} , Fe^{2+} , Hg^{2+} , Mg^{2+} , Mn^{2+} , Ni^{2+} , Sr^{2+} , Zn^{2+} [14]; Cd^{2+} , iodoacetate [16]; ethylenediaminetetraacetic acid (EDTA), K^+ [17]; Na^+ , Triton X-100, Tween 20 [18]; phenylmethylsulfonyl fluoride (PMSF), 4-bromophenacyl bromide [19]; $\text{Bi}(\text{NO}_3)_3$, N-ethylmaleimide and sodium deoxycholate [20]

are reported to be inhibitors. Interestingly, sodium dodecylsulfate (SDS), urea and 2-mercaptoethanol are reported to be both activating [21, 22] and inhibiting [17, 18] compounds.

The production of enzymes is typically performed in submerged fermentation, less often via solid state fermentation, typically under mesophilic conditions, moderate pH and temperature (30–50°C, mostly in 37°C; pH range of 3–9, mostly at 7) in chemically defined ((NH₄)₂HPO₄ as N-source, KH₂PO₄ as K-source) or complex media (yeast extract as N and K source), lactose, maltose glucose or starch as C-source, using chiefly *Bacillus* species [23–27]. Additionally, agro-wastes are also used as substrates and inducers as coconut oil cake, wheat/rice bran, spent brewing grain, cassava bagasse, jackfruit or tamarind seed powder, palm kernel, olive oil or mustard oil cake and rice husk [27].

3. Phosphatase

Phosphatases belong to the enzyme group responsible in the hydrolysis of ester-phosphate bonds which releases phosphates. These are sub-classified as phosphomonoesterases (EC 3.1.3.x), phosphodiesterases (EC 3.1.4.x), enzymes that hydrolyze phosphorus-containing anhydrides (EC 3.6.1.x), P-N bonds (EC 3.9.1.x) and various groups that act on this bonds. From an application point of view, these are grouped as alkaline, acid phosphatases and inorganic diphosphatases. The microbial producers of these enzymes are numerous, including *B. subtilis* [28], *Escherichia coli* [29] and *Pseudomonas aeruginosa* [30] for alkaline phosphatase; *Acidithiobacillus thiooxidans* [31], *E. coli* [32] and *Lactobacillus curvatus* [33] for acid phosphatase and *Geobacillus stearothermophilus* [34], *Rhodobacter capsulatus*, *Rhodospseudomonas palustris* [35] for Inorganic diphosphatase.

The large portfolio of phosphatases works in a broad range of pH and temperature. For the pH, the phosphatases are reported to work optimally between 2.5 [36] and 12.5 [37]. As for the temperature, active ranges are reported to be between 5 [38] and 95°C [39], while optimally, the enzyme works between 20 and 70°C [40, 41]. With different pockets or binding sites, there is also a range for the molecular weight, from 32.5 [42] to 128 [43] kDa.

Several agents are reported to inhibit the phosphatases. These are ascorbate, dithiothreitol, NaF, molybdate, NaBH₄, sodium lauryl sulfate, tartrate [31], 2-mercaptoethanol, BaCl₂, CaCl₂, hexametaphosphate, HgCl₂, MnCl₂, p-chloromercuribenzoate (PCMB), PMSF, tripolyphosphate and ZnCl₂ [33]. In contrast, some organic acids, for example, citrate, pyruvate, succinate [32], 1,10-phenanthroline, EDTA [33] have been found to stimulate enzyme activity.

4. Lipase

Lipases (EC 3.1.1.x) are enzymes degrading lipids. In literature, most of the studied and reported lipases are triacylglycerol lipases (EC 3.1.1.3), while additionally there are carboxylesterase (EC 3.1.1.1) which hydrolyze carboxylic ester bonds, arylesterase (EC 3.1.1.2) also acting on carboxylic esters but more specifically on phenolic esters, phospholipase A2 (EC 3.1.1.4) again hydrolyzing carboxylic esters specifically on phosphatidylcholine. It should be noted that distinguishing each of these enzymes is rather challenging as they have similar activities.

The producing organisms span the fungi and bacteria, in particular *B. subtilis* [44], *E. coli* [45] for EC 3.1.1.1 (carboxyl esterase), *Gluconobacter oxydans* [46] and *Lactobacillus casei* [47] for EC 3.1.1.2 (arylesterases) and *Acinetobacter calcoaceticus*, *B. subtilis*, *Chromobacterium viscosum*, *Micrococcus freudenreichii*, *Lactobacillus delbruckii*, *P. aeruginosa* and *Streptococcus lactis* [48] for EC 3.1.1.3 (triacylglycerol lipase)

Bacterial lipases has a pH working range between 4 [49] and 12 [50], while optimum pH is reported to vary between 6 [51] and 11 [52]. As for the temperature, there is a large range between 0 [53] and 100°C [54], while the optimum temperature for enzyme activity vary between 10 [55] and 90°C [50]. A span of molecular weights is reported for this enzyme (bacterial variants) from 11 [56] to 840 [57] kDa.

Metals ions such as Cu²⁺, Fe²⁺, Fe³⁺, Hg²⁺, Zn²⁺, Ag⁺, Co²⁺, Ni²⁺, Na⁺ and ascorbic acid are reported to have inhibitory effect on the carboxylesterase activity [58, 59] as well as sodium dodecyl-sulfate (SDS), diisopropylfluorophosphate, eserine, sodium fluoride [60] and phenylmethyl-sulfonyl fluoride (PMSF) [61]. Organic solvents such as acetone, EDTA, ethanol, isopropanol, PMSF and SDS [49] are reported to inhibit triacylglycerol lipases [62, 63]. Under lab conditions, Triton X-100, Tween-20, Tween-40, Tween-80 [64], 1,4-dioxane, acetone, dimethyl sulfoxide, ethanol and tetrahydrofuran [65] are reported activators to carboxyl esterases. Interestingly, acetone, Brij 52, cholic acid, deoxycholic acid, isopropanol, Dimethyl sulfoxide (DMSO), lithocholic acid, rhamnolipid and sodium deoxycholate are also reported as activators for triacylglycerol lipases [66].

For production of enzyme, apart from the above-listed organisms, see **Table 1**.

Microorganism	Media	Conditions	Production mode	References
<i>Anaerovibrio zipolytica</i> 5s	g/100 mL: 0.6 g Difco yeast extract; 0.75 g casein hydrolysate; 15 mL 0.3% (w/v) dipotassium hydrogen phosphate; 15 mL 0.3% (w/v) potassium dihydrogen phosphate; 0-1 mL 0.1% (w/v) resazurin and 10 mL 5% (w/v) glycerol, 0.5% (w/v) cysteine HCl and 6% (w/v) sodium bicarbonate	38°C	Batch via 300 mL vessels	[67]
<i>Bacillus coagulans</i> BTS-3	peptone (0.5%), yeast extract (0.5%), NaCl (0.05%), CaCl ₂ (0.005%) and olive oil (1.0%, emulsified with gun)*, pH 8.5	48 h, 55°C, 170 rpm,	Batch via 250 mL erlenmeyers (50 mL working volume)	[68]
<i>Pseudomonas</i> sp.	Ground soybean (2.0%), corn-steep liquor (2.0%), soluble starch (1.0%), K ₂ HPO ₄ (0.5%) and NaNO ₃ (0.5%) and the pH 9.0	30°C, 72 h, 150 rpm	Batch via 500 mL erlenmeyer (working volume of 50 mL)	[69]

* Besides olive oil, coconut oil, castor oil, groundnut oil, mustard oil, sunflower oil, Tween 20, Tween 80, cottonseed oil and soybean oil is studied as a carbon source. Beside peptone and yeast extract, gelatin and urea is also studied as organic nitrogen sources. Besides ammonium sulphate, ammonium nitrate, potassium nitrate and L-asparagine are also studied as inorganic nitrogen sources.

Table 1. Lipase production studies and the reported conditions.

5. Phytase

Phytases are enzymes that hydrolyze phytic acid which is an organic phosphorus source and makes inorganic usable phosphorus. Bacterially produced phytases are 3-phytase (EC 3.1.3.8), 4-phytase (EC 3.1.3.26) and protein-tyrosine-phosphatase (PTP, EC 3.1.3.48). Besides PTP, the other enzymes differentiate at which carbon they attack and take out the phosphorus in phytic acid. Several reports are available on the production of phytases. The organisms used are *Aerobacter aerogenes*, *B. amyloliquefaciens*, *B. subtilis*, *Enterobacter* sp., *E. coli*, *Klebsiella aerogenes*, *Lactobacillus amylovorus*, *Pseudomonas* sp., *Selenomonas ruminantium* [70] for three and four phytases and *B. subtilis*, *M. tuberculosis*, *S. aureus* [71–73]; typically grown under complex media (tryptone, yeast extract and NaCl and sugars, for example, lactose as inducer)

The activity of bacterially produced phytases change with pH, ranging from 2 [74] to 10 [75], while the optimum pH range is narrower (from 2.7 [76] to 8.5 [77]). As for the temperature, optimum working range is between 20 [78] and 80°C [79] due to the presence of some thermophilic organisms [70, 79]. The molecular weight range is found to be between 12.8 [80] and 700 [70] kDa, again depending on the producing host.

Similar to the other enzymes, several metal ions are reported to inhibit the phytase activity. These include Ba²⁺, Cd²⁺, Cu²⁺, Li⁺, Mg²⁺, Mn²⁺ and Zn²⁺ [77, 81], while EDTA is considered as an activator compound [75]

6. Urease

An important enzyme for plant nutrition, in particular for N-cycle is Urease (EC 3.5.1.5), catalysing the conversion of urea to carbon dioxide and ammonia:



This enzyme is produced by bacteria, fungi as well as plants. Some bacterial producers are listed as *A. aerogenes*, *Arthrobacter oxydans*, *Bacillus pasteurii*, *Brevibacterium ammoniagenes*, *Brucella suis*, *E. coli*, *Helicobacter pylori*, *Proteus mirabilis*, *Providencia stuartii*, *S. ruminantium*, *Sporosarcina pasteurii*, *Staphylococcus saprophyticus* and *Ureaplasma urealyticum* [82–84], while the following organisms are reported to produce acid urease: *Arthrobacter mobilis*, *Lactobacillus fermentum* and *Streptococcus mitior* [82]. These are typically grown in batch mode, under complex (yeast extract, peptone and glucose) or chemically defined medium conditions, mesophilic temperatures, with urea as the inducer of the enzyme production [85, 86].

The pH range whereby the enzyme works optimally is 2–9 [87–90], while optimum temperature ranges from 20 to 70°C [91–94]. Molecular weights can vary from 11.1 [82] to 600 [90] kDa. Listed inhibitors are methylurea, thiourea, acetohydroxamic acid, phenylphosphorodiamidate, H₃PO₄, 2-mercaptoethanol, boric acid, Iodoacetamide, Iodoacetic acid, N-Ethylmaleimide, 5,5'-Dithiobis (2-nitrobenzoic acid) (DNTB) [95]; 12-hydroxytetradecanoic acid, 3-hydroxytetradecanoic acid, 6-hydroxytetradecanoic acid [96, 97] and several metal ions [98, 99]. Glycerol, n-octylglucoside, polyethylene glycol (PEG), sodium dodecyl sulfate (SDS),

Triton X-100 have some activatory effect in certain amounts [100]. It is worth noting that urease is nickel-containing metalloenzyme, as a result of which requires to a certain level nickel metal [101], as usual higher concentrations have inhibitory effect [99].

7. Cellulase

Cellulase (EC 3.2.1.4) is an important enzyme, naturally produced by bacteria, fungi and protozoa, in particular by necrophilic microorganisms, and is responsible to hydrolyze (1-4)-beta-D-glucosidic linkages in cellulose, which is by far the most abundant organic compound, totalling to almost 50% of the biomass synthesised by photosynthetic fixation of CO₂. Cellulases also degrade cellulose available in lichenin and cereal beta-D-glucans. As such, it is a key enzyme in degradation of the most abundant polymer on earth. The bacterial producers are listed as *Acetivibrio cellulolyticus*, *B. Subtilis*, *B. Amyloliquefaciens*, *Cellulomonas fimi*, *Pseudomonas fluorescens*, *Ruminococcus albus*, *Thermobifida fusca*, *Thermotoga maritima* [102–104].

Ag⁺, Hg²⁺, Mn²⁺, iodoacetamide, N-bromosuccinimide [105]; Cu²⁺, Pb²⁺, Fe²⁺, Sn²⁺, ethylenediaminetetraacetic acid (EDTA) [106]; NiCl₂, SrCl₂ [7], sodium dodecyl sulphate (SDS) [107]; Cd²⁺, Co²⁺, Zn²⁺ [108] and 4-hydroxybenzoic acid, syringaldehyde, trans-cinnamic acid, vanillin [109] are shown to inhibit bacterial-originated cellulases. Arabitol, dithiothreitol, erythritol, glycerol, histamine [106]; N-ethylmaleimide [110]; CH₃COONa, NH₄Cl, NH₄NO₃ [111] and Ca²⁺ [107] are shown that activate enzyme. For production of the cellulase enzyme, reported conditions are listed in **Table 2**.

Bacteria	Media	Conditions	Production mode	References
<i>Bacillus</i> sp. AC1	Yeast 2.5 g/L, Tryptone 2.5 g/L, carboxymethyl cellulose (CMC) (low viscosity) 2.5 g/L, (NH ₄) ₂ SO ₄ 1 g/L, KH ₂ PO ₄ 0.5 g/L, K ₂ HPO ₄ 0.5 g/L and MgSO ₄ 0.2 g/L	30°C, 2 d	Submerged fermentation	[112]
<i>Bacillus</i> sp. NZ	Carboxymethyl cellulose 5 g/L, peptone 5 g/L, yeast extract 5 g/L, KH ₂ PO ₄ 1 g/L, MgSO ₄ ·7H ₂ O 0.2 g/L, NaCl ₃ g/L	45°C, 24–48 h, pH 9–10	Submerged fermentation via 250 mL erlenmeyers	[113]
<i>Bacillus subtilis</i> CBTK 106	10 g of banana fruit stalk with Na ₂ HPO ₄ ·2H ₂ O 1.1 g/L, NaH ₂ PO ₄ ·2H ₂ O 0.61 g/L, KCl 0.3 g/L, MgSO ₄ ·7H ₂ O 0.01 g/L	35°C, 72 h, pH 7.0, initial moisture content is 65%	Submerged fermentation via 250 mL erlenmeyers	[114]*

* Besides banana fruit stalk, wheat bran, rice bran and rice straw was tested as a substrate, but banana fruit stalk showed more cellulase activity. Also in this article with same media solid state and submerged fermentation was compared.

Table 2. Producing conditions of cellulase from bacteria.

8. The use of enzymes for plant nutrition

The use of enzymes for plant nutrition is typically mentioned within compost preparation context and optimization and/or speeding of this process. In an early work, Hankin et al. [115] studied several microorganisms from the extracellular enzyme production perspective from composting leaves and concluded that depending on the substrate available the microbial community produced tailor-made enzymes, and this production process was highly dependent on temperature [115]. The portfolio of enzymes produced covered all major enzymes. The temperature of the compost core increased significantly, when compared to the outer regions contacting with air. In general, Amylase is typically seen as one of the necessary enzymes to speed-up composting, yet of low importance. As for the use of the phosphatase enzyme for plant nutrition, there are several studies focusing on the soil phosphatase (both alkaline and acidic) activity, hinting the soil-bacteria collaboration. Tiquia et al. [116] reported the dynamics and enzyme activity during composting of poultry litter and yard trimmings, focusing on 19 different enzymes of different microbial groups in soil [116]. Similarly, Garcia et al. [117] after detailed biochemical analysis of biochemical parameters reported that highest phosphatase activity is found on sewage sludge [117].

The relation with enzymatic activity and compost state is so tight that enzymatic activity has been studied as indicator of composting process. Mondini et al. [118] reported the results of such study, whereby they concluded that drying the compost expectedly decreased the activity, but more importantly, measuring the activity of four enzymes (β -glucosidase, arylsulphatase, acid and alkaline phosphatase) in air-dried compost would be a fast and reliable method to follow composting process. Similar outcomes have been reported in Margesin et al. [119], focusing this time to lower temperatures. Herrmann and Shann [120] concluded that cellulase activity could be used as an indicator of stability, while lipase activity indicated compost maturity [120].

Lee et al. reported the positive effect of compost on lettuce growth as two to three times increase in fresh weight of the lettuce. They focused in particular to phosphatase and dehydrogenase activity [121]. Focusing more on the fats mixtures and the effect of lipase on co-composting sludges, Gea et al. [122] reported 85% reduction in fat content, with an initial fat content of 30%. The authors note that due to hydrophobic nature of the fats, the moisture content needs to be maintained above 40%. Krzywy-Gawrońska [123] focused on urease and dehydrogenase activity in compost-fertilized soil, in a 2-year field trial, and found increased level of organic carbon, nitrogen and phosphorus in fertilized soil, clearly pointing to highly nutritious soil.

9. Conclusions

Enzymes are key players in a plethora of biological processes, plant nutrition is no exception. Depending on the soil content and residues that it carries, different enzymes play key roles in rendering soil nutrient-rich; an immediate application is the composting process and PGPR-soil-plant interactions. This important area calls for further research not only on the plant side but also on the enzyme side and more importantly on applications to specific soil types. This

knowledge will further facilitate decreased use of chemical fertilizers and will create avenues for organic farming practices.

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